Signs of alveolar inflammation in non-smoking Swedish wood trimmers

U Johard, A Eklund, M Dahlqvist, A Ahlander, R Alexandersson, U Ekholm, G Tornling, U Ulfvarsson

Abstract

Wood trimmers are periodically exposed to mould and may develop extrinsic allergic alveolitis (EAA). To investigate if there were any signs of EAA in wood trimmers with low exposure, 19 non-smoking wood trimmers underwent bronchoalveolar lavage (BAL), spirometry, and measurement of diffusion capacity (TL_{CO}). The group was subdivided into those with (n = 9) and without serological antibodies against mould. In 14 workers the TL_{CO} was measured both at the beginning and at the end of a week at work. Twenty five healthy non-smokers served as BAL controls and 19 healthy non-smokers as lung function controls. The median exposure of total dust was well below the Swedish threshold value, and the exposure of mould and bacteria was also low. The cell concentrations and the proportions of the various alveolar cells did not differ between the groups. The concentrations of the soluble components albumin, fibronectin, and hyaluronan were, however, significantly increased (p <0·001 for all) in the workers. No difference was found in lung function between the workers and the controls, and the TL_{CO} was not impaired during a week at work. The groups of seropositive and seronegative workers did not differ in any of these parameters. The results are interpreted as a low intensity alveolar inflammation. The presence of precipitating antibodies against mould did not predict any greater risk of developing a more intense inflammation. Analysis of soluble non-cellular BAL components seems to reflect a discrete ongoing alveolar inflammation better than cell counts only.

Inhalation of organic materials—for example, mould spores—may cause extrinsic allergic alveolitis (EAA) with respiratory symptoms, chest *x* ray film changes, impairment of lung function, and reduced diffusion capacity.[1] The alveolitis is generally characterised by an increased proportion of lymphocytes,[2] and this lymphocytosis may persist a long time after exposure to mould has ceased.[3] Also, soluble components of the alveolar fluid such as albumin, fibronectin, hyaluronan, and procollagen III N-terminal peptide have been reported to appear in increased concentrations in bronchoalveolar lavage (BAL) fluid, especially in the acute phase of the alveolitis.[4]

Wood trimmers may develop EAA after inhalation of moulds that from time to time grow on the timber.[5] Precipitating antibodies in the peripheral blood are indicators of such exposure.[6] The pathogenetic and predictive roles of the precipitins are still, however, a matter of controversy.[2 7] It has earlier been reported that wood trimmers may develop a restrictive impairment of lung function and that this impairment depends on the duration and the degree of exposure to moulds and dust.[8] We decided to look for early signs of alveolar inflammation and performed BAL on wood trimmers in a sawmill in northern Sweden where the exposure was regarded as low. The aim was to correlate signs of·inflammatory activity from BAL with lung function, diffusion capacity, and the presence of precipitating antibodies against moulds in the peripheral blood. We analysed the alveolar cell profile to detect an accumulation of inflammatory cells, and measured albumin to reflect the alveolar capillary permeability.[9] Fibronectin concentration and angiotensin converting enzyme activity were used as markers of macrophage activation.[9 10] Correspondingly, the concentrations of hyaluronan and procollagen III N-terminal peptide served as signs of fibroblast activity.[11 12]

Material and methods

STUDY POPULATION

Nineteen healthy male volunteers (mean age 39 range 21–56) working as wood trimmers at a sawmill in

Department of Thoracic Medicine, Karolinska Hospital, Stockholm, Sweden
U Johard, A Eklund, G Tornling
Department of Work Science, Royal Institute of Technology, Stockholm, Sweden
M Dahlqvist, R Alexandersson, U Ekholm, U Ulfvarsson
Department of Lung Medicine, Hospital of Sundsvall, Sundsvall, Sweden
A Ahlander

Sundsvall in northern Sweden entered the study. All were non-smokers (n = 13) or ex-smokers (n = 6); the mean time after stopping smoking was 19 years (range 12–31). All had a normal chest x ray film. As shown in table 1 nine of them (mean age 39, range 26–56) had precipitating circulating antibodies against one or several moulds known to be able to produce "wood trimmer's disease".[6] The presence of antibodies was investigated by a double diffusion technique.[13] The remaining 10 workers (mean age 38, range 21–50) had no precipitating antibodies. The mean duration of employment as wood trimmers was 14 years (range 3–35) for the seropositive group and 10 years (range 1–19) for the seronegative group.

