

Threshold levels of purified natural Bos d 2 for inducing bronchial airway response in asthmatic patients

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Summary

Background Provocation tests are invaluable in establishing threshold levels and a causal relationship between atopic asthma and a certain allergen source, especially in relation to work-associated exposure. Purified major allergens open possibilities for a more accurate assessment of sensitization. **Objective** To determine the threshold dose of purified major bovine dander allergen Bos d 2 in bronchial provocation in comparison with the standard allergen and a set of other parameters of allergy.

Method Nine consecutive patients referred to hospital for confirming the bovine origin of their occupational asthma were subjected to bronchial provocation tests with purified natural Bos d 2 and a standard bovine dander allergen. Additional tests included bronchial histamine challenge, measurements of total IgE, specific IgE antibody determinations and skin prick tests (SPT) with both allergens.

Results In the provocation tests with Bos d 2, a 15% decrease in the forced expiratory volume in 1 s (FEV₁) and/or peak expiratory flow (PEF) values in eight out of nine patients confirmed the predominant role of Bos d 2 in the sensitization. The threshold dose of Bos d 2 varied from 0.1 µg to > 100 µg (median ± median absolute deviation = 4.5 ± 3.9 µg). A positive SPT was induced by a median dose of 13.9 ± 9.8 µg of Bos d 2. Bronchial response to histamine and IgE antibodies against Bos d 2 showed the highest correlations to the provocations results.

Conclusions The efficacy of Bos d 2 in bronchial provocation in patients with occupational cattle-associated asthma was confirmed and the range of the threshold level was determined. There were individual variations, but the response in provocation remains the reference method for identification of the cause of occupational atopic asthma. SPT and the measurement of specific IgE antibodies, preferably with purified or recombinant major allergens, increase the accuracy of the diagnosis.

Keywords allergen, asthma, Bos d 2, bovine, provocation test, threshold

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Introduction

A positive bronchial provocation test can be considered conclusive evidence of the association between bronchial asthma and the work-related exposure to a certain allergen [1]. Quantitative provocation tests are also helpful in estimating environmental concentrations of allergens critical for producing symptoms in exposed sensitized persons. Many of the earlier provocation studies were done using standard allergen extracts containing, in addition to allergens, numerous irrelevant proteins. More recently, the advances in the laboratory technology have provided tools to prepare and use in the tests individual allergens, either highly purified from natural sources or produced *in vitro* as recombinant proteins [2].

The possibility of side-effects and the complexity of the provocation test have led to efforts to evaluate alternative tests for this purpose. *In vitro* measurement of IgE antibodies and skin prick tests (SPT) have been the most common approaches to verify the causal relationship between clinical symptoms and an allergen. Although allergen-specific IgE is critical for triggering immediate allergic reactions [3], clinical studies have shown variable results concerning the relationship between the level of specific IgE or SPT and the intensity of the bronchial response to the allergen [2, 4–10]. Under ideal conditions, SPT and IgE antibody tests provide highly concordant results [11] but discrepancies have also been reported [12, 13].

Occupational exposure to animal dust is associated with a high risk for the development of respiratory allergy, especially in laboratory animal facilities [14, 15]. In Finland, bovine epithelial allergens are responsible for most cases of animal-induced occupational asthma [16]. The predominant allergen in cow dander is Bos d 2, a protein of the lipocalin family [17]. This family comprises most of the aeroallergens of mammalian origin [18], and therefore studies on the parameters of immune

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response associated with the sensitization to Bos d 2 might provide clues to the immunopathogenesis of lipocalin allergy [19].

Extensive testing, including bronchial provocation, is used in Finland for a case of asthma to be recognized as an occupational disease justifying compensation from a national insurance plan. This offered us a possibility to measure the potency of the highly purified natural cow dander allergen Bos d 2 and to compare the results of the provocation tests with other indicators of allergy.

