Bronchoalveolar Lavage Cytology in Ponies with Chronic Airway Disease

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Chronic Airway Disease

Chronic airway disease of horses is a poorly understood clinical syndrome ranging in severity from a mild bronchitis with overproduction of mucus, through bronchoconstriction to chronic dyspnea commonly known as "heaves". The group of conditions has become known as chronic obstructive pulmonary disease.[1] The etiopathogenesis of these conditions remains largely unknown and it is unclear if the horse with bronchitis eventually becomes the horse with heaves.

Functional changes in the lungs of horses with chronic airway disease are well described and include decreased dynamic compliance, increased resistance to air flow, increased intrapleural pressure changes during breathing and hypoxemia.[2-4] However, as *Breeze*[5] points out, studies of lung function have never concurrently investigated lung pathology and it is likely that the same functional changes can result from diverse pathologic conditions. Indeed, we have clearly shown that a clinical syndrome with changes in lung function such as occurs in spontaneous chronic obstructive lung disease can be produced by 3-methylindole intoxication[6] or by aerosol ovalbumin challenge of sensitized horses.[7] The etiopathogenesis of these two diseases is quite different but the end result of both is peripheral airway obstruction and similar changes in lung function. *Breeze*[5] has described changes in the lungs of horses with spontaneous chronic obstructive lung disease ranging from chronic bronchiolitis with acinar overinflation to extrinsic allergic alveolitis similar to that described in „farmer's lung" of humans.

Clearly, the equine clinician needs methods of evaluation capable of subdividing horses with chronic airway disease into pathophysiologic subclassifications. A better understanding of the pathophysiology of the various forms of chronic airway disease will lead to more accurate diagnosis.

Bronchoalveolar Lavage

Bronchoalveolar lavage (BAL) allows repeated and safe collection and evaluation of pulmonary cells in patients.[8] BAL was first used in the treatment of cystic fibrosis, asthma and alveolar proteinosis in humans.[9] Later, BAL was used to collect highly purified alveolar macrophages.[10] The major development, resulting in the adoption of BAL as a clinically useful tool in human medicine, has been the demonstration that lavaged cells reflect the cell population derived from lung biopsies in at least two diseases, namely sarcoidosis and fibrosing alveolitis.[11] BAL has contributed greatly to the improved understanding of a variety of interstitial lung diseases in humans.[12-14] Bronchoalveolar lavage was adapted to the Equid by *Viel* et *al.* in 1980.[15] We recently evaluated BAL cytology, pulmonary function and airway reactivity in ponies with a history of recurrent airway obstruction during clinical remission, during airway obstruction and during a recovery phase and in age- and gender-matched controls at the same time periods.

Materials and Methods

We studied 6 ponies with recurrent airway obstruction and 6 age- and gender-matched controls. Ponies were always housed together and were studied on the same day. Measurements were made with principal ponies in clinical remission (period A) during an acute attack of airway obstruction precipitated by housing principal ponies and their controls in a barn (period B) and after a one- and two-week recovery phase (periods C and D respectively).

Airway Reactivity

Airway reactivity was assessed by determining the dose of aerosol methacholine required to reduce dynamic compliance (Cdyn) to 65 % of base line value (ED_{65}Cdyn). Methods used to measure airway reactivity in ponies have been previously described [16] and are described more completely in another manuscript of these proceedings.

Pulmonary Function Measurements

Ponies were tranquilized with xylazine (Rompun, Haver-Lockhart; 0.5 mg/kg body weight) and restrained in stocks. A pneumotachograph and esophageal balloon transducer system measured air flow and transpulmonary pressure respectively. We measured respiratory frequency (f), tidal volume (V_T), minute ventilation ($\dot{V}_E$), pulmonary resistance (R_L), Cdyn, arterial oxygen tension (PaO_2), and arterial CO_2 tension ($PaCO_2$) using methods described in detail elsewhere.[17]

