Fibronectin and Procollagen 3 Levels in Bronchoalveolar Lavage of Asbestos-Exposed Human Subjects and Sheep*

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To evaluate the potential interest of levels of fibronectin and procollagen 3 in bronchoalveolar lavage fluid as markers of fibrogenic activity, we characterized the time course of changes in fibronectin and procollagen 3 levels in the tracheal lobe of sheep exposed to nonfibrogenic and fibrogenic materials. We correlated these observations with those of bronchoalveolar lavage in long-term asbestos workers in various stages of disease activity. Following studies before exposure, the tracheal lobe of three groups of 24 sheep were exposed once to 100 ml of phosphate-buffered saline solution (PBS), to 100 mg of latex beads in 100 ml of PBS, or to 100 mg of chrysotile fibers in 100 ml of PBS. Bronchoalveolar lavages were obtained at 0, 1, 2, 4, 8, and 12 months after exposure, and four or five sheep per group were killed after each lavage for histopathologic analysis. Fibronectin in bronchoalveolar lavage fluid increased significantly only in the asbestos-exposed sheep to values two to three times above controls or latex-exposed sheep and remained elevated during the 12 months of the study. Levels of procollagen 3 in bronchoalveolar lavage fluid were increased significantly only during the first two months following exposure in the asbestos-exposed sheep only. In the asbestos workers without disease, levels of fibronectin and procollagen 3 in bronchoalveolar lavage fluid were comparable to controls, but these levels were significantly elevated in those with asbestos-associated alveolitis or asbestosis. This study documents that the measurement of levels of fibronectin and procollagen 3 in bronchoalveolar lavage fluid assesses fibrogenic activity of alveolitis and should be useful to predict its progression in a fibrotic process. In asbestos workers the potential use of these markers is primarily related to early detection of asbestos-induced pulmonary injury.

Until recently, the assessment of fibrogenic activity of human interstitial pulmonary diseases was limited to the histomorphologic and immunohistochemical analyses of limited samples of pulmonary tissues.1,3 Through bronchoalveolar lavage of diseased areas, it has been documented that human pulmonary diseases with histologic evidence of fibrogenic activity were associated in the bronchoalveolar lavage fluid with increased levels of fibronectin and procollagen 3 peptides, molecules implicated in the biochemical process of pulmonary fibrosis,4,6 and thus of potential interest as markers of fibrogenic activity; however, these observations were limited to the individuals with chronic disease. The time course of these changes in bronchoalveolar lavage fluid have not been studied in spite of increasing interest in the biologic monitoring of humans exposed to environmental materials or therapeutic drugs with known pulmonary toxic effects.

In the present study, we characterized the time course of these changes in fibronectin and procollagen 3 levels in bronchoalveolar lavage fluid from the sheep tracheal lobe exposed to nonfibrogenic and fibrogenic materials. These observations were correlated with those of bronchoalveolar lavage in long-term asbestos workers in various stages of disease activity.

The data clearly document that fibronectin and procollagen 3 levels in bronchoalveolar lavage fluid are increased early in alveolitis with fibrogenic activity, but not in those without, which should contribute to further refine our clinical understanding of disease activity in the chronic inflammatory pulmonary disorders.

Materials and Methods

Animals

Seventy-two male sheep weighing between 25 and 45 kg were used in this study. They were prepared and accustomed to the pulmonary techniques as previously reported.8,10

Experimental Design

The flock was divided into three groups of 24 sheep. Following studies before exposure (control studies), the sheep tracheal lobe was exposed once to 100 ml of phosphate-buffered saline solution (PBS) (control group), to 100 mg of 0.1 μm latex beads (Sigma Chemical Co) in 100 ml of PBS (latex group), or to 100 mg of UICC. Canadian chrysotile asbestos fibers in 100 ml of PBS (asbestos group). These asbestos fibers were relatively uniform and well characterized, with 92 percent less than 0.25 μm in diameter and 20 μm in length. Exposure of the tracheal lobe was carried out via bronchoscopic catheterization of the tracheal bronchus and slow infusion of the suspension into the lobe. The animals were studied prior to exposure and at 1, 2, 4, 8, and 12 months after exposure by bronchoalveolar lavage and by

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Manuscript received April 10; revision accepted August 12. Reprint requests: Dr. Begin, CHUS, Sherbrooke, Québec, Canada J1H 5N4

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histopathologic methods (four or five sheep per group killed each time).

