Early onset airway obstruction in response to organic dust in the horse

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Deaton CM, Deaton L, Jose-Cunilleras E, Vincent TL, Baird AW, Dacre K, Marlin DJ. Early onset airway obstruction in response to organic dust in the horse. J Appl Physiol 102: 1071–1077, 2007. First published December 7, 2006; doi:10.1152/japplphysiol.00264.2006.—Equine recurrent airway obstruction (RAO) has been used as a naturally occurring model of human asthma. However, it is unknown whether there is an early-phase response in RAO. The aim of this study was to determine whether exposure to organic dust induces immediate changes in lung function in RAO-affected horses, which could be mediated by airway mast cells. Six RAO-affected horses in remission and six control horses were challenged with hay-straw dust suspension by nebulization. Total respiratory resistance at 1 Hz, measured by forced oscillation, was increased from 0.62 ± 0.09 cmH2O·1−1·s (mean ± SE) to 1.23 ± 0.20 cmH2O·1−1·s 15 min after nebulization in control horses (P = 0.023) but did not change significantly in the RAO group. Total respiratory reactance at 1 Hz (P = 0.005) was significantly lower in the control horses (−0.77 ± 0.07 cmH2O·1−1·s) than in the RAO group (−0.49 ± 0.04 cmH2O·1−1·s) 15 min after nebulization. Bronchoalveolar lavage fluid (BALF) histamine concentration was significantly elevated 10 and 20 min postnebulization in control horses but not in RAO horses. Minimum reactance at 1 Hz in the early postnebulization period significantly correlated with both prechallenge BALF mast cell numbers (r = −0.65, P = 0.02) and peak BALF histamine concentration postnebulization (r = −0.61, P = 0.04). In conclusion, RAO horses, unlike human asthmatic patients, do not exhibit an early-phase response. However, healthy control horses do demonstrate a mild but significant early (<20 min) phase response to inhaled organic dust. This response may serve to decrease the subsequent dose of dust inhaled and as such provide a protective mechanism, which may be compromised in RAO horses.

equine; lung function; allergen

HUMAN ASTHMATIC PATIENTS develop an early-phase bronchoconstriction within minutes of exposure to inhaled allergens. Subsequently, a late asthmatic response with the continuation of airway obstruction and the development of airway inflammation can also be observed. Mast cells play an important role in the early asthmatic response in both atopic and nonatopic human asthma (9, 42). Allergen can activate mast cells by cross-linking to high-affinity IgE receptors (FcεRI) (40). The activation of mast cells in response to inhaled allergens results in the release of mast cell mediators, including histamine, tryptase, chymase, cysteinyl leukotrienes, and prostaglandin D2 (8, 14). These mediators can induce airway smooth muscle contraction and the observed early-phase asthmatic response (8, 14). Mast cells also release proinflammatory cytokines that, together with other mast cell mediators, have the potential to induce neutrophil and eosinophil infiltration and bronchoconstriction involved in the late-phase asthmatic response (4, 8, 14). Activation of other mast cell receptors, in addition to FcεRI, such as KIT and complement component 3a receptor, can also induce mast cell degranulation or amplify FcεRI-mediated mast cell activation (1, 3, 16, 21).

Recurrent airway obstruction (RAO) is an equine inflammatory airways disease characterized by the development of a late-phase response after exposure to organic dust, typically from hay and straw. The late-phase response comprises airway obstruction, airway inflammation, and airway hyperreactivity. RAO has been suggested to be the most similar naturally occurring disease in animals to human asthma (35) and has been used as model of human asthma (2, 6, 17, 39).

Few studies have investigated the role of mast cells in the pathogenesis of RAO. The degranulation of basophilic blood cells and pulmonary mast cells in vitro in response to mold spores has been demonstrated to be greater in cells derived from RAO-affected horses than in those derived from control horses (13, 19). Exposure to hay and straw for 5 h increased the concentration of histamine in bronchoalveolar lavage (BAL) fluid (BALF) in RAO-affected horses but not in control horses (26). In contrast, a 30-min exposure to hay and straw did not result in a significant increase in BALF histamine concentration (26). However, histaminase activity in the lung (22) may have resulted in the degradation of histamine released during an early-phase response by the time the 30-min sample was collected. Mast cells that contain chymase rather than tryptase appear to play a role in RAO, with the number of chymase-positive mast cells correlating with the severity of neutrophil infiltration (41). Mediators that are released from mast cells, but not activated neutrophils, have been implicated in the induction of bronchoconstriction in RAO (28). Levels of IgE antibodies in response to mold allergens were greater in BALF from RAO horses when in remission, as well as from those demonstrating clinical signs of disease, compared with BALF from healthy horses (18, 33). Enhanced production of IL-4 and IL-13 may elevate allergen-specific IgE generation from B cells during the early stages of RAO (20). IgE-mediated reactions do not appear to be important in the late-phase response in RAO (41).

