Gallium-67 Uptake in the Lung of Asbestos Exposed Sheep: Early Association with Enhanced Macrophage-Derived Fibronectin Accumulation

Raymond Bégin, Guy Bisson, Raymond Lambert, Yvan Côté, Daniel Fabi, Marc Martel, Guy Lamoureux, Marek Rola-Pleszczynski, Moktar Boctor, Daniel Dalle, and Serge Massé

Unité de recherche pulmonaire, Université de Sherbrooke, Sherbrooke, Québec, Canada

To evaluate the time course and mechanisms of enhanced ^{67}Ga lung uptake in asbestosis, we exposed two groups of sheep every 2 wk to either 100 ml saline (controls) or 100 mg UICC chrysotile fibers in 100 ml saline. The sheep were evaluated periodically by pulmonary function tests (PFT), thoracic radiograph (TR), ^{67}Ga lung scan bronchoalveolar lavage (BAL), and transbronchial lung biopsy (TLB). By month 24 of the study, 9/15 exposed sheep had developed the initial alveolitis and had significant changes in PFT, TR, and TLB. The other six exposed sheep differed from controls only by a 75% increase in BAL fibronectin until month 30, where significant changes in albumin occurred and ^{67}Ga scan score increased. The nine sheep with alveolitis had significant sustained increases in ^{67}Ga scan and BAL levels from month 6, associated with a 150% increase in BAL fibronectin and other parameters of disease activity changed from month 18 to 30. We concluded that in the sheep model of asbestosis, significant changes in ^{67}Ga scan, ^{67}Ga BAL counts, and excessive elevation of BAL fibronectin preceded other parameters of disease activity. The data suggest that excessively activated macrophages are primarily responsible for the early ^{67}Ga lung uptake.

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Enhanced gallium-67 (^{67}Ga) lung accumulation in the interstitial lung diseases is considered to be an index of inflammatory activity that can be quantitated and that correlates with cellularity of lung tissue and bronchoalveolar milieu (*1–7*). The mechanisms of excessive ^{67}Ga accumulation in thoracic disease have been partially elucidated and well reviewed recently by Tsan (*8*). In asbestos exposed humans, it is well established that ^{67}Ga lung scan is abnormal in asbestosis (*9–14*). However, among long-term asbestos workers without established disease, we have reported that ^{67}Ga lung uptake was often increased in the absence of lung rales, restrictive profile of lung volumes, or radiographic opacities (*10–13*). Follow-up of the 16 workers originally reported (*10*) has documented that within 3 to 5 yr, 12 of them developed asbestosis (*15*).

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For reprints contact: R. Bégin, MD, CHUS, Sherbrooke, Québec, Canada. J1H 5N4.

In the sheep model of asbestosis, we have reported that when disease was histologically well established, ^{67}Ga lung uptake correlated well with intensity of the inflammatory process and reflected enhanced capillary permeability and macrophage accumulation (*16–19*). We have extended our studies of the sheep model to measure serially ^{67}Ga lung uptake in parallel with other parameters of disease activity during the course of induction of experimental asbestosis. The data document that enhanced ^{67}Ga lung uptake precedes other parameters of disease activity, is not associated with cumulative exposure dose, and correlates best with macrophage derived fibronectin accumulation in the lung.

MATERIALS AND METHODS

Animals

Twenty-six sheep weighing 25–40 kg were enrolled in this study. They were prepared and accustomed to

the pulmonary techniques as previously reported (*16–19*).

Experimental Design

The flock was divided into a group of 11 sheep exposed to phosphate buffered saline (PBS) only, and a group of 15 sheep to 100 mg UICC Canadian chrysotile asbestos fibers in 100 ml PBS every 2 wk. These fibers were relatively uniform and well characterized (*20*), 92% being <0.25 μm in diameter and 20 μm in length. Exposures were carried out after nasotracheal intubation through a slow infusion of the suspension in the trachea. The animals were studied prior to exposure and at 3–6 mo intervals after by thoracic radiographs (TR), pulmonary function tests (PFT), ^{67}Ga scans and lavage, bronchoalveolar lavage (BAL) analyses, and transbronchial lung biopsies (TLB).