Methods

Patients and testing schedule

The patient group comprised nine dairy farmers (six men, three women) referred consecutively to the Pulmonary Clinic of the Kuopio University Hospital for confirmation of the bovine origin of their occupational asthma. The diagnosis of asthma had been established earlier using criteria of the American Thoracic Society, and the association between symptoms and working in cowshed was confirmed with peak expiratory flow (PEF) monitoring (Wright peak flow meter, Airmed Ltd, Harlow, UK). The mean age of the patients was 40 years (range 22–57 years). Four of them were ex-smokers. Pack-year was used as an index of smoking calculated as the number of packs of cigarettes consumed per day times the number of years of smoking. Anti-inflammatory inhalatory medication (steroids) was discontinued 3 days and contact with cattle 1 week before attending the hospital. All patients undergoing bronchial provocation had a forced expiratory volume in 1 s (FEV₁; Spirometer Model M9449, Medimikro Ltd, Kuopio, Finland) of more than 70% of the predicted value. At the time of the investigation, the patients were symptom-free with less than 10% daily variation in the pre-provocation PEF monitoring.

The patients were admitted for the testing to the inpatient ward of the Pulmonary Clinic. On day 1 of the investigation, the stability of the pulmonary function was verified by repeated PEF measurements. On day 2, the histamine provocation test was performed to determine the degree of bronchial hyper-reactivity [20]. On the following consecutive days, bronchial provocation tests were performed at one test per day in the order: commercial standard bovine dander extract, the corresponding diluent, saline solution (Bos d 2 diluent) and Bos d 2 preparation. All SPTs were performed on day 4. All examinations were performed by the same investigator.

The study was approved by the Ethics Committee of the University Hospital of Kuopio, and a written informed consent was given by all patients.

Reagents

The standard commercial cow allergen preparation (Cow epithelia; ALK A/S, Høstholm, Denmark) was used for the bronchial provocation test. The freeze-dried material was reconstituted with the ALK Diluent (ALK A/S) and used as instructed by the manufacturer. Protein concentration of fresh stock solution (1 : 100 w/v), as determined by the method of Bradford [21] using the Bio-Rad Protein Assay (Bio-Rad Laboratories, Hercules, CA, USA) and bovine serum albumin standard was 0.54 mg/mL. Bos d 2 concentration of the

preparate was 5.7 µg/mL [22]. Serial dilutions of the stock solution were made in the same diluent which was also used as a negative control. Histamine hydrochloride (stock solutions of 4 or 16 mg/mL in phosphate buffer, pH 7, Pharmacy of the University of Helsinki, Helsinki, Finland) was used for the examination of bronchial hyper-reactivity. For SPT, the commercial cow allergen extract in glycerol (Soluprick Bos taurus; ALK, A/S, stock solution of 1 : 100 w/v; protein concentration of the batch used 0.32 mg/mL and Bos d 2 concentration 47 µg/mL) and the ALK Diluent were used. Histamine hydrochloride (10 mg/mL) served as a positive control.

The major cow dander allergen Bos d 2 was purified from commercial raw material (Allergon AB, Ängelholm, Sweden) by affinity chromatography and gel filtration, as described previously [23] and stored at –20 °C in Eppendorf tubes. The allergen was diluted in physiologic saline solution immediately before use both for provocation and SPTs.

Bronchial challenge

Bronchial provocation tests were performed as described [20, 24] using an automatic, inhalation-synchronized dosimeter (Spira Elektro 2, Spira, Hämeenlinna, Finland). The allergen aerosol was generated from a 1-mL sample reservoir with the nebulizing pressure adjusted to 2 bar (flow rate 7.5 L/min) and the nebulizing time to 0.4 s causing a mean volume uptake of 6.5 ± 0.3 µL (SD) of the solution per breath. The maximal count of 60 breaths during a period of approximately 5 min was followed by a rest period of 5 min and subsequent spirometric measurements resulting in an interval of 10–15 min between increasing doses. One challenge cycle allowed the uptake of 390 µL of the nebulized solution per antigen dilution. As calculated from ^{99m}Tc-traced radioaerosol uptake studies, about 60% of the cumulative antigen dose were delivered onto the mucosa of the lower airways [24]. A series of 10-fold dilutions of the allergens was prepared immediately before testing and inhalation was started with the highest dilution. The protein concentration of the standard allergen ranged from 0.54 µg/mL up to 540 µg/mL and that of the purified Bos d 2 antigen from 0.01 µg/mL up to 100 µg/mL. FEV₁ and PEF values were determined 15 min after the starting time for each antigen dilution or immediately when symptoms occurred. The cumulative dose of antigen that caused a 15% fall in the patient's FEV₁ (or PEF; patient 8) was considered the threshold dose for a positive bronchial allergen response.

