Effect of contact avoidance or treatment with oral prednisolone on bronchoalveolar lavage surfactant protein A levels in subjects with farmer's lung

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Abstract

Background – Surfactant protein A (SP-A) acts as an immune system modulator in the lungs and may therefore be involved in the pathogenesis of hypersensitivity pneumonitis.

Methods – The levels of SP-A in bronchoalveolar lavage (BAL) fluid were measured in 20 subjects with acute farmer's lung, 16 asymptomatic dairy farmers, and 14 normal controls. Eight patients had a second evaluation after one month of treatment by either contact avoidance (n=3) or oral prednisolone (20 or 25 mg/day, n=5). Chest radiographs and lung function measurements were also obtained in all farmers, twice in those re-evaluated after treatment.

Results – Patients with acute farmer's lung had significantly higher levels of SP-A than asymptomatic farmers and normal controls ($p=0.005$) with mean (SE) values of 1.43 (0.29) $\mu\text{g/ml}$, 0.62 (0.09) $\mu\text{g/ml}$, and 0.68 (0.11) $\mu\text{g/ml}$, respectively. In eight subjects tested after one month of treatment the level of SP-A was unchanged although all were clinically improved. No correlations were seen between levels of SP-A in BAL fluid and numbers of BAL cells, lung function measurements, or chest radiographic scores.

Conclusion – Although the level of SP-A is increased in the BAL fluid of patients with acute farmer's lung, it is not correlated with clinical abnormalities of this disease.

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Farmer's lung, one of the most prevalent forms of hypersensitivity pneumonitis, is caused by bacteria or fungi found in mouldy hay or straw. Ten percent of dairy farmers in endemic regions have precipitating antibodies to *Saccharopolyspora rectivirgula*, the most frequent causative agent;¹ half of these also have a lymphocytic alveolitis.² The mechanisms which lead to sensitisation, the accumulation of lymphocytes in the lungs, and the manifestation of hypersensitivity pneumonitis remain unclear. Recent studies have documented the in-

volvement of cytokines and other inflammatory mediators.³ Alveolar macrophages in this disease have lost their capacity to suppress mitogen induced proliferation of peripheral blood lymphocytes in vitro.⁴ These cells bathe in pulmonary surfactant which may have a central role in downregulating immune reactions in the normal lung by interacting with alveolar cells.⁵⁻⁸

Surfactant protein A (SP-A), the major surfactant associated protein, has dual functions both in the maintenance of surface active properties of surfactant and as an immune system modulator.^{9,10} Specifically, SP-A is a chemotactic agent for alveolar macrophages, acts as an opsonin, and enhances phagocytosis of bacteria and viruses.¹¹⁻¹³ Since farmer's lung is an immune response to a bacterial antigen and since alveolar macrophages are probably involved in the disease, a role for SP-A in hypersensitivity pneumonitis is certainly possible.⁴ Guzman *et al* have shown that alveolar macrophages from patients with hypersensitivity pneumonitis have an increased content of SP-A.¹⁴ The same group also showed in a small number of patients with hypersensitivity pneumonitis (mostly bird fancier's lung) that levels of SP-A in the bronchoalveolar lavage (BAL) fluid were increased.¹⁵ These authors concluded that SP-A may not only be involved in the pathophysiology of hypersensitivity pneumonitis, but also that levels of this protein in the BAL fluid could be useful as a marker of disease activity in hypersensitivity pneumonitis and other interstitial lung diseases.

The present study was conducted to document further the increase of SP-A in farmer's lung and to look at the effect of treatment (contact withdrawal or corticosteroids) on the BAL fluid levels of this protein. If the hypothesis that SP-A could be a marker of disease activity is correct, effective treatment should result in a decrease to more normal levels in BAL fluid. We also wanted to compare SP-A levels in asymptomatic dairy farmers with lymphocytic alveolitis with those in patients with acute disease as this could be useful in differentiating between an asymptomatic bronchoalveolar lymphocytosis and hypersensitivity pneumonitis, and may help in understanding the possible role of SP-A in the accumulation of lymphocytes in the lungs and in the pathogenesis of this disease.