Bronchoalveolar Lavage

Bronchoalveolar lavage was performed as follows. After ponies were tranquilized with xylazine, a 1 m long, 12 mm diameter fiberoptic endoscope (American Cystoscope Makers Inc., Pelham Manor, New York), was inserted through the chronic tracheostoma. Five ml of 0.5 % lidocaine were used to desensitize the airways. The endoscope was wedged in the left lower lobe bronchus and three 100 ml aliquots of body temperature phosphate buffered saline were infused through the biopsy channel and gently aspirated. The BAL fluid recovered was filtered through a single layer of gauze and the volume was measured. Differential cell counts of 500 consecutive cells were performed using an air-dried Wright-Giemsa stained cytocentrifuge preparation (100 g, 10 minutes) (Cytospin, Shandon Southern Instruments Corp., Sewickly, Pennsylvania). Lavage fluid was centrifuged (1500 rpm. 5 minutes) to separate cellular and protein components, and the supernatant

Table 1: Lung function of principal and control ponies at each measurement period

Parameter	Measurement Period			
	A	B	C	D
	Control Ponies			
PaO_2, Torr	82 ± 3	76 ± 5	80 ± 5	86 ± 9
$PaCO_2$, Torr	39 ± 1	43 ± 1	41 ± 1	38 ± 3
Cdyn, 1 · cmH_2O^{-1}	.72 ± .16	.88 ± .18	.82 ± .20	1.06 ± .22*
R_L, cmH_2 · l^{-1} · sec	.52 ± .08	.44 ± .10	.45 ± .08	.73 ± .12
V_T, liters	2.34 ± .23	2.41 ± .32	2.22 ± .32	2.11 ± .19
$\dot{V}_E$, l · min^{-1}	28.0 ± 2.2	25.9 ± 2.1	25.0 ± 1.6	32.5 ± 4.8
f, min^{-1}	12.2 ± .9	11.1 ± .7	12.4 ± 2.4	15.9 ± 2.8
	Principal Ponies			
PaO_2, Torr	79 ± 3	65 ± 4	76 ± 4	85 ± 3
$PaCO_2$, Torr	41 ± 1	42 ± 2	39 ± 4	39 ± 1
Cdyn, l · cmH_2O^{-1}	.57 ± .08	.21 ± .03*	.48 ± .08	.42 ± .07†
R_L, cmH_2O · l^{-1} · sec	.95 ± .12†	2.37 ± .32*†	.86 ± .17	1.07 ± .16
V_T, liters	2.32 ± .36	1.70 ± .10	2.21 ± .31	2.08 ± .39
$\dot{V}_E$, l · min^{-1}	29.5 ± 5.6	40.0 ± 10.9	30.5 ± 4.1	29.5 ± 3.1
f, min^{-1}	14.7 ± 4.9	22.9 ± 5.4	14.3 ± 2.1	16.2 ± 3.5

*) Significantly different from period A.
†) Significantly different from control.

was decanted from the cell pellet for subsequent protein analysis. The cell pellet was resuspended in 5 ml of Hank's balanced salt solution and assayed for total number of cells using a hemocytometer.

Results

Base-line Cdyn and PaO_2 did not differ between principal and control ponies but R_L was higher in principals (Table 1). Pulmonary function values and airway reactivity were unaffected by barn exposure or return to pasture in control ponies. In principal ponies, barn exposure reduced PaO_2, Cdyn, and ED_{65}Cdyn and increased R_L (Figure 1). These variables had returned to base-line values at measurement periods C and D.

Bronchoalveolar lavage was accomplished successfully in all ponies and yielded an average recovery of 50 % of the infused fluid. The white cell counts in BAL fluids are reported in Table 2 and Figures 2 and 3. The total white blood cell counts in BAL were not significantly different in control and principal groups of ponies and were unaffected by barn exposure. In the principal group of ponies, barn

Table 2: Cell counts/μl (X ± SEM) in BAL fluid of principal (P) and control (C) ponies at measurement periods A, B, C, and D.