The duration of organic dust exposure required before RAO-affected horses develop clinical evidence of airway obstruction varies from hours to days (15, 33), and clinical evidence of an

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early/ immediate obstructive response is not apparent. However, the presence of a subclinical early response has not been investigated. If a subclinical early-phase response to inhaled dust does occur in RAO, the proinflammatory mediators released could exacerbate the late-phase response observed in RAO (12, 23). Therefore, modulating or blocking an early-phase response could be an important therapeutic opportunity. We hypothesized that RAO-affected horses undergo an early-phase subclinical airway obstruction mediated by mast cells when exposed to organic dust. The aims of the study were to determine whether an early-phase response to nebulized organic dust challenge occurs in RAO and whether the magnitude of an early-phase response is related to the late-phase inflammatory response.

MATERIALS AND METHODS

The experimental protocol was approved by the Ethical Review Committee of the Animal Health Trust and conformed to the Home Office Animals (Scientific Procedures) Act 1986.

Animals. Six RAO-affected horses [4 geldings, 2 mares; mean age 14 ± 0.7 (±SE) yr and 510 ± 12 kg body wt] and six control horses (4 geldings, 2 mares; mean age 11 ± 0.8 yr and 447 ± 22 kg body wt) were studied after between 15 and 20 wk at pasture. Exposure to organic dust, by stabling with hay and straw, had previously been demonstrated to induce reversible lung dysfunction in the RAO group (increase in respiratory resistance from 0.73 ± 0.06 cmH2O/L11002 to 1.47 ± 0.12 cmH2O/L11002 at 1.0, 2.0, 3.0, and 5.0 Hz by forced oscillatory mechanics (11)). BAL was performed by instilling 500 ml of 0.9% saline at 37°C as described previously (10) but without local anesthesia of the conducting airways. BALF nucleated cell counts were determined manually on cytospin preparations, and BALF histamine concentration. Twenty-four hours after nebulization, BALF neutrophil counts were significantly higher than before nebulization (Table 1). In the control group, BALF lymphocyte counts were decreased 10 min postnebulization (P = 0.016), and BALF total nucleated cell counts and macrophage counts were decreased 10 min (P = 0.003 and P = 0.007, respectively) and 20 min (P = 0.022 and P = 0.020, respectively) postnebulization and returned to baseline values within 24 h (Table 1). Twenty-four hours after nebulization, BALF neutrophil counts were significantly higher than before nebulization in the RAO group (P < 0.0001) but not in the control group (Table 1).

BALF histamine concentration. In the control group, the concentration of histamine in BALF was higher 10 min (P = 0.027) and 20 min (P = 0.036) after nebulization compared with prenebulization (Fig. 1). BALF histamine concentration was not significantly altered after nebulization in the RAO group (Fig. 1).

Pulmonary function. Neither total respiratory resistance nor reactance at 1, 2, 3, or 5 Hz was significantly changed by organic dust nebulization in the RAO group (Figs. 2 and 3). Total respiratory resistance at 1 Hz (P = 0.023) and 2 Hz (P = 0.05) was increased 15 min after nebulization in control horses (Fig. 2). Total respiratory resistance at 3 and 5 Hz was not

RESULTS

BALF cytology. BALF cytology was not significantly altered 10 or 20 min after challenge with organic dust in RAO-affected horses (Table 1). In the control group, BALF lymphocyte counts were decreased 10 min postnebulization (P = 0.016), and BALF total nucleated cell counts and macrophage counts were decreased 10 min (P = 0.003 and P = 0.007, respectively) and 20 min (P = 0.022 and P = 0.020, respectively) postnebulization and returned to baseline values within 24 h (Table 1). Twenty-four hours after nebulization, BALF neutrophil counts were significantly higher than before nebulization in the RAO group (P < 0.0001) but not in the control group (Table 1).
compared with control horses \((P < 0.003)\). BALF mast cell number prenebulization also correlated with peak BALF histamine concentration post-nebulization, sedation and BAL did not result in significant changes after nebulization in control horses (Fig. 2). Total respiratory reactance at 1 Hz \((P = 0.005)\) and 2 Hz \((P = 0.03)\) was significantly lower in the control horses than in the RAO group 15 min after nebulization (Fig. 3). Reactance at 3 Hz was lower 5 and 15 min postnebulization compared with prenebulization in the control group \((P = 0.035\) and \(P = 0.015\), respectively; Fig. 3). Resonant frequency was not altered by organic dust nebulization in the RAO group (Fig. 4). In the control group, resonant frequency was significantly increased 15 min postnebulization compared with before nebulization \((P = 0.025)\) and 24 h postnebulization \((P = 0.009)\) (Fig. 4).