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Methods

SUBJECTS

The study population consisted of 20 cases of acute farmer's lung (17 men, mean (SD) age 43.7 (12.4) years) with a diagnosis based on clinical, radiological, functional, BAL, and serological criteria¹⁶; 16 asymptomatic dairy farmers (15 men, mean (SD) age 47.4 (8.5) years (range 34–61)) who were free from respiratory disease; and 14 normal non-farming controls (eight men, mean (SD) age 24.6 (4.3) years (range 20–35)). The controls were therefore significantly younger than both groups of farmers. All subjects were non-smokers. Of the 20 cases of farmer's lung, eight were studied twice – at the time of diagnosis and after one month of treatment. The treatment consisted of either oral prednisolone 20 or 25 mg per day (five subjects) or of contact withdrawal (three subjects).

The protocol was approved by our institution's review board and all subjects gave written informed consent.

STUDY PARAMETERS

Lung function measurements and chest radiographs were obtained for all farmers. The lung function tests included lung volumes measured by body plethysmography, forced expiratory flows and single breath carbon monoxide transfer factor (TLCO). Standard postero-anterior and lateral chest radiographs were obtained but only the former were analysed for the current study and were read blindly by one of the authors (MD). The radiographs were scored as previously described.¹⁷ Briefly, lung fields were subdivided into six regions (upper, middle and lower for each lung). Each of these regions was given a score of 0–4 for the presence and intensity of infiltrates. With this system normal lungs have a score of 0 while a score of 24 (top score for all six regions) signifies marked diffuse infiltrates throughout both lung fields.

All subjects underwent bronchoscopy with bronchoalveolar lavage (five aliquots of 60 ml 0.9% warmed saline). The lavage fluid was kept on ice until centrifugation at 400 *g* for 10 minutes at 4°C. Cells were counted with a haemocytometer and differential cell counts were obtained on DiffQuik (Canlab, Baxter division) and esterase stained preparations. Levels of SP-A in BAL fluid were measured by ELISA as described previously.^{18,19} The concentration of inorganic phosphorus in the BAL fluid was measured and used as an indicator of the level of surfactant phospholipids as described previously.²⁰

STATISTICAL ANALYSIS

The data were expressed as mean (SE). To compare SP-A levels in asymptomatic farmers and subjects with farmer's lung with a control group a one-way ANOVA was performed and comparisons between groups were made using Tukey's approach. Estimates of the differences with their respective 95% confidence intervals are given in the results section. As normality and variance homogeneity assumptions were

met, the *F* test estimate from original data was used. Comparisons between asymptomatic farmers and subjects with farmer's lung were performed using Student's *t* tests. Data from subjects with farmer's lung at diagnosis and after one month of treatment were analysed with Student's paired *t* tests. To measure relationships between SP-A levels and lung function measurements, radiological score, or cells in the BAL fluid, Spearman correlation coefficients were used. All reported *p* values were considered significant at the 0.05 level. The data were analysed using the statistical package program SAS (SAS Institute Inc, Cary, North Carolina, USA).

Results

Lung function data for asymptomatic farmers and patients with farmer's lung, both at diagnosis and after one month of treatment in eight subjects, are given in table 1. The results are reported as percentage predicted.^{21,22} As expected, the carbon monoxide transfer factor was the most severely altered parameter with 16 of the 20 patients with acute disease having a TLCO of <80% predicted. Lung function measurements improved with treatment in most subjects. For example, TLCO improved by >10% in six of the eight subjects studied before and after treatment. Mean (SE) chest radiographic scores for the asymptomatic farmers and those with acute farmer's lung were 0.62 (0.36) and 10.4 (1.7), respectively. Individual and mean (SE) chest radiographic scores before and after treatment where available (*n*=7) are given in fig 1 and show improvement in lung infiltrations after one month of treatment in five of the seven patients. Although all subjects in the group of eight were improved after one month of treatment, the increase in lung TLCO and the decrease in radiographic scores did not reach statistical significance (*p*=0.090 and 0.056, respectively).

The total cell numbers and differential cell counts for the three groups of subjects are given in fig 2. Cell counts before and after treatment for the eight subjects who had two evaluations are given in fig 3.