Measurement of specific IgE/IgG and total IgE

Commercial tests were used for the determination of total serum IgE (CAP-IgE RIA, Pharmacia-Upjohn, Uppsala, Sweden) and IgE specific for the standard cow allergen (CAP-RAST, Pharmacia). The levels of Bos d 2-specific IgE and IgG antibodies were measured with enzyme-linked immunosorbent assay (ELISA) similar to that described previously in detail [25]. For measuring of IgE antibodies, microwell plates (MaxiSorp, Nunc, Roskilde, Denmark) were coated with Bos d 2 at a concentration of 5 µg/mL in the coating buffer. Serum samples were tested at a dilution of 1 : 3 in duplicate. For the detection of Bos d 2-bound IgE, rabbit anti-human IgE (Dako A/S, Glostrup, Denmark; A0094) was used. After adding biotinylated goat anti-rabbit Ig (Dako A/S; E0432) and Vectastain ABC Elite Kit (Vector Laboratories, Burlingame, CA, USA; PK-6100)

reagents (avidin DH and biotinylated horseradish peroxidase H), colour reaction was developed and measured as reported previously. The results were presented as optical density values (OD) minus the reagent blank value. For measuring specific IgG antibodies the plates were coated with Bos d 2 at a concentration of 1 µg/mL and incubated with sera diluted at 1:100. The secondary antibody was peroxidase-conjugated rabbit anti-human IgG at a dilution of 1:1000 (Dako A/S; P214). Colour reaction was then developed and measured as above.

Skin prick tests

Skin prick tests were performed according to Nordic recommendations [26] on the back of the patients in duplicate. The standard allergen (the same batch was used throughout the study) was tested at the dilution of 1:100 (w/v), the purified Bos d 2 as six 10-fold dilutions with the strongest concentration of 100 µg/mL. Histamine hydrochloride (10 mg/mL) was used as a positive control. Negative controls were ALK Diluent and physiologic NaCl (for Bos d 2). After 15 min, the weals were marked and documented with a strip of tape. The longest and the midpoint orthogonal diameters were measured and the mean diameter was calculated. The precision of SPT was assessed by comparing the diameters of the duplicates. A Pearson correlation coefficient $r = 0.82$ ($n = 26$) and coefficient of variation (CV) = 25% were obtained. Mean values of the duplicates were calculated and values of ≥ 3 mm were considered positive. For Bos d 2, the concentration of the allergen inducing a positive reaction was calculated for each patient.

Statistical analysis

Statistical analyses were performed as indicated in the Results with an Apple MacIntosh version of the SPSS 6.1.1 statistical software package (SPSS Inc, Chicago, IL, USA) and with the StatView program (Abacus Concepts, Berkeley, CA, USA). Values in Results are shown as means $\pm$ SD or as medians $\pm$ median absolute deviation (MAD). Non-parametric tests (Spearman correlation coefficient, r_s) were used to assess the significance of the difference between the log values of two parameters. Bronchial reactivity to Bos d 2 or to the standard bovine allergen were used as dependent variables in multiple linear regression analyses to assess the predictive strength of the other tests.

Results

Patients' characteristics

The anamnestic data of the patients are summarized in Table 1. All patients of this study had been exposed to cow allergens for a relatively long time, with a mean of 23.4 ± 12.4 years before developing symptoms, which occurred at a relatively high age, with a mean of 38 ± 11 years. The mean age of the patients at the time of the investigation was 40 ± 10 years.