Thoracic Radiograph

Each sheep was positioned on a mobile cart with a wooden board and a grid cassette under the thorax. The x-ray source was placed at a 30° caudal angle, 2 ft from the cassette. The intubated animal was held at total lung capacity using a giant syringe and radiographs taken at exposure factors 80 kV, 20 mAs and 0.02 sec. Each radiograph was read independently by two of us (R.B. and M.B.) and scored according to the International Labor Organization (ILO) classification of radiographic profusion in the pneumoconiosis as previously used (*10–15*).

Pulmonary Function Tests

The methods used in PFT assessment of the sheep have been published (*16–19*). Briefly, transpulmonary pressure was monitored with a naso-esophageal, 7-ml balloon catheter and an airway catheter connected to a Hewlett-Packard 270 differential pressure transducer. Gas flow at the airway opening was measured by connecting the cuffed endotracheal tube to a Fleisch No. 2 pneumotachograph attached to a flow integrator recorder system and a Mink data processing system* for on-line analysis and storing of the data. Each PFT measurement was obtained following a three inspiratory capacity (IC) constant-volume history. Total lung capacity (TLC) was defined as the lung volume at a transpulmonary pressure of +35 ± 5 cm H_2O and residual volume (RV) as the lung volume at a transpulmonary pressure of −35 ± 5 cm H_2O. The static expiratory lung compliance (C_{st}) was determined by multiple-step syringe deflation between TLC and functional residual capacity (FRC).

Gallium-67 Scan and Lavage

Each sheep received 2 mCi of [^{67}Ga]citrate† in the exteriorized carotid loop and 48 hr later, they were scanned from neck to pelvis with a Dyna 4c/15-61 camera‡ coupled to a Cromenco system 3 microprocessor.§ Grading of the ^{67}Ga pulmonary uptake was done by the computer on a 17-point scale: grade 0 was equal to the average background radioactivity as measured in the neck area, grade 16 representing the maximal liver uptake and the in-between grades were equally distributed between grades 0 and 16. Six lung fields were graded and averaged to yield an index of ^{67}Ga lung uptake between 0 and 16 as previously reported in humans and in the model (*10–15*). Because of the diffuse nature of the disease process as seen on scans and thoracic radiograph, we did not analyze the data by region but used an average index of ^{67}Ga lung uptake. After each scan, BAL was carried out in a randomly selected subsegmental bronchus and radioactivity of a 5-ml sample of the 100 ml effluent placed in a LKB-Wallac model 1271 automatic gamma counter¶ and radioactivity between 70 and 210 keV (59.2% of ^{67}Ga emission) was reported in counts per minute per ml (cpm/ml).

Bronchoalveolar Lavage and Fluid Analysis

Most of the techniques in BAL procedures and analyses have been previously described (*16–19,21*). The BAL effluent 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. In the supernatant, albumin and fibronectin were measured by the immunochemical methods of Killingworth and Savory (*22*), using the Behring laser nephelometer instrument.** For sheep albumin, specific antiserum raised in rabbits was obtained commercially.†† Sheep BAL fibronectin was purified by affinity column followed by chromatography and antisheep fibronectin antibodies prepared in rabbits as described (*21*). All results were expressed per ml of BAL fluid. All values of humoral components of BAL were also analyzed in terms of ratio to the albumin content of BAL supernatant.

Transbronchial Lung Biopsy

Under topical local anesthesia and without fluoroscopic guidance, TLB were obtained following the technique previously published (*16*). Subsegmental bronchi were randomly selected and TLB was immediately placed in 10% buffered formaldehyde fixative and processed as routinely done for human lung tissues. All sections were stained with hematoxylin-eosin and selected biopsies were also stained with Masson-trichrome. Morphologic observations were made without knowledge of other parameters under study. Grading of the severity of parenchymal alteration and degree of cellularity was made as an estimation compared with earlier series of TLB (*16–19*). Biopsies were thus assigned a grade of inflammatory alterations from 0 (normal) to 1 (mild), 2 (moderate), and 3 (severe).

Statistic Analysis

All results are expressed as mean ± s.e.m. The data were tested by Student's t-test for differences between groups. A p value of <0.05 was considered significant in this study (*23*).

