Alterations in Bronchoalveolar Lavage Fluid but Not in Lung Function and Bronchial Responsiveness in Swine Confinement Workers

Kjell Larsson, M.D., F.C.C.P.; Anders Eklund, M.D., F.C.C.P.; Per Malmberg, M.D.; and Lars Belin, M.D.

Testing of lung function and bronchial reactivity, bronchoalveolar lavage (BAL), and a skin prick test with a standard panel and six "swine" extracts obtained from swine and swine environment were performed in 20 randomly selected nonsmoking swine confinement workers. In addition, blood samples for detection of antibodies by the diffusion in gel–enzyme-linked immunosorbent assay (DIG-ELISA) technique and precipitating antibodies were drawn. Air samples for measurement of dust and endotoxin levels were collected. All the farmers regarded themselves as healthy. The results were compared with reference groups consisting of urban nonsmoking subjects who had not been exposed to pig farming environment. The pig farmers had normal lung function and the bronchial reactivity was not different from the reference group. In the BAL fluid of the farmers, the concentration of total cells and granulocytes was increased while the concentrations of lymphocytes and macrophages were normal. The BAL fluid concentrations of albumin, fibrinectin, and hyaluronan were elevated in the farmers.

Skin prick tests with swine extracts were negative in all farmers. Antibodies (assessed by DIG-ELISA) against swine dander, swine dust, and pig feed were increased and precipitating antibodies against swine dander were found in 14, against pig food in five, and against swine confinement dust in three of the 20 pig farmers. The concentration of airborne total dust was 7.4 mg/cu mm and the endotoxin concentration was 37 (22 to 60) ng/cu mm during tending the pigs and increased, during feeding, to 13.8 mg/cu mm and 315 (194 to 716) ng/cu mm, respectively. There was no correlation between exposure and lung function or lavage findings. In conclusion, randomly selected pig farmers had signs of airway inflammatory reaction and activation of the immune system without alteration in lung function and bronchial reactivity.

METHODS

Subjects

A random sample from a complete Swedish national register of farmers rearing swines in the vicinity of Stockholm were asked to participate in the study. Of 32 male nonsmoking farmers selected, seven refused to participate in the study, two were excluded because of asthma, and three were not possible to reach. In one of the two asthmatic farmers, airways symptoms were not related to pig farming, while in the other, there was a probable relationship between exposure to swine dust and symptoms. Thus, 20 farmers from 18 farms accepted the investigation. The farmers who were investigated had a mean age of 47 years (range, 23 to 65 years) and had been working with pig farming for 23 years (range, 5 to 40 years) on average. The main working task was pig farming for all the participating farmers and no one kept cattle or worked with dairy farming. The farmers spent on average 3 h (range, 1 to 5 h)

ACE = angiotensin-converting enzyme; BAL = bronchoalveolar lavage; CI = confidence interval; ELISA = enzyme-linked immunosorbent assay; IQR = interquartile range; FEF = peak expiratory flow

Workers in swine confinement buildings are exposed to dust containing feed and fecal particles, dander from swines, bacteria, fungi, endotoxin, and to gases such as ammonia, carbon dioxide, and hydrogen sulfide. Swine confinement workers have an increased frequency of airways symptoms of which cough and phlegm are the most common (for review see Donham) but wheeze and tightness of the chest have also been observed. Slight obstructive decrements in lung function have been reported, and the functional deterioration is related to airways symptoms. A slight decrease in FEV1 over work shift, which correlated better with airborne endotoxin levels than with other exposure variables, has been described. Two studies have been reported on bronchial responsiveness in pig farmers, but the results are inconclusive. A preliminary report has shown an increased concentration of neutrophils in bronchoalveolar lavage (BAL) fluid in swine confinement workers.

The aims of the present study were to investigate if swine confinement workers have signs of airways inflammation and alterations of lung function and bronchial responsiveness. Furthermore, we estimated the immunologic response by skin prick tests and measurements of serum antibodies.
each day, seven days a week, in the swine confinement buildings tending and feeding the pigs. Seven of the farmers were ex-smokers (mean smoking history, 11 pack years [range, 1 to 23 pack years]) and had stopped smoking on average nine years (range, 2 to 16 years) prior to the study. The others were "never-smokers." All had normal chest roentgenograms. All participants gave their informed consent. The study had the approval of the local Ethics Committee.