The control group for the lavage study consisted of 25 non-smoking healthy male volunteers without known history of occupational exposure to moulds. Their mean age was 25 (range 19–31), and all had a normal chest x ray film. The control group for the lung function study consisted of 19 healthy non-smoking office workers from a factory close to the sawmill. Their mean age was 42 (range 26–55) and they all had a normal chest x ray film.

All workers were interviewed personally by means of a questionnaire, which focused on symptoms from the respiratory mucous membranes and signs of alveolitis such as dyspnoea, fever, and pain from joints and muscles. They were also asked for any other exposure outside the working site that might cause signs of alveolitis (for example, bird breeding). One person in the lung function study group with bronchial asthma, which had started before the employment, was excluded from the study. No one had any history of exposure known to cause respiratory disease.

The study had the approval of the local ethics committee and all subjects gave informed consent.

BRONCHOALVEOLAR LAVAGE (BAL)

After premedication with morphine-scopolamine (Kabi, Stockholm, Sweden) and local anaesthesia with lignocaine (Xylocain, Astra, Södertälje, Sweden) a flexible fibreoptic bronchoscope (BF Type 4B2, Olympus, Tokyo, Japan) was wedged in a subsegmental bronchus of the right middle lobe. Sterile saline solution at 37°C was instilled in five aliquots of 50 ml and gently removed by suction as described earlier.[13] The fluid was collected in a plastic siliconised bottle kept on ice. The recovered volume was measured and data are presented as per cent of instilled volume.

HANDLING OF ALVEOLAR CELLS

The BAL fluid was strained through a double layer of Dacron net. After centrifugation at 400 g for five minutes at 4°C, the supernatant fluid was poured off and stored at −70°C until analysed further. The cells were resuspended in Hank's balanced salt solution, and a total cell count was performed in a Bürker chamber. The viability was evaluated by means of excluding trypan blue. A differential cell count was performed after the preparation of centrifugal smears in a Cytospin 2 apparatus (Shandon, Runcorn, UK) at 500 rpm for three minutes. Cells were stained according to May-Grünewald Giemsa and 500 cells were counted.

BIOCHEMICAL ANALYSES OF BAL FLUID

Albumin was determined by rocket immunoelectrophoresis[14] using rabbit antihuman albumin (Dakopatts, Copenhagen, Denmark) as antiserum and human serum albumin (Kabi, Stockholm, Sweden) as standard and was expressed in mg/l. Intra and interassay coefficients of variation (CVs) were 3% and 7% respectively.

Fibronectin was assayed in unconcentrated BAL fluid by a double sandwich enzyme linked immunosorbent assay (ELISA) developed in our laboratory.[15] Serum fibronectin of nephelometric quality from Behring-Hoechst (Frankfurt am Main, Germany) was used as a standard. Concentrations of BAL fibronectin were expressed in µg/l. The detection limit was 10 µg/l. Intra and interassay CVs were 4% and 6% respectively.

Hyaluronan was analysed in the unconcentrated BAL fluid by a radiometric assay in principle by the method of Engström-Laurent *et al*[16] using the Pharmacia HA-kit (Pharmacia, Uppsala, Sweden). The

Table 1 Serological data for sawmill workers (n = 9) with precipitating antibodies against fungi

Worker	Rhizopus rhizopodiformis	Mucor hiemalis	Aspergillus umbrosus	Aspergillus fumigatus	Penicillum frequentas	Pullularia pullularis	Paecillomysis varioti	Cladosporium herbatum
1	+	+	−	−	−	−	−	−
2	+	+	−	−	−	−	−	−
3	+	+	−	−	−	−	−	−
4	+	+	−	−	−	−	−	−
5	+	+	−	−	−	−	−	−
6	+	+	−	−	−	−	−	−
7	+	+	+	−	+	−	−	−
8	+	+	+	+	−	+	+	+
9	−	−	−	−	−	−	+	−

+ = Antibodies found; − = antibodies not found.

detection limit was 10 μg/l. Intra and interassay CVs were 4% and 6% respectively.

Angiotensin converting enzyme (ACE) activity was analysed according to Lieberman[17] in concentrated BAL fluid as described earlier[9] and expressed in U/l (nmol hippuric acid/min/l). Intra and interassay CVs were 4% and 4% respectively.