Human Controls

Twenty-one manual workers without exposure to asbestoses were tested within the period. They were matched for age, height, sex, smoking, and work habits to the asbestos workers; seven were current or ex-smokers, and 14 were lifetime nonsmokers. The mean age of control subjects was 57 ± 5 years (range, 50 to 70 years). None of them was exposed to environmental dust at risk of pneumoconiosis. In this control population, total cells in bronchoalveolar lavage fluid averaged 171 ± 24 × 10^9/ml, with macrophages at 159 ± 22 × 10^9/ml, and neutrophils at 0.86 ± 0.30 × 10^9/ml.

Asbestos Workers

The 34 asbestos workers were between the ages of 42 and 70 years (mean, 58 ± 4 years). All had been exposed to Canadian chrysotile asbestos only in the mines and mills of the eastern townships of Quebec for an average of 35 ± 5 years (range, 24 to 42 years). Twenty-eight were nonsmokers for more than two years, and the six others were either current or recent ex-smokers. Many of these patients were included in our studies of airway function in asbestos workers, or computerized tomographic scanning studies and were among 226 asbestos workers in a study of clinical features of the associated alveolitis. In this present study, we specifically report on fibronectin and procollagen III in bronchoalveolar lavage fluid from these 34 workers as we relate these findings to serial measurements of these markers in the sheep model of asbestosis.

Clinical Evaluation

All patients underwent a medical history, complete physical examination, standard high-kilovoltage posteroanterior, lateral, and oblique chest films, detailed tests of pulmonary function, and 67Ga lung scan as previously reported.

Subsets of Asbestos Workers

On the basis of criteria reported in detail,8,9 the asbestos workers were divided into three categories of disease: (1) group A consisted of seven workers without evidence of asbestoses-induced pulmonary damage; they did not have the diagnostic criteria for asbestosis, and their 67Ga pulmonary uptake and pulmonary pressure-volume curve were within normal limits;8,9 (2) group B was eight asbestos workers with evidence of asbestoses-induced alveolitis documented at biopsy in three of three workers;9,10 in whom the biopsy was obtained; they did not have the diagnostic criteria for asbestosis but had enhanced 67Ga pulmonary uptake and rigid pulmonary pressure-volume curve; and (3) group C was 19 asbestos workers who met the diagnostic criteria for asbestosis. The clinical data and bronchoalveolar lavage cellularity of these patients are summarized in Figure 1.

Bronchoalveolar Lavage and Fluid Analysis

Most of the techniques in the procedures and analyses of bronchoalveolar lavage have been previously described.8,9,11 The effluent from lavage was passed through four layers of cheesecloth to remove mucus, and the cells were pelleted by centrifugation. Cells were counted in a hemocytometer, and cell viability was determined by the trypan blue exclusion technique. Cytocentrifuge smears served to identify the cellular populations recovered with the Wright-Giemsa and naphthyl acetate esterase stains.12 In the supernatant, albumin and fibronectin were measured by the immunochemical methods of Killingsworth and Savory,13 using a laser nephelometry instrument (Behring LN modular system). Sheep albumin, specific antiserum raised in rabbit were obtained commercially (Cappell Lab. Inc.). Sheep fibronectin from bronchoalveolar lavage fluid was purified by affinity column followed by chromatography and antisheep fibronectin antibodies prepared in rabbits as described.9,11 Procollagen III from bronchoalveolar lavage fluid was measured as type 3 procollagen N-terminal peptides by radioimmunoassay, as reported by Low et al.,11 based on the method originally