### DISCUSSION

In contrast to our hypothesis, an early-phase response to organic dust consisting of decreased lung function and increased BALF histamine concentration was identified in control horses rather than in the RAO group. The results also differ from studies in human subjects, where asthmatic but not nonasthmatic individuals demonstrate an early-phase response to inhaled allergen.

The study design included sedation before nebulization of the organic dust. This procedure may have decreased the effects of organic dust on lung function. The measurements taken before nebulization were performed not only to determine baseline lung function but also to account for any effects of sedation and the procedure of BAL on lung function. In the control horses and RAO-affected horses in remission before nebulization, sedation and BAL did not result in significant changes.

### Correlations

\begin{table}
\begin{tabular}{|c|c|c|c|}
\hline
 & Pre & +10 min & +20 min & +24 h \\
\hline
\textbf{Total nucleated cell count, number/μL}} & & & & \\
Control & 275±29 & 122±28* & 142±23* & 395±57 \\
RAO & 280±83 & 283±111 & 163±46 & 523±232 \\
\hline
\textbf{Macrophage, %}} & & & & \\
Control & 53±6 & 52±7 & 49±12 & 50±13 \\
RAO & 49±8 & 53±8 & 45±5 & 34±11 \\
\hline
\textbf{Lymphocyte, %}} & & & & \\
Control & 39±7 & 39±3 & 41±9 & 38±9 \\
RAO & 44±8 & 42±8 & 49±5 & 37±12 \\
\hline
\textbf{Neutrophil, %}} & & & & \\
Control & 2±2 & 2±1 & 3±2 & 6±6 \\
RAO & 2±2 & 2±2 & 3±2 & 26±11*† \\
\hline
\textbf{Mast cell, %}} & & & & \\
Control & 6±2 & 6±5 & 6±5 & 4±3 \\
RAO & 4±2 & 3±2 & 3±3 & 2±1 \\
\hline
\textbf{Macrophage count, number/μL}} & & & & \\
Control & 145±17 & 64±15* & 66±9* & 204±37 \\
RAO & 139±43 & 155±64 & 72±21 & 147±49 \\
\hline
\textbf{Lymphocyte count, number/μL}} & & & & \\
Control & 110±15 & 47±10* & 60±12 & 151±28 \\
RAO & 127±42 & 117±46 & 82±26 & 186±70 \\
\hline
\textbf{Neutrophil count, number/μL}} & & & & \\
Control & 6±1 & 2±1 & 4±1 & 20±7 \\
RAO & 4±1 & 4±2 & 4±1 & 18±114* \\
\hline
\textbf{Mast cell count, number/μL}} & & & & \\
Control & 14±1 & 8±3 & 10±4 & 11±6 \\
RAO & 9±2 & 7±2 & 4±1 & 7±2 \\
\hline
\end{tabular}
\caption{Bronchoalveolar lavage fluid cytology in control horses and horses affected by RAO before and after nebulization of organic dust}

Values are means ± SE. RAO, recurrent airway obstruction. *Significantly different compared with prenebulization (Pre) \((P \leq 0.02)\). †Significantly different compared with control horses \((P = 0.003)\).
changes in lung function at the time points measured. However, in RAO-affected horses with airway obstruction, sedation with \( \alpha_2 \)-agonists has been demonstrated to decrease airway resistance (5).

The technique of forced oscillation was used to measure lung function instead of simultaneous measures of transpulmonary pressure and flow rate, as the forced oscillation technique is noninvasive and can identify frequency-dependent changes (44). Lung function measurements with the two techniques have previously been demonstrated to closely correlate (43). The forced oscillation technique not only measures the reactance and resistance of peripheral intrathoracic airways but also of extrathoracic airways, including the larynx. Alterations in lung function measured in control horses after nebulization may therefore not be a result of small airway obstruction. However, the observations of 1) a negative frequency dependence in total respiratory resistance and 2) a more negative reactance at frequencies below resonant frequency in control horses 15 min postnebulization are consistent with distal small airway obstruction rather than upper airway obstruction in humans (34). Saline nebulization was not used as a control. However, we have previously determined that lung function is not altered up to 10 min after saline nebulization in either control or RAO horses (Deaton and Marlin, unpublished observations).