Procollagen III N-terminal peptide was assayed in unconcentrated BAL fluid by a radioimmunoassay (RIA) in principle according to Rodhe and co-workers[12] using a commercial RIA kit (Behring-Hoechst, Frankfurt am Main, Germany). Concentrations were expressed in μg/l. The detection limit was 0·2 μg/l. Intra and interassay CVs were both 11%.

LUNG FUNCTION STUDY

The wood trimmers and the lung function control group were studied by static and dynamic spirometry (single breath wash out with nitrogen (N_2) and carbon monoxide (CO)). The examination of the two groups was mixed and performed, unless otherwise stated, at the end of a week of work. The equipment for determinations of dynamic and static lung volumes and transfer factors consisted of a dry rolling seal spirometer (VG 2000 S with Diffusimat 2000, Mijnhardt, Holland) with a helium analyser based on the thermoconductivity principle. The CO was analysed by means of an infrared gas spectrophotometer. All volumes were corrected to BTPS (body temperature and pressure, saturated with water vapour). The equipment was calibrated at least once a week with 1 l and 3 l precision syringes. The results are given as per cent of predicted values.[18]

Static spirometry was performed with the helium dilution technique. The residual volume (RV) and the total lung capacity (TLC) were determined.

Dynamic spirometry gave values for vital capacity (VC) and forced expiratory volume in one second (FEV_1). At least three measurements were taken for each person, according to the guidelines stated by ATS.[19] The highest values for FEV_1 and VC were used in the statistical analysis.

Diffusion capacity (TL_{CO}) was measured by the single breath CO technique.[20] In 14 workers (five with and nine without precipitating antibodies) the TL_{CO} was determined both after a work break of two days and in the afternoon at the end of the week after four or five days of work.

Measured values from the dynamic and static spirometry and transfer factor were compared with reference values obtained from the summary equations given by Quanjer *et al.*[18]

ESTIMATION OF EXPOSURE

Total dust

The exposure to dust was measured by personal sampling equipment carried by 16 workers. Dust was sampled on cellulose acetate filters (Millipore Corp, Boston, USA) with a diameter of 25 mm and a pore size of 0·8 μm placed in an open faced cassette through which air was pumped at a flow rate of 2 l/min. Sampling was done over an entire eight hour shift. Dust concentration, expressed in mg/m³ air, was calculated from the change in weight of the filter divided by the volume of air sampled.

Moulds and bacteria

In 15 workers airborne mould spores and bacteria were collected by personal air sampling on polycarbonate filters (Nucleopore Corp, Pleasanton, California, USA) with a pore size of 0·4 μm and a diameter of 25 mm. The filters were placed in presterilised plastic filter cassettes connected to portable pumps (Casella, Bedford, UK) calibrated for an airflow of 1·0 l/min. The duration of the sampling time was 1·5 hours. The handling of the filters has been described in detail before.[21] Briefly, the filters were rinsed in extraction fluid in a shaking apparatus for 15 minutes. Part of the suspension was used for determination of viable micro-organisms by the plate count technique. One ml of 10 fold dilution was incubated in fungal medium and 1 ml in bacterial medium. The plates were incubated at 24 and 45°C (fungi) and 24, 40, and 55°C (bacteria and actinomyces) for seven days. The total number of colonies were counted. The results are given in colony forming units (cfu)/m³ air. Part of the suspension was stained with acridine orange and filtered through a black stained nucleopore filter. Microscopical investigation was made after drying. The total amounts of moulds and bacteria were counted and expressed as numbers/m³ air.

STATISTICAL ANALYSIS

As the BAL parameters were not normally distributed, the results are given as medians and interquartile ranges (iqr) unless otherwise stated. Levels of statistical significance were evaluated using the non-parametric Mann-Whitney test and Spearman's rank correlation coefficient.

Results

SUBJECTIVE SYMPTOMS

Ten of the 19 (53%) workers had had symptoms in accordance with acute allergic alveolitis during the past year (dyspnoea, cough, wheezing, fever, and pains in muscles or joints starting some hours after work and ceasing some hours later). Five of them were seropositive and five were seronegative. The average duration of employment for those with symptoms was 14 years and for those without symptoms 10 years.