Figures 1, 2, and 3. Clinical, functional, radiographic and bronchoalveolar lavage cellularity data of our patients. In group N (normal controls), there were 21 manual workers without asbestos exposure. Group A was seven asbestos workers without any evidence of asbestos-induced pulmonary damage; group B was eight workers with decreased 67Ga pulmonary uptake and rigid pulmonary pressure-volume (P-V) curve without criteria for established asbestosis; group C was 19 asbestos workers who met diagnostic criteria for asbestosis.8,9,10 67Ga pulmonary uptake, parenchymal opacities, and rales were scored as previously reported.8,9,10 Data on pulmonary function are reported as percent predicted of Bates et al.8,9

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described by Rohde et al.³ All results were expressed per milliliter of bronchoalveolar lavage fluid. All values of humoral components of lavage fluid were also analyzed in terms of the ratio to the albumin content of bronchoalveolar lavage fluid supernatant.

**Histopathology**

At months 1, 2, 4, 8, and 12 of the study, four sheep in each group were killed and the lungs removed from the thoracic cavity. The tracheal lobe was identified, and nine samples of the lobe were obtained each time for microscopic examination. The pulmonary samples were processed as routinely done for human pulmonary tissue.

**Statistical Analysis**

All results are expressed as mean ± SE. The data were tested by Student’s t-test for differences between groups. A value of p < 0.05 was considered significant in this study.

**RESULTS**

**Sheep**

**Bronchoalveolar Lavage.** The results of analysis of bronchoalveolar lavage fluid are presented in Figure 2. In the saline-exposed sheep, total cells from lavage averaged 250,000/ml and did not vary significantly in the course of the experiment. Similarly, the levels of macrophages, neutrophils, fibronectin, and procollagen 3 did not change. In the latex-exposed sheep, we have previously reported a clear increase in total cells from lavage at month 1 to eight times the control value,³¹,³³ and at month 12, this had returned to the level of saline-exposed sheep. From that point and after, there was no significant difference in levels of macrophages, neutrophils, fibronectin, and procollagen 3 between latex-exposed and saline-exposed sheep. In the asbestos-exposed sheep, total cells were clearly increased through the experiment; and one year after exposure, total cells in bronchoalveolar lavage fluid remained at 150 percent of controls, macrophages at 150 percent of controls, and neutrophils at 285 percent of controls. Fibronectin in bronchoalveolar lavage fluid was significantly elevated only in the asbestos-exposed sheep, and the levels increased throughout the experiment. Procollagen 3 in lavage fluid increased significantly early and returned to control levels at month 4 after exposure (Fig 2). Albumin was increased significantly only after month 4, and it paralleled the second increase in fibronectin (data not shown).

**Pulmonary Histopathology.** In the saline-exposed

![Figure 2](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21505/ on 06/26/2017)
sheep, the histopathology of the tracheal lobe remained normal during the experiment. In the latex-exposed sheep at month 1, there was a pneumonia-like infiltrate of the tracheal lobe; the infiltrate was predominantly alveolar and was largely composed of macrophages without distortion of the pulmonary architecture. At month 4, the infiltrate had regressed, and areas of residual cell accumulation constituted less than 5 percent of the total lobe (Fig 3). After month 4, the histopathology of the latex-exposed tracheal lobes had returned to normal (Fig 4). In the asbestos-exposed sheep at month 1, there was also a pneumonia-like infiltrate composed of macrophages and neutrophils, with considerable distortion of the architecture of the lung and airway; at month 4, the infiltrate had considerably regressed to some 20 percent of the initial extent; it was located predominantly in the peribronchiolar and endobronchiolar areas, with persistent severe distortion of the small airways (Fig 5). At month 8, the infiltrate continued to regress but peribronchiolar and endobronchiolar fibrosis could be detected; at month 12, the infiltrate was at 5 to 10 percent of the initial extent, but peribronchiolar and endobronchiolar fibrosis were severely distorting the pulmonary architecture (Fig 6).