All farmers were interviewed by using a standardized questionnaire and by an oral interview with regard to the presence of airway symptoms such as cough, phlegm, nasal obstruction, and throat irritation. No subject had cardiac disease. One farmer had rhinoconjunctivitis during pollen season but no symptoms from the lower airways. Two farmers with asthma were not invited since our intention was not to investigate the incidence of airways disease in pig farmers but to study alterations in the airways induced by the pig farming environment. The coexistence of airways disease (whose etiology is actually unknown) would have complicated the interpretation of the results.

The reference group regarding lung function consisted of 20 healthy, nonsmoking, office workers (men) with a mean age of 47 years (range, 39 to 66 years) with no history of exposure to farming environment. The lung function parameters were compared with the predicted values given by Hedenström et al.9-11 The reference values for the bronchial provocation tests were obtained from healthy subjects (11 female) with a mean age of 37 years (range, 23 to 51 years) with no exposure to a farmer's environment.

Our reference values for comparisons regarding the findings in BAL fluid were obtained from 25 never-smoking healthy subjects (12 female) with a mean age of 25 years (range, 18 to 35 years). All subjects in the reference group were "never-smokers" and had no history of farming exposure.

Reference group for the diffusion in gel–enzyme–linked immunosorbent assay (DIG-ELISA) analyses were 25 nonfarming healthy blood donors. Ten of these serum samples served as controls also for the precipitin analyses.

Procedure

On one day lung function was measured, a bronchial methacholine challenge and a skin prick test were performed, and blood samples for antibody detection were drawn. Approximately one week later, BAL was performed. The exposure to airborne contaminants were measured during 1 h of work in the swine confinement building within two weeks from the day of the BAL, in all but three farmers in whom the intervals between BAL and exposure measurements were longer (25 days in two subjects and 77 days in one subject).

Lung Function: Lung volume (total lung capacity [TLC] and residual volume [RV]) were measured at a breathing frequency of 25 breaths/min in a volume chart body plethysmograph (Erich Jaeger Gmbh, Würzburg, Germany) connected on line to a computer. The TLC and RV were stated as the mean value of three measurements. Ventilatory capacity and forced expiratory flows were measured with a low-resistance rolling-seal spirometer (OHIO model 840, Airco, Madison, Wis) connected on line to a computer. The diffusing capacity (transfer factor) was determined by the carbon monoxide single breath method described by Cotes (Morgan transfer test, PK Morgan Ltd, Chatham, UK). The pulmonary function testing has been described in detail elsewhere.12-14

Peak Flow Measurements: Peak expiratory flow (PEF) was measured during seven days in a peak flow meter (mini-Wright, Clement Clarke Ltd, London, UK) in the morning immediately after getting up, at noon, and in the evening before going to bed, at all three occasions in the sitting position. This over-day variation in PEF was compared with a reference group consisting of nine healthy subjects (six women) with a mean age of 41 years (range, 28 to 50 years) with no exposure to farming environment. The variability of PEF over the day was expressed as the greatest difference between the day in percent of the "grand" mean value, ie, the mean PEF value for all measurements on all days. For calculation of the intra-individual PEF variability in the group, the standard deviation of the greatest PEF difference over the day was calculated in each subject. The standard deviation of these 20 standard deviations was then calculated and expressed in percentage of the "grand" mean PEF value of the group. In addition, PEF was measured before and after a 1- to 2-h working period in the swine confinement building. The PEF value stated was always the best value out of three blows.

Bronchial Responsiveness: The methacholine challenge was performed with inhalation of the diluent followed by inhalation of increasing doses of methacholine starting at 0.5 mg/ml; each increment represented a doubling of the dose. The bronchial challenges were performed with a jet nebulizer (MA2 nebulizer, Astra Meditee, Göteborg, Sweden), which at a driving pressure of 390 kPa has an output of 0.38 ml/min (0.013 ml/min). The mass median aerodynamic diameter of dried nebulized was 1.7 µm according to measurements (with an Aerodynamic particle sizer, APS-3300, TSI Inc, St Paul, Minn). The nebulizer was connected to the top of a double cone-shaped metal tube with a height of 48 cm to which an additional airflow was connected and corrected to total airflow of 0.4 L/s. The subjects inhaled the nebulized solution, which has been dried during the sedimentation through the tube, through a mouthpiece at the bottom of the metal tube at tidal breathing with a frequency of 0.25 Hz using a metronome to guide the breathing. At the outlet of the metal tube, a back valve was connected to limit the inspiratory flow to the supplied air flow of 0.4 L/s. The dose of inhaled methacholine was individually measured. The FEV₁ was measured with a dry spirometer (Vitalograph, Buckingham, UK) and airway resistance was measured with the flow-interruption method (AW-test Erk Jaeger GmbH, Würzburg, Germany) starting 1 min after cessation of the inhalation. The result was expressed as the concentration and the cumulated methacholine dose causing a 20 percent decrease in FEV₁, (PC20FEV₁, and PD20FEV,) and the concentration and the cumulated dose causing a 33 percent increase in airways resistance (PC33Raw and PD33Raw). Also, the slope of the relation between change in FEV₁, vs (the linear) cumulated dose was calculated by regression analysis.