RESULTS

Subsets of Asbestos Exposed Sheep

At month 24 and after, the group of 15 sheep exposed to asbestos had significantly higher pathologic scores, higher radiographic scores, lower vital capacity, lower static lung compliance, lower arterial PO_2 (data not shown), and these changes were accentuated in the year after. Within the group, these changes were the effect of disease limited to some of the sheep. We then separated the group of asbestos exposed sheep into two subsets: a subset of six sheep who had minimal or no TLB and TR changes and were within the 95% tolerance limit of PFT of PBS exposed sheep—this subset is reported as without asbestos alveolitis hereafter—and a second subset of asbestos exposed sheep composed of nine sheep with changes on TLB, TR, and PFT consistent with those of the initial asbestos alveolitis previously reported in this model (*17*)—this subset is reported as with asbestos alveolitis hereafter. Gallium-67 lung uptake indices and BAL analyses were not used in this separation of the two subsets of asbestos exposed sheep.

Pulmonary Function Tests

In Fig. 1, we present the results of PFT. Total lung capacity (TLC), vital capacity (VC), functional residual capacity (FRC), residual volume (RV), and static lung compliance (C_{st}) in control sheep increased slightly over time, reflecting the continuous growth of these animals enrolled in the study at the age of 12 mo. In the asbestos exposed sheep without alveolitis, the only significant change was a decrease in C_{st} from month 24 ($p < 0.05$). In the sheep with asbestos alveolitis, the change in C_{st} was accentuated more and also accompanied by significant changes in VC from month 24 ($p < 0.05$).

Thoracic Radiographs

All TR of control animals were and remained without infiltrates or pleural lesions throughout the experiment (Fig. 2). In the sheep without asbestos alveolitis, the same observation was recorded till month 24 where one of the six sheep had a 1/0 infiltrate in two lung fields, which appeared in a second sheep at month 30 and were present in 3/6 at month 36. In the sheep with asbestos alveolitis, scores of parenchymal opacities of 1/0 or greater were present in each of the nine sheep and the average score went from 2.7 ± 1.7 at month 24 to 7.3 ± 2.7 at month 36 ($p < 0.05$).