The levels of SP-A in the BAL fluid of the three groups are given in fig 4. Patients with farmer's lung had significantly higher levels of SP-A than asymptomatic farmers and normal controls with mean (SE) values of 1.43 (0.29) µg, 0.62 (0.09) µg, and 0.68 (0.11) µg, respectively (*p*=0.005). Differences and 95% confidence intervals were as follows: acute versus controls, 0.75, 95% CI 0.80 to 1.42; acute versus asymptomatic farmers, 0.81, 95% CI 0.16 to 1.45. Asymptomatic farmers and normal controls had very similar levels of SP-A (difference –0.06, 95% CI –0.76 to 0.65). No correlations were found between SP-A levels in the BAL fluid and BAL cells, lung function measurements, chest radiographic scores, or age (table 2). SP-A levels remained high after one month of treatment in the eight subjects with farmer's lung who were studied twice (fig 5). No correlation was seen in this group

Table 1 Mean (SE) results of pulmonary function tests expressed as percentage predicted in the asymptomatic farmers (AS) and those with farmer's lung (AC)

Lung function	AS (n = 16)	AC (n = 20)	AC before treatment (n = 8)	AC after treatment (n = 8)
FEV ₁	113.25(3.67)	71.75(3.82)	74.50(6.26)	84.75(6.12)
FVC	104.43(2.72)	72.15(2.94)	72.12(3.45)	86.37(4.16)
FEV ₁ /FVC	108.25(1.55)	96.33(3.73)	100.33(5.42)	94.00(6.19)
TLCO	107.18(4.26)	63.05(4.35)	54.37(3.35)	70.25(7.23)

FEV₁ = forced expiratory volume in one second; FVC = forced vital capacity; TLCO = lung carbon monoxide transfer factor.

Figure 1 Individual radiographic score before and after one month of treatment for seven of the eight subjects with acute farmer's lung who were studied twice. Although the score decreased (improved) in five subjects the difference did not reach statistical significance.

between changes in SP-A levels and changes in radiographic scores, BAL cells, or TLCO (r values of 0.31, -0.14 and -0.45 with p values of 0.50, 0.74 and 0.26, respectively).

The amount of inorganic phosphorus in the BAL fluid was similar for all groups of subjects including those lavaged after one month of treatment (fig 6), indicating that concentrations of phospholipids were not increased in patients with hypersensitivity pneumonitis compared with asymptomatic farmers and healthy controls.

Discussion

As previously reported, these results show that farmer's lung is associated with a high intensity lymphocytic alveolitis and that some asymptomatic farmers have a moderate increase in BAL cells, especially lymphocytes.² The lack of improvement in BAL cellularity after one month of treatment was also as previously reported.²⁴

The results of this study show that SP-A levels in BAL fluid of patients with acute farmer's lung are increased compared with normal controls. Since the level of inorganic phosphorus was not increased in patients with acute farmer's lung, the increase in SP-A does not reflect an overall increase in surfactant but an increase in the SP-A/phospholipid ratio. These data confirm findings in other forms of hypersensitivity pneumonitis and, in addition, show that SP-A levels remain high after effective treatment of farmer's lung.¹⁵ Although the number of subjects studied before and after treatment is small ($n = 8$), the lack of correlation between lung function, lung infiltrations, and the persistent increase in SP-A after either form of treatment does not support the hypothesis that SP-A levels in BAL fluid could be used as a marker of disease activity.

The lack of correlation between age and SP-A levels and the similarity in SP-A levels in the controls and asymptomatic farmers suggest that higher SP-A levels in patients with acute farmer's lung cannot be explained by the age difference between the groups.

Although SP-A has immunoregulatory functions, its exact role in farmer's lung remains

Figure 2 Total and differential counts for the cells recovered by bronchoalveolar lavage in the three groups studied presented (A) as the number of cells per ml BAL fluid recovered and (B) as a percentage of the total cells. Patients with acute farmer's lung had an increased number of cells with a high percentage of lymphocytes while asymptomatic farmers had intermediate values between those with acute farmer's lung and normal subjects. N = normal controls; AS = asymptomatic farmers; AC = patients with acute farmer's lung.