**Human Subjects**

**Clinical Data.** On the basis of clinical, radiologic, and functional data, 19 of the 34 subjects were recog-
Figure 6. Histopathologic findings in asbestos-exposed tracheal lobe at month 12, demonstrating less intense inflammatory process than at month 4 (Fig 5), but lesions of small airways did not regress and became fibrotic (hematoxylin-eosin, original magnification ×63).

nized as having asbestosis. In comparison with the other 15 workers and the controls, this group had identical age and smoking habits. They had a significantly higher rale score, higher score of radiographic pulmonary opacities, lower vital capacity, lower total lung capacity (TLC), lower diffusing capacity for carbon monoxide, higher 67Ga pulmonary uptake, and more rigid pulmonary pressure-volume curve (Fig 1) (p < 0.05 in all). The workers of group B differed (by definition) from group A only in terms of rigidity of the pulmonary pressure-volume curve and increased 67Ga pulmonary uptake. Eight had a roentgenogram scored at 0/0 or 0/1; none of them had bilateral rales. The clinical data in the asbestos workers of groups A, B, and C are essentially in keeping with our previous work.

Bronchoalveolar Lavage. In Figure 7, we present the results of analysis of fibronectin and procollagen in bronchoalveolar lavage fluid. In the controls, the ratio of fibronectin/albumin in bronchoalveolar lavage fluid was 33.7μg/mg ± 6.4μg/mg, and the ratio of procollagen 3/albumin was 1.22 ± 0.22 ng/mg. In the asbestos workers without asbestosis and with normal pulmonary pressure-volume curve and normal 67Ga pulmo-

Figure 7. Levels of fibronectin and procollagen 3 in human bronchoalveolar lavage fluid reported as ratios to albumin (A). Groups N, A, B, and C are as in Figure 1.

nary uptake (group A), the fibronectin/albumin ratio was 33.1μg/mg ± 6.0μg/mg, and the procollagen 3/albumin ratio was 1.03 ± 0.24 ng/mg. In group B, the fibronectin albumin ratio was 45.7μg/mg ± 34μg/mg (not significant), and the procollagen 3/albumin ratio was 2.3 ± 0.8 ng/mg (p<0.05 vs controls and group A). In the workers with asbestosis, the fibronectin/albumin level was at 71.4μg/mg ± 32μg/mg and was significantly higher than controls and group A (p<0.05); the procollagen 3/albumin ratio was at 2.0 ± 0.8ng/mg and was comparable to levels in group B (p<0.05 vs controls and group A). Analysis of the data excluding smokers did not significantly change the results.

Discussion

The present report extends earlier investigations on mechanisms of pulmonary injury associated with the presence of respirable particles in the bronchoalveolar milieu. In the animal model, this study documents that asbestos-associated alveolitis which progresses to peribronchiolar and endobronchiolar fibrosis (the fundamental lesion of asbestosis) is associated with significant early increase in the level of procollagen 3 in bronchoalveolar lavage fluid and a sustained increase in the fibronectin level, which do not occur in the nonfibrosing and regressive latex-associated alveolitis. In asbestos workers with clinical and lavage evidences of alveolitis, levels of procollagen 3 and fibronectin are similarly increased in bronchoalveolar lavage fluid, which should improve our understanding of disease activity in asbestos workers.

In the sheep tracheal lobe, this study documents that after a single high-dose exposure to asbestos, there are severe derangements of the bronchoalveolar milieu and activation of a chronic sustained alveolar, peribronchiolar and endobronchiolar inflammatory process which evolves to fibrosis. On bronchoalveolar lavage the increased macrophage and neutrophil cellularity is associated with increased fibronectin, a glycoprotein
produced locally by the macrophage, known to be chemotactic and an attachment factor for fibroblasts and a stimulant for fibroblast replication in association with another macrophage-derived fibroblast growth factor (MDFGF). In the asbestos-associated alveolitis in sheep, we have reported enhanced production of MDFGF and macrophage-derived neutrophil chemotactic factor (MDNCF). Because these three macrophage-derived factors (fibronectin, MDFGF, and MDNCF) are currently thought to have major roles in the pathogenesis of pulmonary fibrosis, our data from sheep suggest that these factors contribute to maintain the alveolitis on a long-term basis and lead to its progression to asbestosis. The mechanisms which activate the macrophage to produce these factors are likely related to the prolonged retention of asbestos fibers in the bronchoalveolar milieu, as suggested by our previous study of alveolar clearance of chrysotile in the sheep model. In the model, we have shown that one year after cessation of exposure, 11 percent of the fibers shorter than 8 μ, 32 percent of the fibers with length in the range of 8 μ to 20 μ, and 50 percent of the fibers longer than 20 μ were still recoverable by pulmonary lavage. The persistence of these fibers in the bronchoalveolar milieu may constitute the determinant factor for maintenance of activated macrophages and alveolitis.

