

Detection of airborne reindeer epithelial antigen by enzyme-linked immunosorbent assay inhibition

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Hypersensitivity reactions to reindeer epithelial (RE) allergens have been recently demonstrated among reindeer herders. To determine the concentration of airborne RE antigens a method based on inhibition of the enzyme-linked immunosorbent assay (ELISA) was developed. Dust samples were collected in workshops where reindeer leather was processed and the workers had inhaled dry epithelial dust during their working shifts. Specific IgE to RE allergen could be detected in one of 5 workers. RE antigen concentrations varied from 0.01 $\mu\text{g}/\text{m}^3$ to 3.9 $\mu\text{g}/\text{m}^3$ in the air of the workshop. All workers except one claimed work-associated respiratory symptoms.

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Occupational allergic diseases caused by animal-derived proteins are common in exposed persons. This has prompted the development of new methods to monitor occupational and environmental impurities in order to evaluate the risk of sensitization and also to identify etiological factors in occupational allergies. Immunochemical detection of airborne allergens has been applied to laboratory animal facilities, cowsheds, and environments polluted by air-conditioning systems (1, 4, 6, 9).

More than 7000 in northern Finland are exposed to reindeer epithelium (RE) in their daily work as reindeer herders (2). According to our earlier studies, positive skin tests to RE are rare among reindeer herders working outdoors (2, 5). However, RE can be expected to cause sensitization more effectively indoors where its concentration is higher. Hundreds of Lapps are working under such conditions while producing handicrafts of reindeer leather.

In the present study, we have developed an enzyme-linked immunosorbent assay (ELISA) inhibition test for measuring airborne concentrations of RE antigens (REAg). Measurements were performed in workshops where reindeer leather was handled. Clinical examination included skin prick tests (SPT) and lung function tests and, in addition, specific IgE against RE was measured.

Material and methods

Subjects

Clinical examinations accompanied by lung function tests and SPT were performed on 5 randomly selected workers (4 women and 1 man) who prepared products of reindeer leather in workshops. Their age ranged from 31 to 65 years. At the time of the examination a blood sample was obtained, and the serum was stored at -20°C and thawed for use as needed.

Lung function tests

The following tests were performed: forced vital capacity (FVC) as well as forced expiratory volume in 1 s (FEV_1) were determined, and $\text{FEV}_1\%$ ($\text{FEV}_1/\text{FVC} \times 100\%$) was calculated. FVC and FEV_1 were measured with a spirometer (Vicat[®], Mijhardt). Spirometric exhalations were repeated until 3 acceptable recordings were available, of which the highest was used. A bronchodilator (rimiterol hydrobromide, Pulmadil[®]) was given to each subject who had a $\text{FEV}_1\%$ less than 75% .

Skin prick test (SPT)

The SPT allergens were: cat, cow, dog and horse epithelium, house dust mite (*D. pteronyssinus*), birch pollen, meadow grass pollen and mugwort pollen

(Allergologisk Laboratorium, ALK, Copenhagen, Denmark), at a concentration of 10 HEP (histamine equivalent prick) units, and RE (Pharmacia Diagnostics, Uppsala, Sweden) at a concentration of 1:100 w/v.

The SPT was applied on the inner forearm, and the results were read after 15 min. Histamine hydrochloride (10 mg/nk [ALK]) was employed as a positive control and 50% glycerol in water as a negative control. A reaction of at least half the size of that produced by histamine was considered positive.

Specific IgE to RE

Specific IgE to RE was measured by using a commercial enzyme allergosorbent test (EAST) (Pharmacia Diagnostics, Uppsala, Sweden) according to the manufacturer's instructions.

Antigen preparation

REAg was prepared as described previously with minor modifications (10): the freeze-dried raw material of reindeer hair and skinscrapings was purchased from Allergen AB (Ängelholm, Sweden). This material was defatted in diethyl ether (1:25 w/v) at 4°C for 24 h, dried at room temperature, and extracted in Coca's solution (1:25 w/v; 0.5% sodium chloride, 0.275% sodium hydrogen carbonate, 0.4% phenol, pH 7.2 at room temperature for 72 h). The solution was clarified by filtering through Whatman No. 3 filter and was then centrifuged at 36600 g for 0.5 h. The supernatant was dialysed (molecular

weight cutoff 3.5 kDa) in a vast excess of 0.05 M ammonium hydrogen carbonate, pH 7.8 at 4°C for 72 h, filtered through Gelman Acrodisc (pore size 0.45 µm) and lyophilised. The preparation was stored at -20°C. The protein content of the preparation was 1.21 mg/ml according to the method of Lowry et al. (3) using bovine serum albumin (Sigma Chemical Co., USA) as a standard.

