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Nasal sensitization of dairy farmers to bovine epithelial and urinary antigens

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SUMMARY

Nineteen dairy farmers with nasal symptoms associated with working in cow houses participated in the study. Nasal challenge with bovine epithelial antigen (BEA) and bovine urinary antigen (BUA) was made before and after the indoor feeding season. Nasal challenge made before the indoor feeding season with BEA was positive in five patients and three of them showed positive reaction in nasal challenge also with BUA. After the indoor feeding season the results in nasal challenge with BEA were approximately equal to BEA and four of them showed positive response in nasal challenge to BUA. However, we did not find any significant increase in sensitivity in nasal challenge to BEA or BUA after the indoor feeding season. In addition to these patients, two patients who were excluded from nasal challenge before the indoor feeding season showed positive results in nasal challenge after the indoor feeding season with both BEA and BUA.

Our results suggest that BUA in addition to BEA may have significance to nasal symptoms.

INTRODUCTION

Respiratory symptoms are common among the dairy farmers (Vohlonen et al., 1985). Bovine epithelium is an important occupational allergen in the dust which is a complex mixture of mainly organic materials (Rautalahti et al., 1987). Twenty per cent of Finnish dairy farmers suffer from allergic rhinitis diagnosed on the basis of positive response in nasal challenge with cow dander extract (Terho et al., 1985).

In addition, the farmers are exposed in their working environment to epithelial allergens and urinary allergens of the cattle. However, clinical significance of bovine urinary antigens is quite an unknown field. Urinary proteins of laboratory animals, such as rat, rriouse, cat and dog, have been shown to cause respiratory symptoms to laboratory personnel or to animal keepers (Newman et al.,

1977; Hoffman, 1980; Schumacher, 19-80; Lee et al., 1982; Twiggs et al., 1982; Lutsky et al., 1985). Recently, urinary antibodies have been shown in dairy farmers (Virtanen et al., 1988). The exposure to bovine-derived urinary and epithelial allergens is more extensive during the indoor feeding season in winter time than in summer, when the cattle is kept mainly outdoors. The effect of seasons on nasal challenge reaction is unknown.

The aim of this study was to reveal if the bovine urinary antigens have any significance to nasal allergic reactions in Finnish dairy farmers, and to compare the allergenic properties of urinary antigens to those of epithelial antigens in nasal challenge. We wanted to find out if bovine urinary antigens caused positive reactions in nasal challenge in patients with negative reaction with bovine epithelial antigens in nasal challenge but who, however, suffer from nasal symptoms associated with working in cowhouses. We also estimated if the increased exposition to the bovine allergens during indoor feeding season had any influence to the process of sensitization.

PATIENTS AND METHODS

Nineteen dairy farmers (9 women and 10 men, mean age 41 years) with nasal symptoms associated to working in the cowhouse were chosen for the study and were examined at the Otorhinolaryngological Department of the Kuopio University Hospital. Before accepting the patients to the study, clinical examination including ultrasonography of the maxillary sinuses was made. Two patients could not be subjected to nasal challenge before the indoor feeding season because of maxillary sinusitis. All patients underwent RAST and skin prick testing to cow dander (RAST, Pharmacia Diagnostic; skin prick test allergen by Allergologisk Laboratorium A/S, Denmark). The results of the tests were considered positive as follows:

1. RAST value 2 or higher (scale 0-4);
2. skin prick test result++ or stronger according to Scandinavian standards, scale from - to ++++, +++ being histamine control equivalent and ++ being half of histamine control (Aas et al., 1972).

Skin prick testing to cow was made only once, after the indoor feeding season, and it was positive in 12 cases. All RAST-positive patients had also positive skin prick test and one patient with RAST value of 0 had highly positive result in skin prick test.

For nasal challenge the bovine epithelial antigen (BEA) was prepared using freeze-dried raw material of bovine epithelium purchased from Allergan AB (Angeholm, Sweden) as described previously (Virtanen et al., 1988). The material was defatted in diethyl ether (1:10 w/v) at 4 °C for 24 h, dried at room temperature and extracted for 72 h at room temperature in Coca's solution, pH 7.2 (1: 10 w/v; 0.5 sodium chloride, 0.275% hydrogen carbonate, 0.4% phenol). The

solution was centrifuged at 36,000 g for 30 min, and the supernatant was dialyzed (MW cut-off 3,500 Da) for 72 h at 4 °C in a vast excess of 0.05 M ammonium hydrogen carbonate (pH 7.8), filtered through a Gelman Acrodisc (pore size 0.45 μm) and lyophilized. Storage was at -20 °C.