Table 2   *General characteristics of the BAL fluid from 19 non-smoking sawmill workers with and without precipitating antibodies against mould and 25 healthy non-smoking volunteers (controls)*

			Total		Differential cell count			
Group	Recovery (%)	Viability (%)	cell count (× 10^6)	Cell conc (× 10^6/l)	AM (%)	Ly (%)	PMN (%)	Eos (%)
Sawmill workers:								
All	68	81	10	72	87	11	1	0
(n = 19)	(55–72)	(70–82)	(7–17)	(52–93)	(83–92)	(7–16)	(0–2)	(0–0)
Seropositive	69	74	10	91	87	12	1	0
(n = 9)	(43–72)	(70–81)	(10–17)	(66–93)	(82–90)	(7–17)	(0–3)	(0–0)
Seronegative	62	81	10	62	89	9	1	0
(n = 10)	(56–71)	(78–91)	(7–17)	(51–87)	(86–92)	(7–14)	(1–2)	(0–0)
Controls:	68	84	15	91	89	9	1	0
(n = 25)	(65–75)	(81–87)	(12–22)	(68–112)	(87–93)	(6–11)	(1–2)	(0–1)

Data are given as medians with interquartile ranges. No statistically significant differences were found. Recovery = per cent recovered fluid of instilled volume; viability = per cent viable of total cells; total cell count = total number of cells recovered; cell conc = calculated cell concentration in the recovered volume; AM = alveolar macrophages; Ly = lymphocytes; PMN = polymorphonuclear neutrophils; Eos = eosinophils.

BAL DATA

The recovery, viability, total cell numbers, total cell concentrations, and the proportions of the various types of alveolar cells did not differ significantly between workers and controls (table 2). Nor did any of the variables differ between the seropositive and the seronegative workers.

The median concentration of albumin was 53 mg/l (interquartile range 40–66) for all workers compared with 34 mg/l (iqr 24–42) in the controls. The difference was statistically significant (p < 0·001; figure A). The median concentration of fibronectin (figure B) was 80 µg/l (iqr 65–106) in the workers and 41 µg/l (iqr 26–50) in the controls (p < 0·001). The median hyaluronan concentration (figure C) was 42 µg/l (iqr 34–46) in the workers and 27 µg/l (iqr 16–31) in the controls (p < 0·001). Statistically significant differences (p < 0·001 for all components) were also seen between the controls and the seronegative workers. A somewhat weaker, but still statistically significant, difference (p < 0·01 for all components) was found between the controls and the seropositive workers. No statistically significant differences were found in the concentrations of albumin, fibronectin, and hyaluronan between the two subgroups of workers. The median ACE activity was 394 U/l (iqr 265–506) in the workers and 380 U/l (iqr 310–460) in the controls (non-significant). The two subgroups of workers did not differ in this respect either. Procollagen III N-terminal peptide was not detectable in any of the BAL fluids.

LUNG FUNCTION

Table 3 shows the values for VC, FEV_1, TLC, RV, and TL_{CO}. None of the parameters differed significantly between the workers and the controls. Nor was there any difference between the two subgroups of workers.

In the group of workers where the TL_{CO} was measured at the beginning and at the end of a week of work, the median value at the beginning of the week was 92·4% (iqr 77·4–102·9) of the predicted value and at the end of the week 97·8% (iqr 92·3–114·9). This difference was not significant. Furthermore, no difference was noted between the seropositive and the seronegative workers at the beginning or at the end of the week.

EXPOSURE LEVELS

The median daily exposure of total dust was 0·25 mg/m^3 air (iqr 0·22–0·34) and of viable mould spores 3700 cfu/m^3 air (iqr 2100–4900). The median total number of fungi was 13 × 10^4/m^3 air (iqr 71 × 10^3 – 26 × 10^4). Median exposure of living bacteria was 260 cfu/m^3 air (iqr 0–1400) and the median total number of bacteria was 15 × 10^4 (iqr 81 × 10^3 – 29 × 10^4). No significant correlations were seen between any of these parameters and any of the BAL parameters.

Discussion

In the present study the total cell count and the proportions of different cells in the bronchoalveolar lavage fluid did not differ between the workers and the control group. The workers, however, had increased concentrations of the soluble BAL components albumin, fibronectin, and hyaluronan. Thus there were signs of a low intensity alveolar inflammation in wood trimmers exposed to low concentrations of dust, moulds, and bacteria.