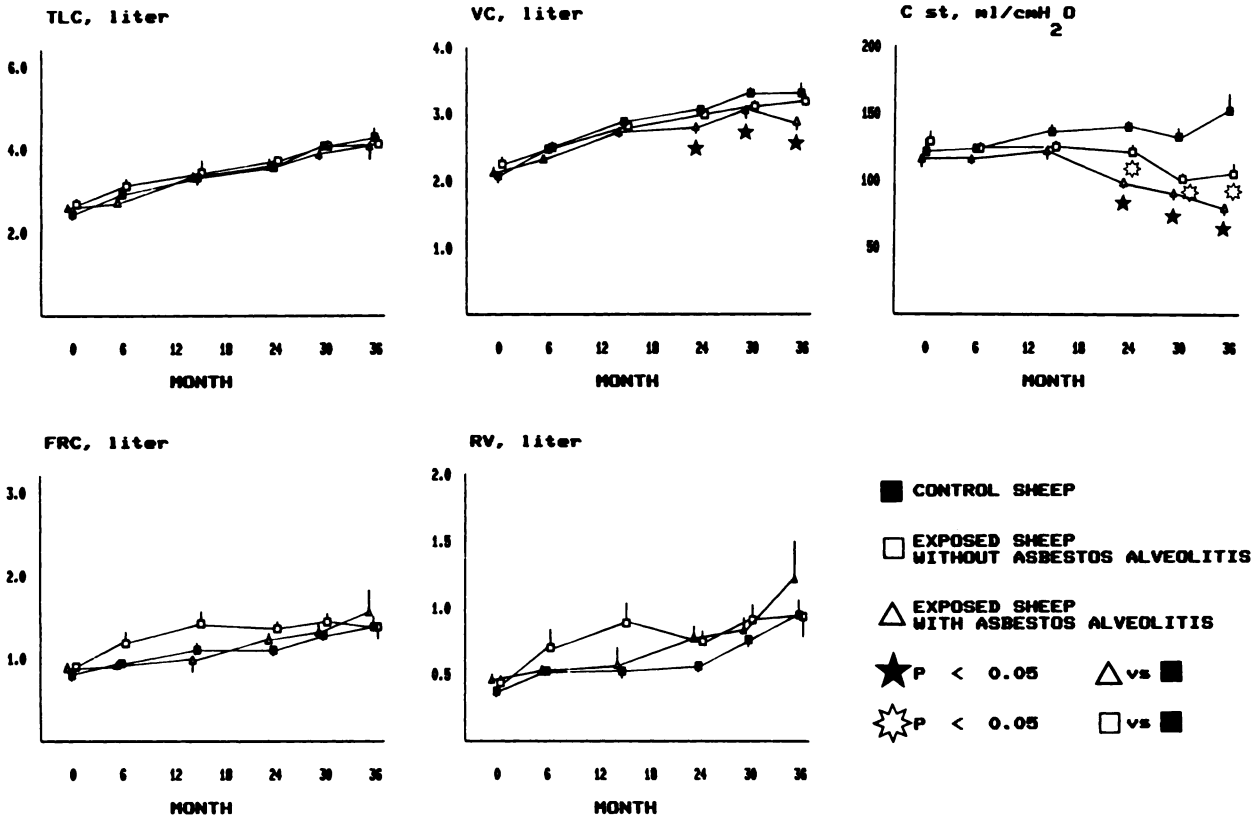

FIGURE 1
Pulmonary function tests

Gallium-67 Lung Uptake

In the control sheep, ^{67}Ga scan uptake index varied from 0.9 to 1.5, averaging 1.25 ± 0.18 and did not change significantly over time. In the sheep without asbestos alveolitis, the index was in the control range except at month 30 where it was significantly higher at 2.5 ± 0.5. In the sheep with asbestos alveolitis, the index was significantly higher from month 6, averaging 2.2 ± 0.5 and increased further at month 30 to 3.0 ± 0.31 ($p < 0.05$). The radioactivity counts on BAL (Fig. 2), also clearly separated the sheep with asbestos alveolitis from those without and controls ($p < 0.05$) starting at month 9. Representative normal and abnormal ^{67}Ga thoracic scans are presented in Fig. 3.

BAL Analyses

In Fig. 4, we present results of BAL analyses. In controls, there was no significant change in all parameters over time. In the asbestos exposed sheep without alveolitis, the results of cellularity were comparable to controls at all points whereas albumin was significantly increased at months 30 and 36, 99 ± 7 and 90 ± 10 compared with 64 ± 4 μg/ml for controls ($p < 0.05$). In the sheep without alveolitis, fibronectin in BAL was increased significantly from month 9 at 175% controls and it increased further in parallel with the albumin leakage from month 30. In the sheep with asbestos alveolitis, significant changes in cellularity occurred at month 24 and after; whereas albumin started to increase at month 24 and fibronectin at month 9. In the latter sheep, BAL fibronectin averaged 250% controls from month 9 to 24 and increased further with the albumin leakage at months 30 and 36 (Fig. 3).

Lung Biopsies

In the control sheep, lung biopsies remained normal. In the asbestos exposed sheep without alveolitis, the only significant change was focal peribronchiolar fibrosis. In the sheep with the asbestos alveolitis, there was in addition to the focal peribronchiolar fibrosis, a diffuse macrophagic and neutrophilic alveolitis extending from the peribronchiolar area to the adjacent interstitium and alveoli (Fig 5).

DISCUSSION

In the sheep model of asbestos studied at mid-course, we have previously documented good correlations between ^{67}Ga lung uptake and pathologic score of disease activity (*10*). In the present study, we have obtained repeatedly measurements by BAL and scan of ^{67}Ga lung uptake during induction of the disease process and have found that enhanced uptake of the marker assessed independently by scan and BAL occurred before the disease process can be detected by other methods. The enhanced ^{67}Ga lung uptake correlated then only with excessive accumulation of fibronectin in BAL (Figs. 1-3). Because fibronectin is a glycoprotein produced locally by macrophages and associated with fibrogenic activity (*24-26*), we suggest that activated macrophages