Bronchoalveolar Lavage: The BAL technique has been described earlier.12 Bronchoscopy was performed through the mouth with a flexible fiberoptic bronchoscope (Olympus Type 4B2) under local anesthesia with 2 percent lidocaine (Lignocaine, Xylocaine, Astra, Södertälje, Sweden) before premedication with benzodiazepine and atropine. The bronchoscope was wedged in a middle lobe bronchus and 250 ml of sterile saline solution at 37°C was instilled in five aliquots of 50 ml. After each instillation, the fluid was gently aspirated and collected in a siliconized plastic bottle kept on ice.

BAL Fluid Analyses: Total and differential cell counts were carried out essentially as described earlier.12 After straining the fluid through a single layer of gauze, cells were pelleted at 400 g for 5 min at +4°C. The supernatant was kept frozen at −70°C until analysis. The pellet was resuspended in TRIS-Hanks balanced salt solution at a pH of 7.4 and the cells were counted in a Bürker chamber. Smears for differential cell counts were prepared by cytocentrifugation. After staining with May-Grünewald Giemsa, 400 cells were counted.

Albumin in the BAL fluid was analyzed by rocket immunoelectrophoresis14 using commercial antisierum (Dakopatts, Denmark) and human serum albumin as standard (Kabi Vitrum, Sweden). Angiotensin-converting enzyme (ACE) activity was measured in the concentrated BAL fluid according to Lieberman15 as described earlier12 and expressed as U/L (nmol hippuric acid/min/L). Hyaluronic acid was analyzed by a radioimmunoassay in principle as described earlier16 using a test kit ("Pharmacia HA test"). Fibronectin was assayed by a double sandwich ELISA developed in our laboratory17 using unlabeled and horseradish peroxidase-labeled rabbit antihuman fibronectin (Dakopatts, Copenhagen, Denmark) and standard
serum fibronectin of nephelometric quality (Behring-Hoechst, Frankfurt am Main, Germany).

**Skin Prick Tests and Antibody Measurements:** Skin prick tests were performed with six different extracts from swine and swine environment: urine (1:2), plasma (1:4), and dander from swines, two types of pig food, and dust from swine confinement buildings. Swine urine was dialyzed and concentrated ×5 using 20 mM ethylene glycol. This concentrate was mixed with an equal volume of 100 percent glycerol and 1 percent phenol diluent. Swine plasma was used after being diluted four times in 50 percent glycerol and 0.5 percent. One gram of acetone-washed epithelial scraping from deep frozen pig tails, two types of cereal and fishpowder-based pig food, and dust collected from swine confinement buildings were extracted with 10 ml of phosphate-buffered saline solution and diluted by the addition of an equal volume of 100 percent glycerol and 1 percent phenol to a final concentration of 1:20.

Skin prick tests were also performed with a standard panel consisting of extracts from birch, timothy, mugwort, dog, cat and cow dander, *D. pteronyssinus*, Alternaria, and Cladosporium (Spectralgen 100,000 BU/ml, Pharmacia, Uppsala, Sweden).

Precipitating antibodies were determined by the double-diffusion-in-gel method of Ouchterlony. The amount of antibodies against different swine, mold, and bacterial antigens was estimated using a DIG-ELISA. Antigen extracts from swine urine, swine dander, dust from swine confinement buildings, pig food, Rhizopus, *Aspergillus fumigatus*, Alternaria, Penicillium, and *Micropolyspora faeni* were used in these tests.