Albumin is only synthesised in the liver, and the increased concentrations of this substance found in the lavage fluid from the wood trimmers is probably caused by an increased alveolar capillary permeability,[22] although a decreased clearance from the
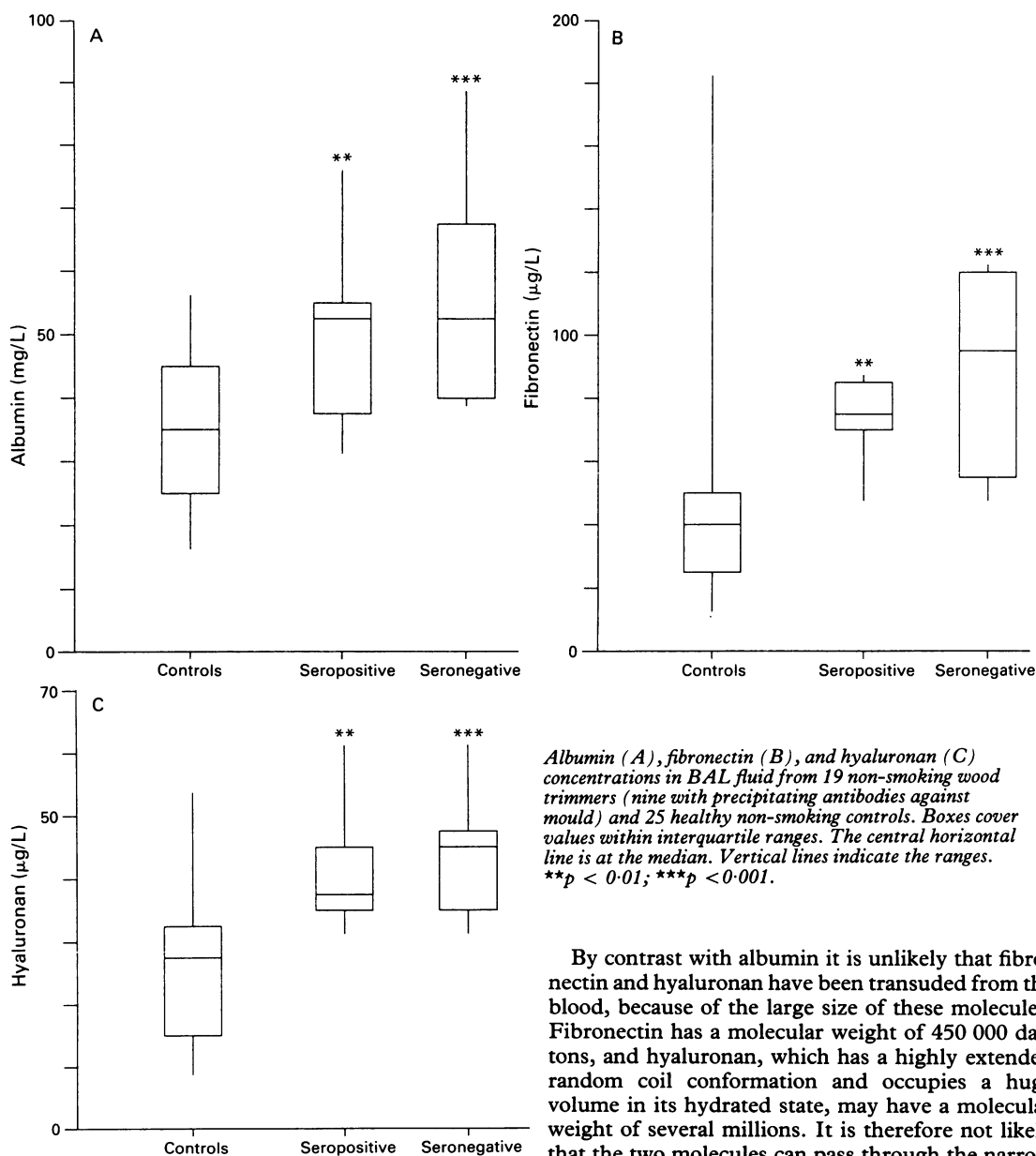
Albumin (A), fibronectin (B), and hyaluronan (C) concentrations in BAL fluid from 19 non-smoking wood trimmers (nine with precipitating antibodies against mould) and 25 healthy non-smoking controls. Boxes cover values within interquartile ranges. The central horizontal line is at the median. Vertical lines indicate the ranges. **p < 0.01; ***p < 0.001.