	Measurement Period			
	A	B	C	D
	Control Ponies			
Total cells	184 ± 59	159 ± 43	188 ± 39	224 ± 109
Neutrophils	4.0 ± 1.5	27 ± 17.1	2 ± 0.5	3 ± 1.0
Macrophages	64 ± 13	54 ± 17	69 ± 12	84 ± 25
Lymphocytes	101 ± 49	73 ± 28	107 ± 30	124 ± 74
Eosinophils	0.2 ± 0.2	0.3 ± 0.13	1.1 ± 0.4	8.4 ± 7.0
Mast cells	11 ± 4.5	4 ± 1.1	6 ± 2.0	5 ± 1.6
Epithelial cells	3 ± 1.4	1 ± 0.4	4 ± 2.1	1 ± 0.5
	Principal Ponies			
Total cells	199 ± 61	316 ± 49	487 ± 234	316 ± 121
Neutrophils	5.8 ± 2.3	184 ± 43.6*†	32 ± 19.9	44 ± 31
Macrophages	57 ± 13.6	33 ± 11	93 ± 32	86 ± 34
Lymphocytes	120 ± 48	69 ± 15	255 ± 139	163 ± 90
Eosinophils	2 ± 0.8	9 ± 6.2	101 ± 65	14 ± 9.2
Mast cells	8 ± 2.3	9 ± 2.6	6 ± 1.7	5 ± 1.6
Epithelial cells	6 ± 1.9	14 ± 12	5 ± 2.1	3 ± 1.2

*) Indicates significant difference within group P.
†) Indicates significant difference between group P and C at the same measurement period.

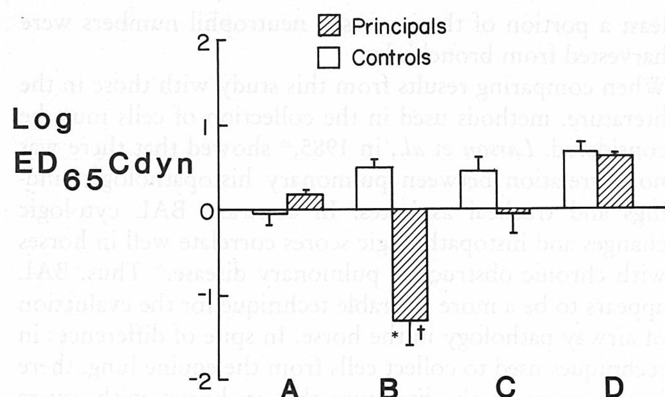

Fig. 1: Log dose of methacholine required to decrease dynamic compliance to 65 % of base-line value ($ED_{65}Cdyn$) in principals (hatched bars) and control ponies (open bars).
* Indicates significant difference within principal group.
† Indicates significant difference between groups at the same measurement period.

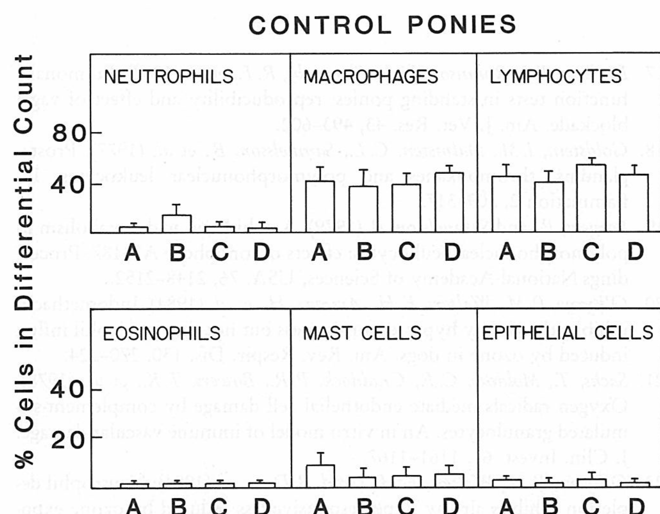

Fig. 2: Differential cell counts (%) of bronchoalveolar lavage (BAL) fluid in control ponies at measurement periods A, B, C, and D.