Six out of nine patients (67%) reported classical symptoms of atopic allergy during their childhood or physician-confirmed cases of asthma in their nuclear family as an indication of a genetic allergic disposition.

In three patients, SPTs revealed reactivity only against cow epithelium-derived allergens. One patient exhibited reactivity against one additional animal species (horse) and five patients against a number of other allergens. In patients with multiple allergies, cow reactivity was accompanied by reactivity to dog, horse or cat.

Bronchial challenge

The results of the bronchial airway challenges are shown in Table 2. All patients tolerated the challenge procedures without complications. A late reaction presenting with symptoms of asthma was observed in one patient (no. 5) 8 h after the termination of the bronchial exposure with the purified Bos d 2. A 20% decrease in PEF was recorded, followed by a further decrease to a 44% fall at 12 h.

Eight of the nine patients with verified cow asthma (89%) exhibited bronchial reactivity also against the purified major allergen Bos d 2, and a nasal response was observed in three patients. The median $\pm$ MAD threshold dose of Bos d 2 provoking a 15% fall of the FEV₁ or PEF (PD₁₅) was 4.5 ± 3.9 µg. There was considerable variation between individual patients – in the eight patients reacting to Bos d 2 the range was 0.1–27.5 µg, a 275-fold difference between the lowest and highest dose. The median threshold dose of the standard allergen was 8.1 ± 7.8 µg and the range 0.3–54.5 µg (180-fold difference). One patient (no. 9) showed neither *in vivo* nor *in vitro* reactivity against the purified Bos d 2. To the standard allergen this patient responded at the median threshold dose of 8.1 µg. The ratio between threshold doses of the standard allergen and

Table 1. Patients' characteristics

Patient (no.)	Age (years)	Sex	Atopy*	Exposure time (years)	Age at the time of asthma diagnosis (years)	Smoking history	Smoking (pack-years)†	Sensitization‡
1	37	F	–	11	36	ExS	2	A, C, D, Dn, G
2	43	F	+	40	28	ExS	1	C, D, G
3	42	F	+	25	40	NS		Dn, M
4	29	M	–	10	28	NS		H
5	57	M	+	40	56	NS		–
6	44	M	+	19	43	ExS	7	A, B, H
7	44	M	+	30	43	NS		–
8	45	M	–	NA	44	NS		–
9	22	M	+	12	20	ExS	5	B, C, Dn, H, G
Mean $\pm$ SD	40 $\pm$ 10			23.4 $\pm$ 12.4	38 $\pm$ 11			

*According to anamnestic data. †Packs of cigarettes per day times the number of years of smoking. ‡Tested with SPT, all were positive for the standard cow dander preparation (ALK). A, alder (*Alnus*); B, birch pollen; C, cat D, dog; Dn, dandelion (*Taraxacum*); H, horse; G, grass pollen; M, mugwort (*Artemisia vulgaris*); NS, non smoker; ExS, ex smoker; NA, not available; SD, standard deviation.

Bos d 2 (Table 2) is an indirect indicator of the role of Bos d 2 in the induction of symptoms. The ratio was clearly exceptionally high in patient nos 3 and 5, suggesting a major role for Bos d 2. On the other hand, in patient no. 9, Bos d 2 apparently had very little to do with the symptoms.

The correlation between the threshold doses of the standard allergen and Bos d 2 is depicted graphically in Fig. 1(a). Statistically the correlation between the two preparations remained non-significant ($r_s = 0.60$; $0.10 > P > 0.05$).

In six patients (67%), a 15% fall of the FEV₁ value was observed after exposure to histamine hydrochloride at a dose of less than 1.6 mg, which is considered to represent a threshold dose for non-specific airway hyper-reactivity.

Skin tests and antibodies

Table 2 also reveals the results of skin tests and antibody measurements. Reactivity in the bronchial provocation tests against the standard allergen ($n = 9$) or against the purified Bos d 2 ($n = 8$) was accompanied by a positive SPT (weal size > 3 mm) against the corresponding allergen. The median $\pm$ MAD value of Bos d 2 concentration corresponding to a positive reaction was $13.9 \pm 9.8 \mu\text{g}$ ($n = 9$). The medians of the weal size in the skin tests were 5.5 ± 1.0 mm ($n = 9$; range 2.0–7.5 mm) with the purified Bos d 2 allergen (100 $\mu\text{g}/\text{mL}$) and 5.0 ± 1.0 mm (range 3.5–8.25) with the standard allergen.