By contrast with albumin it is unlikely that fibronectin and hyaluronan have been transuded from the blood, because of the large size of these molecules. Fibronectin has a molecular weight of 450 000 daltons, and hyaluronan, which has a highly extended random coil conformation and occupies a huge volume in its hydrated state, may have a molecular weight of several millions. It is therefore not likely that the two molecules can pass through the narrow interepithelial pores, which are 0·6–1·0 nm diameter.[23] The increased concentrations of these substances in the BAL fluid suggest that the substances are produced by activated cells in the alveolar space or in the interstitium. Fibronectin has been shown to be synthesised by cultured alveolar macrophages,[10] whereas the synthesis of hyaluronan has been shown to occur mainly in cultured fibroblasts.[11] Increased concentrations of fibronectin in BAL fluid have been found in sarcoidosis.[24 25] Also, increased concentrations of hyaluronan have been reported in

alveoli may also cause increased concentrations. It has previously been reported that the amount of albumin in lavage fluid is raised in the alveolitis seen in sarcoidosis.[9] This increase is significantly more pronounced in clinically active disease, when the alveolitis is probably more intense. The albumin concentration may be an even better indicator of the intensity of the alveolitis than the percentage of BAL lymphocytes.[9]

Table 3 Lung function and diffusion capacity in 19 non-smoking sawmill workers with and without precipitating antibodies against mould and 19 healthy non-smoking controls

	VC	FEV,	TLC	RV	TL_{CO}
All workers:	99·2	96·5	96·7	88·7	102·9
(n = 19)	(95·9–107·7)	(81·0–107·4)	(88·7–100·8)	(79·6–118·5)	(95·0–114·9)
Seropositive	99·0	93·3	98·0	94·9	97·9
(n = 9)	(97·6–101·6)	(89·5–103·0)	(93·6–103·1)	(86·0–125·8)	(94·2–105·7)
Seronegative	100·4	98·2	92·8	84·9	109·9
(n = 10)	(88·7–109·6)	(80·1–112·8)	(85·0–100·8)	(73·9– 94·1)	(97·1–119·5)
Controls:	102·8	100·2	97·6	102·0	94·6
(n = 19)	(94·5–107·2)	(94·1–108·5)	(93·1–105·0)	(82·5–111·3)	(84·0–109·5)

Data are given in per cent of predicted values as medians with interquartile ranges; VC = vital capacity; FEV$_1$ = forced expiratory volume in one second; TLC = total lung capacity; RV = residual volume; TL_{CO} = diffusion capacity for CO. No statistically significant differences were found.

bleomycin induced alveolitis in rats,[26] in sarcoidosis,[25] and in extrinsic allergic alveolitis.[4] In addition, concentrations of fibronectin and hyaluronan show a significant correlation both in healthy subjects and in alveolitis in sarcoidosis.[25] We found no such correlation, possibly due to the low number of our study population. High and parallel increases of fibronectin and hyaluronan may be a prerequisite for the development of fibrosis.[25]

Increased concentrations of ACE in BAL fluid have earlier been reported in alveolitis caused by sarcoidosis[9 27] and also in other inflammatory reactions in the alveoli—for example in smokers.[25] We found no such increase in the wood trimmers, indicating that there was no pronounced inflammatory reaction.

Bjermer *et al*[4] reported raised concentrations of lavage type III procollagen peptide in patients with acute episodes of farmer's lung and noted that the concentration was normal at clinical remission 6–14 months later. They proposed that the increased concentrations may signal an increased risk of developing fibrotic changes. By contrast, O'Connor *et al*[27] could not find any correlation between lavage type III procollagen peptide concentration and the severity of the sarcoid disease or any relation between the initial concentration in the BAL fluid and later lung function deterioration. We found no increase in procollagen III N-peptide in the BAL fluid recruited from the wood trimmers with normal lung function. Thus the inflammatory process in the alveolar space does not seem to be intense.

The outcome of a low intensity alveolitis is uncertain. It can hypothetically either progress to fibrosis with restrictive impairment of lung function or remain a harmless physiological response to inhaled materials. In a study on farmer's lung, a disorder similar to wood trimmer's disease, a more pronounced alveolitis was shown than in our study.[2] At follow up six to seven years later, however, no restrictive impairment was seen, indicating that the alveolitis may not be a good predictor for the development of

fibrosis. Also, none of the workers in our study showed any changes on chest *x* ray film, impaired diffusion capacity, or decreased spirometric values, despite a duration of employment exceeding 10 years.