**Measurements of Exposure to Airborne Contaminants:** Total dust and endotoxin were sampled by means of personal samplers. The farmer carried two filter cassettes in the breathing zone, directed obliquely downwards on the chest. The cassettes were equipped with cellulose acetate filters (Millipore AAWP filters, Cork, Ireland) with a diameter of 25 mm. In addition, one cassette was equipped with a cyclone (Cyclone, SKC, cut-off 5 μm) making estimations of the respirable fraction possible. The airflow was measured with a rotameter before and after sampling and was adjusted to 2 L/min. The cassettes were used open faced. Measurements were performed during 1 h in the swine confinement building during tending of the pigs (tending values) and during feeding the pigs (feeding values).

Total dust was measured by a normal weighing procedure after 24 h of conditioning, using a balance (Mettler ME 22 balance, Mettler, Greisensee, Switzerland) and reference filters. Endotoxin was measured by the Limulus amebocyte lysate assay. The filters from the airborne dust samples were shaken in 10 ml of pyrogen-free water in a washer (Stomacher). Serial dilutions and a negative and positive control were tested. The last dilution, resulting in a stable clot, was compared with commercial *E. coli* lipopolysaccharide (Sigma, St Louis, Mo).

**Statistics**

The BAL data, results from measurements of bronchial reactivity, and exposure data are presented as median values with interquartile ranges (iq). Otherwise data are presented as mean values (SD) or with 95 percent confidence interval (CI). Statistical comparisons with regard to lung function were made by Student's *t* test. All other statistical analyses were made by Mann-Whitney *U* test and Spearman's rank correlation. A p value <0.05 was considered significant.

**Results**

**Symptoms**

None of the swine confinement workers had severe symptoms involving the airways. Mild airways symptoms such as cough/phlegm and nasal obstruction/throat irritation induced by working in the swine confinement building were reported by three and two farmers, respectively.

**Lung Function**

The lung function of the swine confinement workers did not differ significantly with regard to any of the parameters measured when compared with our own reference group (Fig 1). In the swine confinement workers, the 95 percent CI for all lung function parameters included 100 percent of predicted value, ie, were not different from the predicted values. In the reference group, the 95 percent CI of ME50 and DC0 exceeded 100 percent of predicted value (Fig 1).

The PEF values were 99 percent (93 to 104 percent, 95 percent CI) in the swine confinement workers and 102 percent (94 to 111 percent, 95 percent CI) of predicted value in the reference group. The mean PEF variability (in percentage of grand mean value) during the day was small (5.6 percent in the farmers and 5.0 percent in the reference group) and did not differ significantly between the two groups (Fig 2). The mean standard deviation for the intraindividual variability (in percentage of grand mean value) was 2.1 percent in the farmers and 2.6 percent in the reference group. There was no significant difference between PEF measured before (605 [89, SD] L/min) and after (602 [91] L/min) the work shift in the swine confinement building (Fig 2).

Bronchial reactivity was similar in the pig farmers and the reference group and did not differ significantly between the groups (Table 1).

**BAL Analyses**

In the BAL fluid of the farmers, there were signifi-
In the pig farmers, no differences with regard to the findings in BAL fluid were found when nonsmokers were compared with exsmokers.

**Skin Prick Tests and Antibody Measurements**

Skin prick test was positive against timothy and dog dander in one subject. In all other participants, skin prick tests were negative.

The amount of antibodies as assessed by the DIG-ELISA technique was higher in the farmers than in the reference group with regard to swine dander, swine dust, and pig food (p<0.001). No differences between the groups were found with regard to swine urine or the microorganisms investigated. No significant correlations between the amount of antibodies against swine antigens and the time of exposure or BAL bindings were found.

Precipitating antibodies to antigens in swine dander were found in 14, to pig food in five, and to swine confinement dust in three of the swine confinement workers. One worker gave a strong immunoprecipitate to *A fumigatus*. In the ten blood donors who served as controls, faint immunoprecipitates to swine dander antigens were found in four.

**Exposure Measurements**

For total dust, there was a significant difference between tending, 7.4 (4.1 to 13.7) mg/cu mm, and feeding, 13.8 (10.0 to 27.3) mg/cu mm, (p<0.01). Also endotoxin in the air samples was higher during feeding than during tending, 315 (194 to 716) ng/cu mm and 37 (22 to 60) ng/cu mm, respectively (p<0.001).