Rabbit antisera to RE

Rabbits were immunized with RE allergen fortnightly for a 4-month period each time receiving 1.0 mg of protein. The antigen solution was emulsified with Freund's incomplete adjuvant. Injections were given subcutaneously. Blood was drawn several times and antiserum was stored at -70°C.

Air sampling and antigen extraction

Airborne samples were collected by a volumetric filter method according to the Finnish standard SFS 3860 using constant airflow of 2–20 l/min through a MF – Millipore AAWP03700 or a comparable filter (FAS: SFS). Stationary filter holders were used. Test subjects 1 and 2 (see Table 1) were preparing leather boots in a small room (6 m²) and subject 3 was making boots in another room (12 m²). These rooms did not have artificial ventilation. Subjects 4 and 5 made reindeer leather souvenirs in a workshop (48 m²) which had artificial ventilation. Subject 5 used a pressure blower to clean the products causing visible dust in the room air.

Table 1. Clinical data of 5 subjects exposed to REA and dust concentrations in their working places

Data	Subjects					Mean
	1	2	3	4	5	
Age (years)	63	37	65	44	31	48
Sex (F/M)	F	F	F	F	M	
RE-exposure (years)	50	11	17	12	6	19.2
Symptoms	W	W,C	—	W	R	
Smoking	S	S	NS	S	EX	
SPT						
inhal. allergens	—	birch dog ++	birch	—	—	
RE	—	—	—	—	—	
FVC (l)	2.65 (99)	4.45 (118)	3.40 (123)	2.55 (75)	5.20 (100)	
FVC ₁ (l)	2.35 (102)	3.95 (121)	2.70 (117)	2.10 (71)	4.75 (121)	
FEV ₁ %	89	88	79	82	91	
Total IgE (U/ml)	15	12	17	31	6	16.2
(U/ml)						
RE-ABE (U/ml)	0	0	0	0	0.8	
ELISA-inhib. REAg µg/m ³	3.91	3.91	0.77	0.018	0.01	
Total dust mg/m ³	2.2	2.2	0.2	0.9	5.1	

REAg=reindeer epithelium (antigen); W=bronchial wheezing, C=conjunctival itching, R=rhinitis; S=smoker, NS=non-smoker, EX=ex-smoker, SPT=skin prick test, FVC=forced vital capacity, FEV₁=forced expiratory volume, FEV₁ %=(FEV₁/FVC×100%), (l)=percents of predicted values, RE-ABE=specific IgE to RE-antigen (RAST-test).

The filters were weighed to measure the total dust concentration and then cut into pieces and suspended in 3 ml of phosphate buffered saline (PBS, pH 7.4) containing 0.02% (w/v) thiomersal (BDH Chemicals Ltd, England). The suspension was ultrasonicated 5 times for 1 min in ice to release the particulate material from the filter. After centrifugation at 36600 g for 30 min the supernatant was collected and stored at -70°C .

ELISA inhibition procedure

A specific inhibiting activity of REAg was measured by ELISA inhibition as previously described (8): a microELISA plate (Nunc-Immuno Plate I, Denmark) was coated overnight with REAg at a concentration of 0.10 $\mu\text{g}/\text{ml}$ protein in coating buffer (0.05 M carbonate buffer, pH 9.6) in volumes of 200 $\mu\text{l}/\text{well}$ at 4°C . Next morning the plate was washed with phosphate-buffered saline (PBS, pH 7.4) and incubated with diluent at 37°C for 0.5 h. The diluent was PBS (pH 7.4) containing 0.5% gelatine (w/v) and 0.5% (v/v) non-ionic detergent, Nonidet P40 (BDH Chemicals Ltd, UK). After incubation, the plate was washed and stored at -70°C . Before use, the plate was allowed to warm up to room temperature and was then washed. The standard antigen and 2-fold dilutions of the samples in 100 μl volumes were added in duplicate. Rabbit anti-REAg antiserum at a dilution of 1:55 000, 100 $\mu\text{l}/\text{well}$, was added immediately. The plate was incubated for 1 h at 37°C with an agitation rate diluent at 37°C for 0.5 h. After another wash, biotinylated goat anti-rabbit IgG (Vector Laboratories, USA; BA-1000) at a dilution of 1:1000 was added; and the plate was incubated for 1 h at 37°C . After the next wash Vectastain ABC Standard Kit (Vector Laboratories, USA; PK-4000) reagents (avidin DH and horseradish peroxidase H) were added at a dilution of 1:500 and left at room temperature for 0.5 h. After a further wash, substrate solution (tetramethylbenzidine, 0.1 mg/ml; Merck, Germany) and urea hydrogen peroxide (BDH Chemicals Ltd, UK), corresponding to 0.05% H_2O_2 in 0.1 M sodium acetate buffer (pH 6.0), were added. The reaction was stopped (2 M H_2SO_4) and the absorbance was measured at 450 nm by a Titertek Multiskan spectrophotometer (Flow Laboratories).