Occupational exposure to wood dust may cause chronic obstructive lung disease[28 29] and chronic restrictive changes.[8] In an earlier study[30] it was reported that lung function was impaired if the concentration of total dust exceeded 2 mg/m^3 air. In another study[8] lung function in workers in two sawmills was compared. In one sawmill the exposure to viable moulds was low (mean 2 × 10^3 cfu/m^3 air) and in the other the exposure was higher (5 × 10^4 cfu/m^3). In the second sawmill signs of restrictive impairment were found among the workers and they increased during a week of work. In our study the median exposure to total dust was 0·25 mg/m^3 air and the median exposure to viable moulds was 3 × 10^3 cfu/m^3 air, indicating a low grade exposure. The total dust concentration was well beyond the Swedish threshold limit (3 mg/m^3 air for wood dust) and of the same degree as in the above cited studies, in which no impairment of lung function was found.

The value of analysing precipitating antibodies against moulds in the peripheral blood for prediction of alveolitis or fibrotic development is still a matter of controversy. Some suggest that precipitating antibodies are only a reflection of contact to the corresponding antigen,[29 31 32] whereas others have claimed that precipitins may play a part in the pathogenesis of alveolitis.[7 33] We found no difference in the intensity of the alveolitis between the groups with and without precipitins. Nor did we notice any difference in the grade of alveolar inflammation between symptomatic and asymptomatic workers. By contrast, Chan-Yeung *et al*[34] found signs of alveolitis in symptomatic but not in asymptomatic workers.

We conclude that in this Swedish sawmill with low concentrations of airborne wood dust, moulds, and bacteria the workers had a low intensity alveolar inflammation reflected by increased concentrations

of soluble compounds in the BAL fluid. Neither the lung function nor the diffusion capacity was impaired, however, and no decrement of the diffusion capacity was found during a week of work. The presence of precipitating antibodies against moulds in the peripheral blood did not predict a more intense alveolar inflammation. To estimate the predictive value of BAL signs of an alveolar inflammation with regard to an ongoing fibrotic process prospective studies are necessary.

Requests for reprints to: Urban Johard, MD, Department of Thoracic Medicine, Karolinska Hospital, S—104 01 Stockholm, Sweden. Phone 46-8-7293902, Fax 46-8-332998.

1 Malmberg P, Rask-Andersen A, Palmgren U, Höglund S, Kolmodin-Hedman B, Stålenheim G. Exposure to micro-organisms, febrile and airway-obstructive symptons, immune status and lung function of Swedish farmers. *Scand J Work Environ Health* 1985;11:287–93.

2 Gariépy L, Cormier Y, Laviolette M, Tardif A. Predictive value of bronchoalveolar lavage cells and serum proteins in asymptomatic dairy farmers. *Am Rev Respir Dis* 1989;140:1386–9.

3 Leblanc P, Bélanger J, Laviolette M, Cormier Y. Relationship among antigen contact, alveolitis, and clinical status in farmer's lung disease. *Arch Intern Med* 1986;146:153–7.

4 Bjermer L, Thunell M, Hällgren R. Procollagen III peptide in bronchoalveolar lavage fluid: a potential marker of altered collagen synthesis reflecting pulmonary disease in sarcoidosis. *Lab Invest* 1986;55:654–6.

5 Wimander K, Belin L. Recognition of allergic alveolitis in the trimming department of a Swedish sawmill. *Eur J Respir Dis* 1980;61 (suppl 107):163–7.

6 Belin L. Clinical and immunological data on "wood trimmer's disease" in Sweden. 1980; *Eur J Respir Dis* (Suppl 107) 61:169–76.

7 Cormier Y, Bélanger J, Beaudoin J, Laviolette M, Beaudoin R, Herbert J. Abnormal bronchoalveolar lavage in asymptomatic dairy farmers. *Am Rev Respir Dis* 1984;130:1046–9.

8 Hedenstierna G, Alexandersson R, Belin L, Wimander K, Rosén G. Lung functin and rhizopus antibodies in wood trimmers. A cross-sectional and longitudinal study. *Int Arch Occup Environ Health* 1986;58:167–77.

9 Eklund A, Blaschke E. Relationship between changed alveolar-capillary permeability and angiotensin converting enzyme activity in serum in sarcoidosis. *Thorax* 1986;41:629–34.

10 Rennard SI, Hunninghake GW, Bitterman PB, Crystal RG. Production of fibronectin by the human alveolar macrophage: mechanism for the recruitment of fibroblasts in sites of tissue injury in interstitial lung diseases. *Proc Natl Acad Sci USA* 1981;78:7147–51.

11 Laurent TC, Frasek JRE. The properties and turnover of hyaluronan. In: Evered D, Whelan J, eds. *Function of the proteoglycans. Ciba Foundation Symposium 124.* Chichester: Wiley and Sons, 1986:9–29.