Figure 3 Pre-treatment (AC1) and post-treatment (AC2) values of BAL fluid cell counts for the eight patients with farmer's lung who had two evaluations expressed as (A) the number of cells per ml BAL fluid recovered and (B) as a percentage of the total cells. Although the number of cells decreased in five subjects the difference for the group did not reach statistical significance.

Figure 4 Mean (SE) levels of surfactant protein A (SP-A) in the BAL fluid for the three study groups. Patients with farmer's lung (AC) had significantly higher levels of SP-A than asymptomatic farmers (AS) and normal controls (N).

Figure 5 Individual SP-A levels in BAL fluid before and after one month of treatment in eight patients with farmer's lung. One month of treatment did not significantly change the amount of SP-A in the BAL fluid.

unknown. Increased SP-A activity could enhance alveolar macrophage activation and phagocytosis. The loss of alveolar macrophage lymphosuppression seen in acute hypersensitivity pneumonitis⁴ could result from this activation. The lack of increase in BAL fluid levels of SP-A in asymptomatic farmers with a lymphocytic alveolitis does not support a role for SP-A in the recruitment of these cells into the lung. The surfactant phospholipid profile was generally unchanged except for increased amounts of sphingomyelin in patients with active farmer's lung as previously described.²³ However, alterations in the surfactant

phospholipid profile, as well as production of inflammatory cytokines following antigen challenge, can be counted either as a non-specific marker of epithelial dysfunction with parenchymal inflammation or as an additional contributor to the dysregulation of the cytokine network.^{3 23 25}

The persistence of high levels of SP-A in the BAL fluid after effective treatment suggests that SP-A is not a sensitive marker of disease improvement after one month of follow up. One possibility is that the increased SP-A level represents a defence mechanism with SP-A not being involved in the disease process but turned

Table 2 Spearman's correlation coefficients (R) and p values for the relationships between SP-A levels in BAL fluid and total lavage cell counts, chest radiography scores, and lung function measurements (% predicted) for the 20 patients with acute farmer's lung

	Radiographic score	BAL cell count	FEV ₁	FVC	TLC	TLCO
R	0.20	-0.14	-0.39	-0.43	-0.41	-0.02
p	0.40	0.55	0.09	0.06	0.08	0.94

FEV₁ = forced expiratory volume in one second; FVC = forced vital capacity; TLC = total lung capacity; TLCO = carbon monoxide transfer factor.

Figure 6 Mean (SE) values of inorganic phosphorus in BAL fluid from normal controls (N), asymptomatic farmers (AS), and patients with acute farmer's lung (AC) before (AC1) and after (AC2) treatment. The levels were similar for all groups of subjects including those lavaged after one month of treatment.

on to limit the effects of the disease. The opsonin function of SP-A could help the alveolar macrophage to get rid of the offending antigen. SP-A, by its effects on surface active properties of surfactant, could also help to reduce the lung stiffness responsible for the restrictive functional pattern typical of this disease. If this occurs, the capacity of SP-A in this aspect is obviously incomplete.

Type II epithelial cell hypertrophy/metaplasia and activation, together with interstitial lymphoplasmocytic infiltration, are commonly observed in hypersensitivity pneumonitis.²⁶ Type II epithelial cell upregulation of SP-A expression and production by steroids has been described and may explain, at least in part, the high levels of the protein still observed after one month of treatment in three of the five patients who have taken this medication.²⁷ On the other hand, persistent high levels of SP-A also must be regarded in the context of persistent biological lung impairment in spite of rapid clinical relief. Indeed, chest radiographs and respiratory function tests were only partially improved and lymphocytic alveolitis persisted following one month of treatment. In contrast, the normal levels of SP-A seen in asymptomatic farmers with lymphocytic alveolitis act as a sensitive biological marker of distal epithelium integrity and correlate with the absence of clinical farmer's lung disease. This also highlights the fact that interstitial infiltration by inflammatory cells is a key process in inducing the clinical disease and probably in upregulating SP-A expression/production simultaneously.

In conclusion, the present study shows that SP-A levels are increased in acute farmer's lung but suggests that this protein is not a clear marker of disease severity. The lack of increase of SP-A in asymptomatic bronchoalveolar lymphocytosis would indicate that SP-A is not involved in the altered immune modulation that allows the recruitment of lymphocytes in the lungs in hypersensitivity pneumonitis.

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