For determination of the amount of antigen in the samples, a standard curve of absorbance against the log concentration of the standard antigen was plotted. The results of the samples were calculated from the straight part of this curve. Using the ELISA inhibition method the lowest limit of antigen detection was 100 ng/ml.

Results

Clinical findings of 5 employees preparing reindeer leather products appear in Table 1. Duration of the exposure to REA varied from 6 to 50 (mean 19.2) years. Three workers smoked regularly more than 10 cigarettes daily. Two of 5 subjects had positive SPT to at least one inhalant allergen and one to RE. One subject had $\text{FEV}_1 < 80\%$ and another one had FVC and $\text{FEV}_1 < 75\%$ of predicted values.

Three subjects complained of occasional bronchial wheezing at work and during exercise. One of them had conjunctival irritation and a positive SPT to RE. Another had rhinitis which occurred during the working days. Wheezing was present in 2 subjects who worked in a room with the highest REA concentration. Wheezing was present only in the smokers and none of them had specific IgE to RE. The total IgE varied from 6 to 31 U/ml. One test subject had specific IgE to RE allergen by using EAST.

The total dust concentration varied from 0.2 to 5.1 mg/m^3 . The highest dust concentration was measured during the use of a pressure blower to clean the final leather material (see subject 5 in Table 1). The lowest concentration was detected when subject 3 was handling leather boots. Detectable levels of REAg were present in all air samples (Table 1). The highest concentration of 3.9 $\mu\text{g}/\text{m}^3$ was measured in samples from the 2 smallest rooms where leather boots were made and where there was no artificial ventilation. In contrast, REAg concentration was only 10 ng/m^3 in the room where the dust concentration was highest.

Discussion

The aim of the present study was to develop a method to assess the risk of occupational exposure to allergens in a particular working environment. Increasing awareness of the significance of work-related respiratory diseases is a prerequisite for monitoring the effect of corrective and preventive measures as well as for defining acceptable levels of pollutant in working environments. The target material and methods of monitoring depend on the working environment. In cases where animal-derived allergens can be expected to be the main risk factor, immunochemical methods are an obvious choice (1, 6, 7).

According to earlier experience (7) we adapted the ELISA inhibition test for detecting airborne reindeer allergens in workshops where reindeer leather is being handled. In addition to reindeer herding, hundreds of people in Lapland make their living by manufacturing clothes, boots and souvenirs of reindeer leather. With the limited number of air samples used to evaluate the ELISA inhibition in practice, it be-

came apparent that a) the method is able to detect reindeer epithelial antigens at concentrations present in the workshops, and b) there was wide variation between concentrations. Previous studies in laboratory animal facilities have shown that all allergen concentrations in air samples varied with the number of animals and the work activity in the room (6). In the present study the concentrations seemed to be related to room size rather than to type of activity. It was also apparent that the total dust concentration, although an important parameter for air pollution, was not a reliable indicator of the concentrations of allergenic dust. The most accurate estimation of respiratory exposure would probably be obtained by collecting samples from the breathing zone (7).

In our earlier studies we demonstrated that skin reactions against RE were rare among reindeer herders (2). However, the exposure among herders is probably much lower than among persons working on reindeer leather indoors. Five persons working in the rooms where samples were collected were examined clinically. The laboratory tests revealed that only one had detectable levels of IgE antibodies against RE. Another person who was atopic and allergic to dog had no antibodies despite 11 years' exposure to reindeer allergens. These findings show that the development of IgE antibodies and symptoms of respiratory allergy as a response to exposure to animal allergens are multifactorial processes. However, the concentration of allergens in the air is one of the key factors in these processes, and reliable methods should be developed to measure allergens specific to different working environments. We concluded that the ELISA inhibition method proved suitable for determining airborne concentrations of RE allergens. Allergic reactions against RE can be studied by skin prick test and IgE immunoassay with commercially available reagents. Larger epidemiological studies are needed to establish the relationship between allergen concentrations and clinical symptoms and signs of reindeer allergy.

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