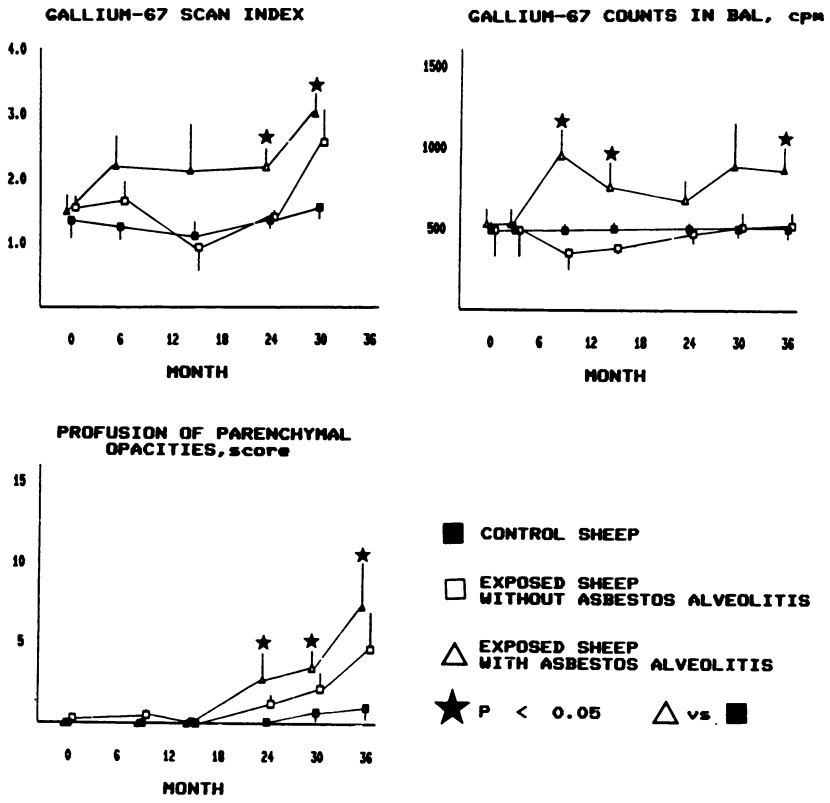

FIGURE 2
Gallium-67 lung uptake and thoracic radiograph. Scan index was obtained as previously reported (*10*). Gallium-67 radioactivity counts in BAL are detailed in Materials and Methods section. Profusion of parenchymal opacities on thoracic radiograph was scored as previously reported (*13*)

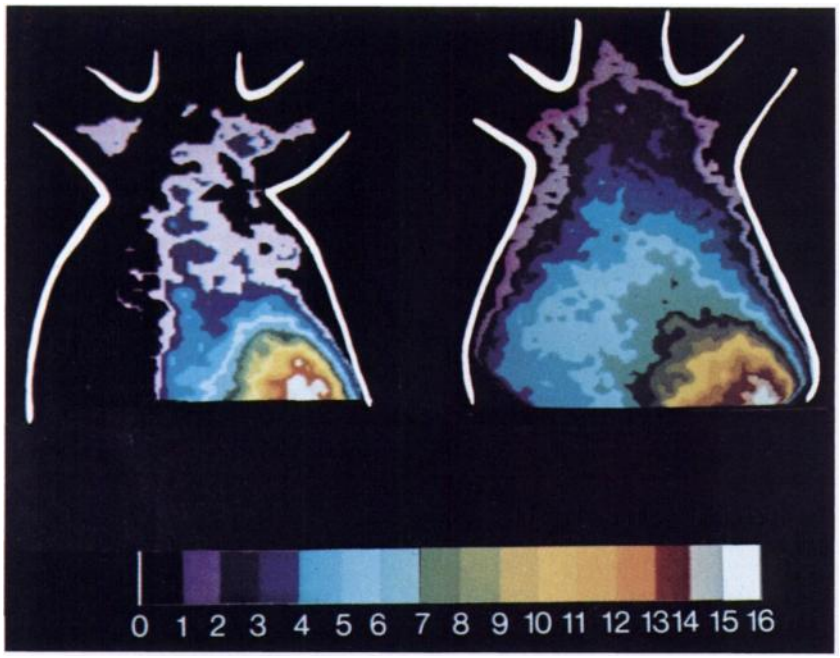

FIGURE 3
Gallium-67 thoracic scan. In left panel, normal scan of asbestos exposed sheep without alveolitis (grade 0.5); in right panel, abnormal scan of sheep with asbestos alveolitis (grade 4)

producing a large amount of fibronectin are likely responsible for the enhanced accumulation of ^{67}Ga in the lung. The observation of a normal ^{67}Ga lung uptake in asbestos exposed sheep without alveolitis in face of enhanced fibronectin accumulation (Figs. 2 and 3) suggest that there is a threshold of macrophage activity below which ^{67}Ga lung uptake remains normal and above which it is enhanced. The threshold hypothesis is based not only on the comparison of the two subsets of asbestos exposed sheep before month 24, but also in