In the assessment of fibrogenic activity of alveolitis, the quantitation of type 3 collagen compared to type 1 in lung biopsies and in postmortem samples from patients with fibrosing alveolitis documented that an increased ratio of type 3 to type 1 collagen was associated with an earlier fibrotic process which responded better to therapy, which would be of interest in the staging of activity of the fibrosing alveolitis; however, this method necessitates lung biopsies and thus is of limited usefulness clinically. The recent development by Rohde et al of a radioimmunoassay of procollagen 3 N-terminal peptide allowed Low et al to demonstrate increased levels of procollagen 3 in bronchoalveolar lavage fluid from patients with idiopathic pulmonary fibrosis and in patients with sarcoidosis, with the highest values in those with idiopathic pulmonary fibrosis, suggesting increased secretion of type 3 collagen. In the sheep model, we document a similar increase in procollagen 3 in lavage fluid, which occurs during the first two months after asbestos exposure, at which time early peribronchial fibrosis could be observed in pulmonary tissues. The absence of similar changes in the latex-associated alveolitis strengthen the contention of its specificity for a fibrosing process; its increased levels occurring early in the fibrotic process are in keeping with immunohistochemical data. Thus, the data of Low et al and our data suggest that measurement of levels of procollagen 3 in bronchoalveolar lavage fluid may be a useful method of assessing fibrogenic activity of alveolitis.

In the study of asbestos workers, we have documented that in those with asbestosis, the alveolitis is characterized by a significant increase in total cells, macrophages, and neutrophils in the bronchoalveolar lavage fluid, which confirms the previous report of Bignon et al. Furthermore, in workers with asbestosis, fibronectin and procollagen 3 in lavage fluid were significantly elevated, which parallels previous results in patients with idiopathic pulmonary fibrosis. These data from bronchoalveolar lavage are also particularly useful in demonstrating that in the workers who did not meet the usual diagnostic criteria for asbestosis, those with enhanced 67Ga pulmonary uptake and rigid pulmonary pressure-volume curve (group B) had significantly higher levels of total cells, macrophages, neutrophils, fibronectin, and procollagen 3 in bronchoalveolar lavage fluid than workers with similar asbestos exposures but with a normal 67Ga scan and pulmonary pressure-volume curve. These data from bronchoalveolar lavage suggest that the alveolitis of asbestos workers from group B has all the features to expect its progression to asbestosis, which has been our observation in 12 of the 16 workers of our original report.

In conclusion, this study documents that the measurement of levels of fibronectin and procollagen 3 in bronchoalveolar lavage fluid assesses fibrogenic activity of alveolitis and can predict its progression in a fibrotic process. The potential use of these markers of fibrogenic activity in asbestos workers is primarily related to early detection of asbestos-induced pulmonary injury in workers with uncertain disease activity. In the reversible interstitial pulmonary disease, these markers of fibrogenic activity should be of interest as indicators to stage the disease and to predict its potential reversibility by therapy.

ACKNOWLEDGMENT: M. Geoffroy, S. Gouin, and S. Péloquin provided assistance.

REFERENCES

5. Rennard SI, Hunninghake GW, Bitterman PB, Crystal RG. Production of fibronectin by the human alveolar macrophage: mechanism for the recruitment of fibroblasts to sites of tissue injury in interstitial lung disease. Proc Natl Acad Sci 1981; 78:7147-51
29 Sébastien P, Bégin R. Alveolar clearance of chrysotile in the sheep model. ILO 6th Int Pneumoconiosis Conf Monogr 1984; 2:1010-20

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