Bovine urinary antigen (BUA) solutions were prepared from the urine of newly slaughtered cows by freeze-drying and suspending the dry powder in ion-exchanged water (1 5 w/v) (Virtanen et al., 1988). The solution was then handled as BEA, beginning from the step of centrifugation.

The protein concentrations of the antigen preparations were measured according to the method of Lowry et al. (1951) using bovine serum albumin as a standard. The protein contents of the preparations used were 11 mg/ml (BEA) and 4.9 mg/ml (BUA). For nasal provocation tests, six 10-fold dilutions of both bovine extracts were made in 0.15 M phosphate-buffered saline at pH 7.2 and with a concentration range of 0.01-1.000 μg/ml.

Nasal provocation test was made first to phosphate-buffered saline (pH 7.4) without antigens (negative reference). Then it was continued by spraying 0.1 ml BEA solution to the left nasal cavity and 0.1 ml BUA solution to the right nasal cavity. If there was no response, the challenge was repeated after an interval of ten min with solutions always 10-fold stronger in allergen concentration ad concentrations 10,000 times that of the first solution. The nasal challenge was considered positive if two of the following symptoms were recorded:

1. hypersecretion,
2. oedema,
3. subjective symptoms: sneezing, nasal irritation or obstruction.

RESULTS

Seven patients had RAST value 2 or higher before the indoor feeding season and there was no statistically significant increase in the values recorded after the indoor feeding season. Actually, there was only one patient who showed rise in RAST value.

Nasal challenge, made before indoor feeding season, with BEA was positive in five patients who all had positive skin prick test but of whom one had negative RAST. However, the patient with RAST 0 had strongly positive cow skin test. Three of these five patients with positive response in nasal challenge with BEA had also positive response in nasal challenge with BUA (Table 1).

After the indoor feeding season there were five patients with positive nasal challenge with BEA and four of them had positive nasal challenge with BUA. In addition to these patients, two more patients who were excluded from the tests before indoor feeding season because of maxillary sinusitis, were tested after the indoor feeding season and both of them had positive nasal challenge response to

Table 1. The concentrations of bovine extracts that caused positive responses in nasal challenge and serum lgE levels before and after indoor feeding season. (-) = negative response to bovine extracts with concentration of 1,000 µg/ml and (*) when nasal challenge was not performed. Skin prick test with cow dander is made after the indoor feeding season. Patient no. 8 was not allergic against cow allergens and got non-specific reactions in nasal challenge.

				before indoor feeding season				after indoor feeding season			
						responses in nasal challenge				responses in nasal challenge	
patient	sex	age	skin test	serum lgE	RAST	BEA µg/ml	BUA µg/ml	serum lgE	RAST	BEA µg/ml	BUA µg/ml
1	M	19	++++	1,143	4	10	100	1,110	4	10	10
2	M	52	++++	100	3	100	-	129	3	1,000	1,000
3	F	40	++++	19	0	1,000	1,000	22	0	1,000	1,000
4	M	43	++	597	3	1,000	1,000	730	3	1,000	1,000
5	M	54	++++	107	2	*	*	102	3	1,000	1,000
6	F	43	++++	61	2	*	*	54	2	100	100
7	F	38	++++	34	2	10	-	34	2	1,000	-
8	M	38	-	69	0	-	1,000	90	0	0.1	0.1

BEA and BUA (Table 1). They had also positive cow RAST and skin prick test. The positive results with BEA and BUA before and after indoor feeding season were usually obtained with approximately the same concentrations of allergen dilutions. Before the indoor feeding season three patients got positive response to BUA and after the indoor feeding, in addition to these three patients, there was one patient with negative response before the indoor feeding season who got now positive reaction to BUA with concentration of 1,000 µg/ml. One patient (patient no. 8) without allergy against cow allergens, had non-specific irritation with urinary allergen before indoor feeding season and with both urinary and epithelial antigens after indoor feeding season (Table 1).