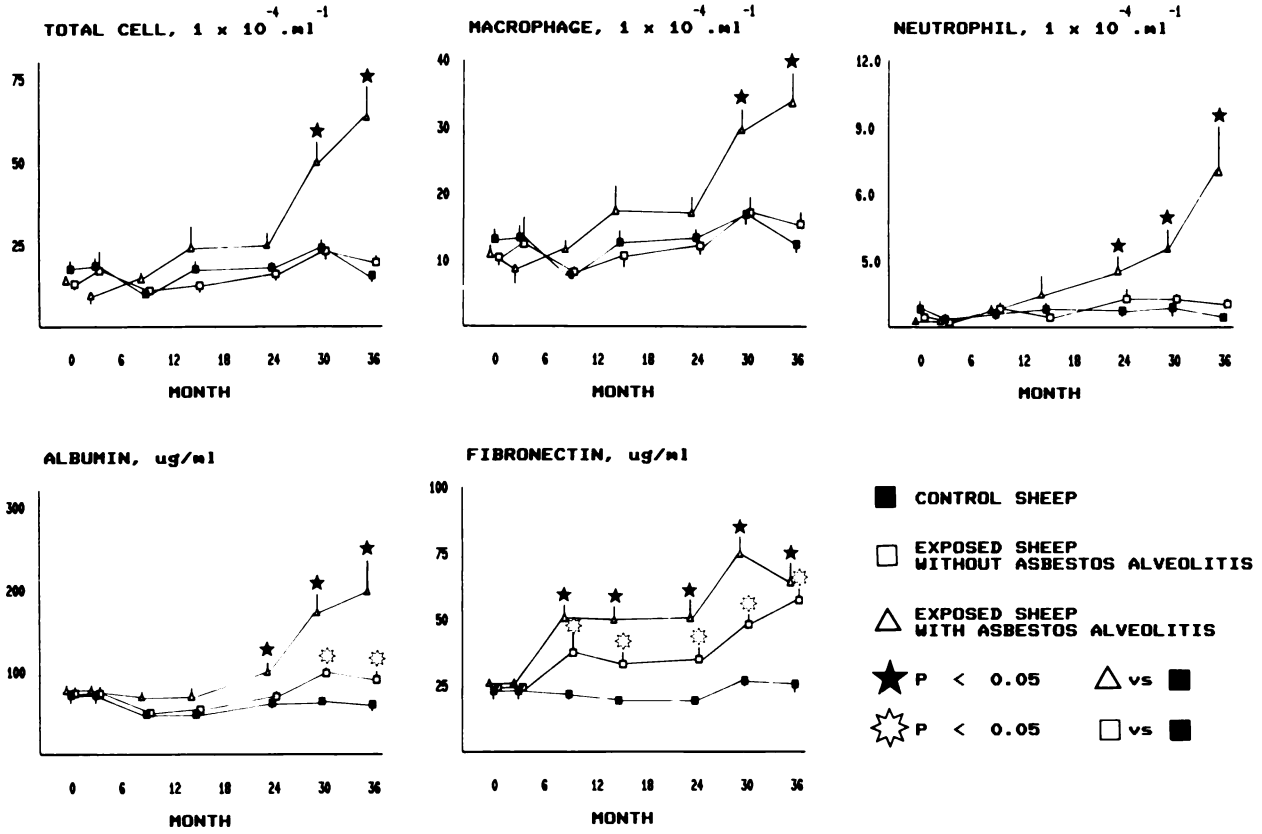

FIGURE 4
Bronchoalveolar lavage analyses. Cellularity is reported as ×10,000 cells per ml of effluent; albumin and fibronectin in μg/ml