<table>
<thead>
<tr>
<th>Table 1—Bronchial Reactivity*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>PC20FEV&lt;sub&gt;1&lt;/sub&gt;, mg/ml</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>PD20FEV&lt;sub&gt;1&lt;/sub&gt;, mg</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>PC33Raw, mg/ml</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>PD33Raw, mg</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Slope</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

*PCFEV<sub>1</sub> and PDFEV<sub>1</sub> denote the concentration and the cumulated dose of methacholine causing a FEV<sub>1</sub> decrease of 20 percent compared with the value obtained after inhalation of the diluent. PC33Raw and PD33Raw are the corresponding concentration and cumulated dose of methacholine causing a 33 percent increase of Raw compared with the value after inhalation of the diluent. The slope expresses the percentual decrease of FEV<sub>1</sub> and increase of Raw per milligram of inhaled methacholine. Median values and Iqr are given. Iqr denotes interquartile range, ie, the 25th to 75th percentiles.
Endotoxin levels in the respirable fraction, ie, when measurements with a cyclone-equipped sampler was performed, were 8 (5 to 29) ng/cu mm when tending and 17 (9 to 53) ng/cu mm when feeding, representing 52 percent and 10 percent, respectively, of the total endotoxin concentration in the air samples. There was no significant difference between the endotoxin levels in the respirable fraction between tending and feeding.

There were no significant correlations between exposure measurements (total dust and endotoxin) and lung function or BAL data.

DISCUSSION

In the present study, randomly selected, nonsmoking, swine confinement workers were studied. Since all farmers considered themselves healthy, selection mechanisms were related to the willingness to accept the BAL procedure rather than concern for disease. In the present study, the lung function was normal in all individual farmers, but considering the limited size of the study sample and the selection of nonsmokers, the possibility that pig farming is associated with small obstructive changes as reported by others cannot be excluded.

To our knowledge, two reports on bronchial responsiveness in pig farmers have been published. One was aimed at relating bronchial responsiveness with airways symptoms and obstructive lung function decrement and did not include a comparison with a reference group. Bronchial reactivity can be evaluated correctly only when comparisons are made with a reference group in which bronchial challenges have been performed identically to the investigated group. Thus, from the study by Iversen et al, it is not possible to draw firm conclusions regarding the prevalence of an increased bronchial responsiveness among pig farmers. In a pilot study of pig farmers who had joined a farmer's health program, a slightly larger decrease

FIGURE 3. Cellular findings in the swine confinement workers (exposed group) and the reference group. The filled bars denote the 25th and 75th percentile and the line within the bars the median values. The barlines denote tenth and 90th percentile and the dots denote values above the 90th and below the tenth percentile. Median values are given to the right of the bars and 25th and 75th percentiles are given to the left of the bars.
in FEV₁ to a low dose of methacholine was found when compared with controls. More than 50 percent of these nonsmoking farmers reported airways symptoms. Thus, the changes were small and difficult to interpret in terms of bronchial responsiveness and there was a possibility of selection of symptomatic farmers. In the present study, randomly selected pig farmers with a low prevalence of symptoms had normal bronchial responsiveness, which is compatible with previous findings in symptomless farmers. Furthermore, the PEF values were normal in both the farmers and the reference group. The fluctuations of PEF during the day were not increased in the pig farmers. High amplitudes of PEF variation during the day are probably an expression of bronchial hyperresponsiveness and the lack of PEF fluctuations supports our observation of a normal bronchial responsiveness in the farmers. No changes in PEF over work shift were found in accordance with earlier findings.

In the alveolar space, the total cell concentration was somewhat increased as reflected in the BAL fluid. This increase was mainly due to an increased concentration of neutrophils, which also has been reported earlier. The neutrophil response in pig farmers differs from the lymphocytosis observed in dairy farmers. Dairy farmers are exposed to large quantities of fungal and actinomycete spores against which these farmers have an increased amount of serum antibodies. Pig farmers are not exposed to mold dust to the same extent and in the present study they had normal amounts of antibodies against several mold species.

High dust levels were observed during feeding with crushed grain mixtures. Grain dust may recruit neutrophils to the alveoli by direct chemotactic activity and by activating the complement system with generation of C5a, which functions as an attractant of neutrophils. In addition, grain dust can stimulate alveolar macrophages to release chemotactic factors. The activation of the complement system with generation of C5a is also induced by endotoxin which is frequently present in grain dust and which was found in the air samples in the present study.