Despite the measurement of statistically significant decreases in lung function in the control group after organic dust nebulization, no clinical signs of increased respiratory effort (such as increased respiratory rate, abdominal effort, or nasal flaring) were observed, suggesting that the degree of airway obstruction was mild. It is plausible that the early-phase response of the control horses may be a protective mechanism. In healthy human subjects, the induction of bronchoconstriction before inhalation of Teflon particles significantly reduced the fraction of particles deposited in the peripheral airways and alveoli (36). Furthermore, the fraction of particles deposited in the peripheral airways and alveoli was considerably diminished by only a relatively small degree of bronchoconstriction (36). Therefore, in control horses, after a mild early-phase airway obstruction, the subsequent dose of inhaled organic dust reaching the alveoli would be decreased, which would reduce the stimulus for the late-phase response. The absence of a significant early-phase response in RAO-affected horses would conversely render them more susceptible to higher dust deposition in the alveoli and peripheral airways and consequently result in an exaggerated late-phase response. The deficiency of a significant early-phase response in RAO horses may reflect the absence of sufficient numbers of pulmonary mast cells to produce adequate amounts of mediators to induce airway smooth muscle contraction. Combining data from both the control and RAO groups, decrements in lung function measured during the early phase were associated with the BALF histamine concentration and BALF mast cell number. Mast cells can release a range of mediators in addition to histamine, such as 5-hydroxytryptamine and leukotriene D4, which are also able to enhance airway smooth muscle contraction in the horse by augmenting cholinergic responses (28).

The decrease in lung function observed in the control horses may not solely be explained by airway smooth muscle contraction. In human asthmatic patients, local allergen rapidly induced hyperemia, edema, and bronchial narrowing (27). In a guinea pig model of human asthma, increased mucous secre-
tion and pulmonary edema occurred immediately after exposure to allergen in sensitized animals (38). Pulmonary edema appeared to reflect airway epithelial cell disruption induced by inflammatory cells (38). In the present study, there was no evidence for mucous accumulation or inflammatory cell infiltration into the airways in control horses after nebulization of organic dust.

The severity of airway inflammation induced by organic dust is dose dependent in both control and RAO horses, and the threshold for induction of inflammation is far greater in the control group (29). The dose of dust suspension utilized in the present study was the highest dose administered to RAO horses by Pirie et al. (29). Three different hay dust suspensions induced marked airway inflammation in RAO horses when administered at the dose used in the present study (29). For BAL performed 24 h after nebulization, an adjacent airway was lavaged to that sampled 10 min after nebulization to minimize the effects of the previous BAL on BALF cytology. Sweeney et al. (37) demonstrated that any effects of a BAL on BALF cytology from an adjacent airway were eliminated within 48 h. Repeating the BAL procedure after 6 and 24 h on different lung regions did not significantly alter BALF cytology in healthy horses (25). Similarly, in the present study, BALF neutrophil numbers were only significantly elevated in the RAO group and not in the control horses 24 h after nebulization.

![Fig. 3](image3.png)

![Fig. 4](image4.png)

![Fig. 5](image5.png)
nebulization and BAL of an adjacent airway. Therefore, the airway inflammation induced 24 h after nebulization in the RAO group is likely to have been induced by organic dust, in agreement with Pirie et al. (29).

The absence of a significant correlation between measures of lung dysfunction associated with the early-phase response and BALF neutrophil numbers 24 h postnebulization suggests that the lack of an early-phase airway obstruction is not the predominant factor in RAO-affected horses developing a late-phase inflammatory response. However, the power of the study may have been insufficient to determine a significant correlation. In addition, the airway inflammation may have partially resolved by 24 h as neutrophil infiltration into the pulmonary epithelial lining fluid occurs within 6 h of organic dust nebulization in RAO horses (29). Inhaled dust may induce the late-phase neutrophilic infiltration in RAO observed in the present study by enhancing the production of proinflammatory mediators from a range of cell types in addition to mast cells, such as alveolar macrophages, lymphocytes, and airway epithelial cells (6, 7, 24, 41). Reduced numbers of macrophages and lymphocytes were evident in control horses immediately after challenge. This reduction was unanticipated, and we have no data to suggest a mechanism for such effects. The inflammatory process is likely to be complex, given the diverse composition of organic dust. Components of organic dust have synergistic effects on the development of airway inflammation when inhaled together (30, 31).

Another important component of the late-phase response in RAO is the induction of reversible airway obstruction. However, in the present study, increases in resistance and decreases in reactance were not detected 24 h after nebulization compared with baseline measurements in either RAO or control horses. Similarly Pirie et al. (29) determined that dynamic lung compliance, maximum change in transpulmonary pressure, and a clinical score of respiratory effort were not altered in RAO horses 5 h after nebulization of hay dust suspension. These findings are likely to reflect the short duration of the challenge compared with continuous exposure to organic dust by stabling with hay and straw. Horses were exposed to organic dust by nebulization of a hay-straw dust suspension rather than stabling with hay and straw so that the dose of organic dust that each horse received could be standardized. To confirm that the early-phase response in control horses has a protective effect on lung function, horses would have to be exposed to organic dust after the induction of the early-phase response.

In conclusion, exposure to organic dust results in a mild early-phase airway obstruction in healthy horses but not in horses affected by RAO. This early-phase response appears to be regulated by mast cell-derived mediators and may be a protective mechanism to decrease the dose of organic dust reaching the peripheral airways.

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