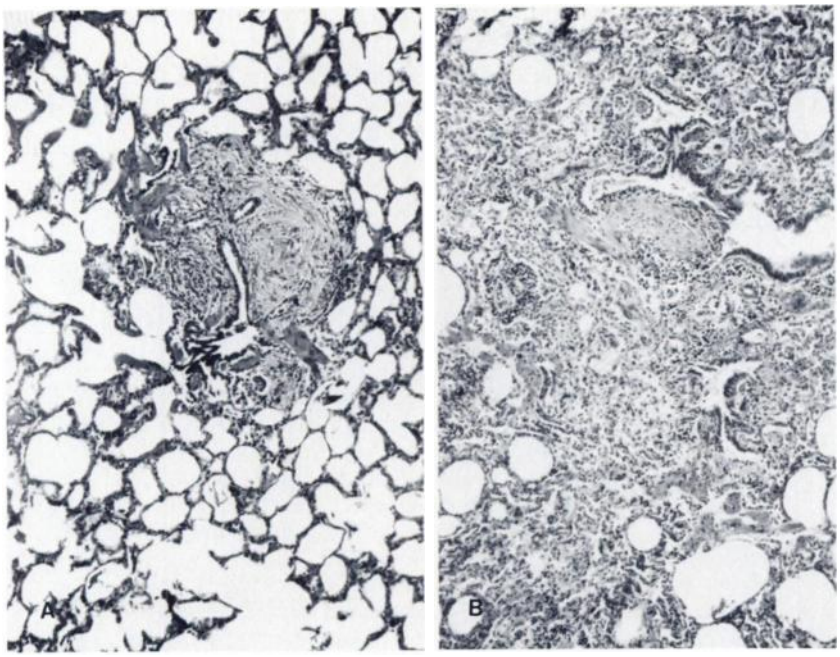

FIGURE 5
Lung biopsies. A: Biopsy of sheep exposed to asbestos without alveolitis; it shows peribronchiolar fibrosis. B: Biopsy of sheep with asbestos alveolitis; it shows peribronchiolar fibrosis and in addition, inflammatory process in adjacent interstitium and alveoli (Hematoxylin-eosin ×63)

comparing the sheep without alveolitis before and after month 24, at least with regard to the scan index. The absence of increased ^{67}Ga BAL count at month 30 in the latter subset may reflect a delay of appearance of changes in BAL which may not reflect as accurately rapid interstitial changes (*17,19*). Thus, these experimental data in the sheep model of asbestosis confirm our previous observations in long-term asbestos workers in documenting that enhanced ^{67}Ga lung uptake is a phenomenon which precedes the usual changes in parameters of detection of disease and further suggest that macrophages actively producing excessive amounts of fibronectin are primarily responsible for enhanced uptake of the marker.

A second observation of interest in this study is the fact that given the same cumulative asbestos exposure, only 9/15 of the sheep had disease activity at month 24. Beside ^{67}Ga lung uptake, the only significant change which preceded the separation of the two subsets of asbestos exposed sheep was a different rate of fibronectin production (Fig. 3). This suggests that disease activity at least at its initial stage may well be determined by the inherent capacity of the macrophage population to produce fibronectin and/or other factors such as the neutrophil chemotactic factor (NCF) and macrophage derived growth factor (MDGF) implicated in the pathogenesis of the disease process (*27,28*). Alternatively, the mechanism whereby fibronectin production is lower in the sheep without alveolitis may be related to individual capacity of clearance of the fibers from the bronchoalveolar milieu (*29*). Thus the mechanism whereby some of the sheep exposed to the same cumulative exposure dose did not develop alveolitis at month 24 may be related either to inherent macrophage dysfunction or faster clearance of the fibers from the alveolar space.

The clinical insights gained by these experimental studies are primarily related to early detection of inflammatory lung diseases where macrophages are the major effector cells. Early macrophage activation can be detected by ^{67}Ga scanning in the pneumoconioses prior to the usual modes of disease detection. As an enhanced ^{67}Ga lung uptake is usually associated with disease progression in the animal model as well as in humans (*10,15*), an abnormal ^{67}Ga scan in asbestos workers should constitute an early warning parameter of inflammatory disease activity and call for close follow-up of the workers and cessation of exposure. An additional line of parallel information on the use of ^{67}Ga scanning in early detection of inflammatory lung disease has come from investigation of early infectious inflammatory lung disease in the immunosuppressed patients where abnormal ^{67}Ga is considered an excellent early indicator of infectious lung disease such as *P. carinii* pneumonia (*30*).

In conclusion, this study in the sheep model of asbestosis clearly documents that enhanced ^{67}Ga lung uptake precedes changes in thoracic radiograph, pulmonary function, and cellularity of BAL in the course of development of asbestos alveolitis. The enhanced ^{67}Ga lung uptake is best related to excessive amount of BAL fibronectin produced by the fiber activated macrophages. Further analyses of factors differentiating the sheep resistant to asbestos biologic activity will increase our understanding of the biologic mechanisms of the disease and lead to new approaches to control disease activity.

FOOTNOTES

* Digital Equipment, Montréal.

† DuPont NEN Medical Products; No. Billerica, MA.

‡ Picker, Northford, CT.

§ Cromenco, Mountainview, CA.

¶ LKB Wallac, Tuskir, Finland.

** Behring LN modular system, Hoechst Behring, Frankfurt, WG.

†† Cappell Lab., Inc., Downingtown, PA.

ACKNOWLEDGMENT

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