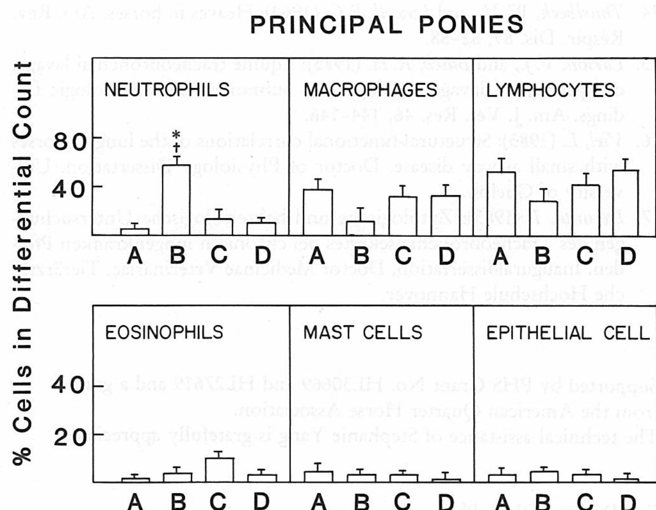

Fig. 3: Differential cell counts (%) of bronchoalveolar lavage (BAL) fluid in principal ponies at measurement periods A, B, C, and D.
* Indicates significant difference within principal group.
† Indicates significant difference between principal and control groups at the same measurement period.

exposure increased the BAL's white blood cell count in all individuals but this apparent change was not statistically significant. At measurement period A, differential white blood cell counts in BAL fluids were similar in control and principal groups of ponies (Table 2 and Figures 2 and 3). Barn exposure and return to pasture had no significant effect on the BAL fluid differential cell count in the control group of ponies. However, in the principal group of ponies, barn exposure significantly increased the number of neutrophils in the BAL fluid from 5.8 ±2.3 cells/μl to 184 ±43.6 cells/μl. At periods C and D, neutrophil counts in BAL fluid of principal ponies had returned to normal. Macrophage, lymphocyte, eosinophil, mast cell and epithelial cell numbers in the BAL fluid of principal ponies were unaffected by barn exposure and return to pasture. However, in two principal ponies at period C, eosinophils comprised greater than 20 % of cells in the BAL fluid, while in one principal pony the per cent eosinophils at period C was greater than 10 %. Peripheral blood white cell counts and differential counts for both groups of ponies were not significantly different. Barn exposure and return to pasture had no effect on these variables.

Discussion

The changes in lung function observed in principal ponies at period B are similar to those previously described.[4] There was an increase in R_L and a decrease in Cdyn and PaO_2. Principal ponies were hyperreactive to aerosol methacholine during acute exacerbations of airway obstruction but not during clinical remission.

Acute disease exacerbation initiated by barn housing was associated with markedly increased numbers of neutrophils in the BAL fluid but neutrophil numbers returned to baseline values when ponies were removed from the barn environment. Since airway hyperreactivity, pulmonary dysfunction, and pulmonary leukostasis followed similar time courses, these data suggest that neutrophils may be involved in the pathogenesis of airway hyperreactivity and airway obstruction.

The mechanism by which increases in lung neutrophil numbers may be related to development of airway hyperreactivity is uncertain. Neutrophils can produce products of both the cycloxygenase[18] and lipoxygenase pathways[19] of arachidonic acid metabolism. Since indomethacin pretreatment prevents airway hyperresponsiveness in ozone-exposed dogs without preventing neutrophil infiltration into the lung,[20] it has been postulated that neutrophils are releasing cycloxygenase products which may be involved in the pathogenesis of airway hyperreactivity. However, other products of neutrophil metabolism may also be involved.[21] Although there is evidence that in ozone-exposed dogs, neutrophils are important in the pathogenesis of airway hyperreactivity,[22] studies in other species suggest that neutrophil infiltration into the lung has no direct influence on the airway hyperreactivity observed.[23] In guinea pigs, ozone-induced bronchial hyperreactivity and neutrophil infiltration into the lung have a different time course.[23] Neutrophil