DISCUSSION

It is generally accepted that epithelium is the major source of bovine-derived allergens. However, it has been reported in several studies that, in addition to epithelial allergens, also saliva and urinary allergens are important (Newman et al., 1977; Lee et al., 1982; Edwards et al., 1983; Anderson et al., 1985; Lutsky et al., 1985; Walls et al., 1985; Platts-Mills et al., 1987). Especially among laboratory animal workers, urinary antigens seem to be an important cause of occupational allergic diseases (Platts-Mills et al., 1987). Urinary antigens have also been considered to be even more reliable indicators of allergy to laboratory animals than epithelial antigens (Lutsky et al., 1985). Viander et al. (1983) reported of dog antigens that urinary antigen is more specific than dander. Virtanen et al. (1990) measured concentrations of specific dust in swineries and found measurable

levels of swine urinary antigens although clearly lower than the levels of swine epithelial antigens. There are no reports of the levels of cow urinary antigens in cowhouses, but it is obvious that also in cowhouses there are measurable levels of urinary antigens as in swineries.

Recently Virtanen et al. (1988) revealed that dairy farmers have antibodies to cow-urinary antigens. Cow allergic farmers had immunoblotting reactivity against urinary antigens while non-allergic farmers showed negative results. Bovine epithelial and urinary extracts share about 2% of their antigen components in ELISA-inhibition and urine derived antigens seem to be antigenetically more close to serum proteins than epithelial antigens are. Most of cow-allergic farmers react in IgE immunoblotting against epithelial as well as urinary antigens (Ylonen et al., 1990). Four major allergens of cow hair and dander have been demonstrated in cow saliva, urine, whey, beef and amniotic fluid. Most of the allergens in cow urine extract have been showed to cross-react with those of cow hair and dander (Prahl, 1981). Thus it can be expected that urinary antigens may cause symptoms to sensitive cow-allergic patients or at least they can increase the amount of allergen exposure. Also in nasal provocation testing cow-allergic farmers might have positive response to urinary antigens. The allergens of cow saliva could also have significance to allergic symptoms in cow-allergic patients because of their relative high content of four major allergens (Prahl, 1981). In the present study seven patients had positive reactions in nasal challenge with bovine epithelial and six of them had also positive reactions to bovine urinary allergen. This result is very uniform with findings from Ylonen et al. (1990).

IgE reactivity against epithelium and urine appears to be directed toward the same allergenic determinants. IgG antibodies against environmental agents have been considered to be indicators of the amount of exposure (Lutsky et al., 1985). In laboratory animal allergy the quantity of IgG antibodies to rat urinary allergens was correlated with the exposure to animals so that the correlations were for handling hours per day, not for the length of exposure in years (Platts-Mills et al., 1987). Also Virtanen et al. (1988) suggested that in the process of sensitization the duration of exposure is of minor importance. A pollen season causes the rise in specific IgE levels with peak values being reached 4-6 weeks later (Yunginger et al., 1973).

According to the previous knowledge it could be supposed that reactivity in nasal challenge with BEA and BUA might increase during the indoor feeding season when the allergen exposure is maximal. In the cowsheds the delivery of allergens may be quite constant and seasonal fluctuation of their concentration is probably moderate. In summertime the cattle is usually kept and handled outdoors and the exposure of dairy farmers to bovine allergens is decreased. However, the results of the present study did not show any detectable increase in the sensitivity in nasal challenge with BEA and BUA after increased exposure.

In the present study also the RAST values did not change significantly. However, the classification of RAST values into classes from 0 to 4 is too inaccurate to show any difference before and after indoor feeding season.

The mechanism of sensitization process is poorly known and so is the role of the amount of exposure. In addition to the duration and intensity of exposure to the allergens, there must be several other factors influencing the sensitization process.

As a conclusion, in the present study positive nasal challenge reactions to BUA parallel to reactions to BEA is presented for the first time. This finding has importance because the BUA is hardly avoidable. Nowadays it has become usual to get the cattle indoors for feeding and milking and also to keep them indoors during summertime. Thus, the exposure to allergens may be remarkable and also continuous the year around. It may be one reason, why we could not show any difference in the reactivity before and after the indoor feeding season.

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