12 Rodhe H, Vargas L, Hahn E, Kalbfleisch H, Bruguera M, Timpl R. Radioimmunoassay for type III procollagen peptide and its application to human liver disease. *Eur J Clin Invest* 1979; 9:451–9.

13 Ouchterlony Ö. Diffusion-in-gel methods for immunological analysis. In: Kallos P, ed. *Progress in allergy 5.* Basel: Karger, 1958:1–78.

14 Laurell C-B. Electroimmunoassay. *Scand J Clin Invest* 1972;29 (suppl 124):21–37.

15 Blaschke E, Eklund A, Hernbrand R. Determination of fibro-nectin and its degradation products in bronchoalveolar lavage fluid. *Scand J Clin Lab Invest* 1990;50:619–25.

16 Engström-Laurent A, Laurent UBG, Lilja K, Laurent TC. Concentration of sodium hyaluronate in serum. *Scand J Clin Lab Invest* 1985;45:497–504.

17 Lieberman J. Elevation of serum angiotensin-converting enzyme (ACE) level in sarcoidosis. *Am J Med* 1975;59:365–72.

18 Quanjer PhH, Dalhuijsen A, Zomeren BC van. Summary equations of reference values. *Bull Eur Physiopath Respir* 1983;19 (suppl 5):45–51.

19 Gardner RM. ATS statement-snowbird workshop on standar-dization of spirometry. *Am Rev Respir Dis* 1978;119:831–8.

20 Cotes JE. Transfer factor (Diffusion capacity). *Bull Eur Physiopath Respir* 1983;19 (suppl 5):39–44.

21 Palmgren U, Ström G, Blomquist G, Malmberg P. Collection of airborne micro-organisms on Nucleopore filters, estimation and analysis-CAMNEA method. *J Appl Bacteriol* 1986; 61:401–6.

22 Reynolds HY. Bronchoalveolar lavage. *Am Rev Respir Dis* 1987;135:250–63.

23 Taylor AE, Garr KA. Estimation of equivalent pore radii of pulmonary and alveolar membranes. *Am J Physiol* 1970; 218:133–40.

24 O'Connor C, Odlum C, van Breda A, Power C, FitzGerald MX. Collagenase and fibronectin in bronchoalveolar lavage fluid in patients with sarcoidosis. *Thorax* 1988;43:393–400.

25 Blaschke E, Eklund A, Hernbrand R. Extracellular matrix components in bronchoalveolar lavage fluid and their relation-ship to signs of alveolitis. *Am Rev Respir Dis* 1990;141:1020–5.

26 Nettelbladt O, Hällgren R. Hyaluronan (hyaluronic acid) in bronchoalveolar lavage fluid during the development of bleomycin-induced alveolitis in rats. *Am Rev Respir Dis* 1989;140:1028–32.

27 O'Connor C, Ward K, van Breda A, McIlgrom A, FitzGerald MX. Type 3 procollagen peptide in bronchoalveolar lavage fluid: Poor indicator of course and prognosis in sarcoidosis. *Chest* 1989;96:339–44.

28 Chan-Yeung M, Lam S, Koerner S. Clinical features and natural history of occupational asthma due to western red cedar. *Am J Med* 1982;3:411–5.

29 Carosso A, Ruffino C, Bugiani M. Respiratory diseases in wood workers. *Br J Ind Med* 1987;44:53–6.

30 Vedal S, Chan-Yeung M, Enarson D, *et al.* Symptoms and pulmonary function in western red cedar workers related to duration of employment and dust exposure. *Arch Environ Health* 1986;41:179–83.

31 Burell R, Rylander R. A critical review of the role of precipitins in hypersensitivity pneumonitis. *Eur J Respir Dis* 1981;62: 332–43.

32 Lee TH, Wraith DG, Bennett CO, Bentley AP. Budgerigar fancier's lung. The persistence of budgerigar precipitins and the recovery of lung function after cessation of avian exposure. *Clinical Allergy* 1983;13:197–202.

33 Roberts RC, Moore VI. Immunopathogenesis of hyper-sensitivity pneumonitis. *Am Rev Respir Dis* 1977;116: 1075–90.

34 Chan-Yeung M, Leriche J, Maclean L, Lam S. Comparison of cellular and protein changes in bronchial lavage fluid of symptomatic and asymptomatic patients with red cedar asthma on follow-up examination. *Clinical Allergy* 1988; 18:359–65.