Thus, the observed alveolar granulocytosis could be due to several mechanisms. The endotoxin "tending levels" in the respirable fraction in the present study were similar to those found in terminals for grain dust
deposition and our “feeding values” were about one third of those found during normal unloading of silos. The exposure in the present study was lower than in previous reports from swine confinement buildings in the southern part of Sweden.

The inflammatory reaction in the alveoli was also reflected by the high concentration of albumin in the BAL fluid. This albumin emanates from blood plasma and enters the airways by leakage through the alveolo-capillary membrane. Also fibronectin, another soluble component of the BAL fluid, occurs in slightly but significantly increased concentration. The production of fibronectin is enhanced in patients with interstitial pulmonary diseases and fibronectin may function as a chemoattractant for fibroblasts, bronchial epithelial cells, and neutrophils. Fibronectin may also attach neutrophils to the alveolar wall. Thus, fibronectin could contribute to the recruitment of neutrophils in the airways of pig farmers. The alveolar macrophage is an important fibronectin-producing cell in the lower airways, but several other cells, including the neutrophils, can produce fibronectin but contribute only with a small fraction of the total fibronectin production in interstitial lung disease.

The increased concentration of fibronectin was not accompanied by an increase of ACE activity in the BAL fluid. The same pattern has been observed in other types of environmental exposure such as in aluminum potroom workers. In dairy farmers, however, both fibronectin and ACE concentrations in BAL fluid were increased. The alveolar macrophages may also release ACE. The dissimilar findings in pig and dairy farmers indicate that fibronectin and ACE are released by different mechanisms or possibly by different cells. It could be argued that fibronectin and ACE leak through the alveolo-capillary membrane like albumin. However, the concentration of fibronectin in plasma has been found to be normal in patients with interstitial lung disorders although they had high BAL concentrations of this substance. This observation and the high molecular weight of fibronectin and ACE compared with albumin speaks in favor of a local production in the alveolar space and against leakage through the membrane. Additionally, the hyaluronan levels in BAL fluid from pig farmers were significantly higher than in the reference group, although the concentrations were only modestly increased. Healthy dairy farmers have normal hyaluronan levels in BAL fluid (Larsson et al., in press). In contrast, the hyaluronan levels in dairy farmers with acute alveolitis were about 15 times higher than the concentrations found in the pig farmers. The main source for hyaluronan in the airways is fibroblasts located in the alveolar interstitium, but it may also be secreted by other cells of mesenchymal origin or by pulmonary endothelial cells. It is not likely that hyaluronan leaks into the airways from the blood since farmers with acute allergic alveolitis have normal plasma levels of hyaluronan while the hyaluronan concentration in the BAL fluid is increased.

The BAL findings reflect an inflammatory reaction in the alveolar space due to exposure in swine confinement buildings. The negative skin prick tests against “swine-related” antigens indicate absence of IgE-mediated allergy against the corresponding allergens. The serum levels of IgG antibodies against two of the swine antigens and the pig food were increased while antibodies against fungi and actinomycetes were normal. In contrast, dairy farmers exhibit increased amounts of serum IgG antibodies against several fungal and actinomycete species. Thus, the different exposure typical for dairy farms and swine confinement buildings has different consequences with regard to antibody formation, BAL findings and airways responsiveness (Larson et al., in press).

ACKNOWLEDGMENTS: We wish to thank Caroline Angleryd, Margitha Dahl, Boine Bernbrand, Björn Sannagård, and Britt-Marie Sundblad for expert technical assistance. This study was supported by grant 88-0405 from the Swedish Work Environment fund and the Swedish Heart-Lung Foundation.

REFERENCES

Bronchiolitis Obliterans after BMT (Paz et al)

21 Ramsdale EH, Morris MM, Hargreve FE. Interpretation of the variability of peak flow rates in chronic bronchitis. Thorax 1986; 41:771-76
29 De Luca AJ, Palmgren MS. Seasonal variation in aerobic bacterial populations and endotoxin concentrations in grain dusts. Am Ind Hyg Assoc J 1987; 48:106-10
30 Olsenchock SA, May JJ, Pratt DS, Morey PR. Occupational exposures to airborne endotoxins in agriculture. Prog Clin Biol Res 1987; 231:475-87
32 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 USA 1981; 78:7147-51
41 Richards BJ, Morris TC. Collagen and mucopolysaccharides production in growing lung fibroblasts exposed to chrysotile asbestos. Life Sci 1973; 12:441-51