Total IgE level was elevated in all patients with a median of 204 ± 80 IU/mL. The levels of specific IgE antibodies against Bos d 2, measured with ELISA and against the standard allergen measured with radioallergosorbent test (RAST) are also shown in Table 2. Shown in Fig. 1(b) is the correlation between the two parameters. The correlation was significant ($r_s = 0.78$; $0.05 > P > 0.01$).

A significant correlation was also found between anti-Bos d 2 IgE and SPT with Bos d 2 ($r_s = 0.72$; $0.05 > P > 0.01$) and between anti-Bos d 2 IgE and anti-Bos d 2 IgG values 2 ($r_s = 0.72$; $0.05 > P > 0.01$).

Diagnostic value of SPT and in vitro tests

A Spearman rank correlation analysis was also used to compare the relationship between the bronchial threshold values and various other parameters of allergy. The results are shown in Table 3. A good correlation was observed between IgE

antibodies against Bos d 2 and the threshold values of the bronchial provocation tests: for Bos d 2, $r_s = -0.73$ ($0.05 > P > 0.01$); for the standard allergen, $r_s = -0.67$

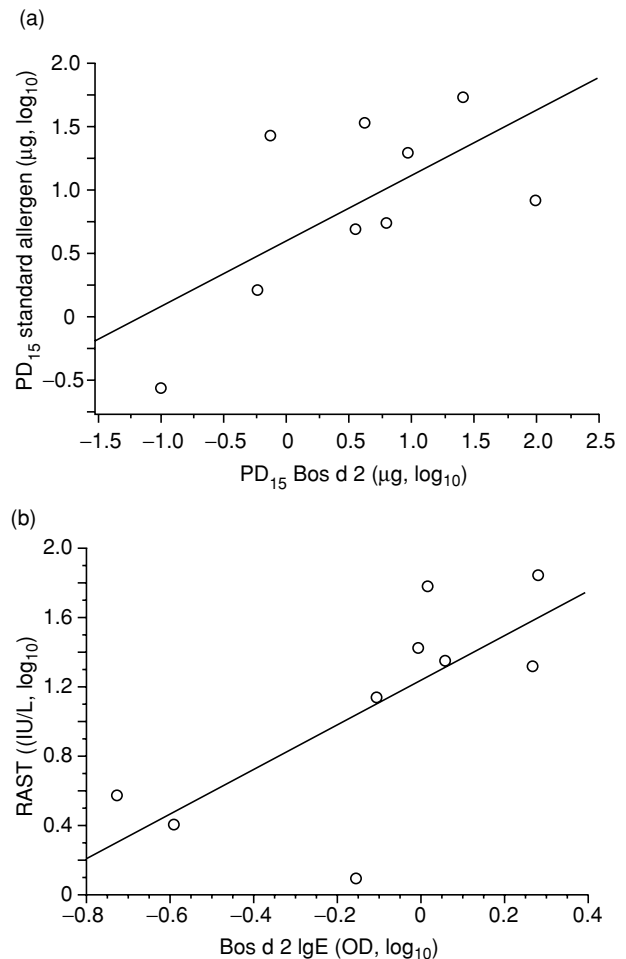


Fig. 1. Comparison of the reactivity of cattle asthmatic patients against standard bovine dander allergen (ALK) and purified natural Bos d 2. (a) Response in bronchial provocation. $r_s = 0.60$, $0.10 > P > 0.05$; (b) Specific IgE antibodies against the standard allergen measured with CAP-RAST and against Bos d 2 measured with an enzyme immunoassay, $r_s = 0.78$, $0.05 > P > 0.01$.

Table 2. Thresholds of bronchial airway challenge and other parameters of allergy in individual patients

Patient (no.)	PD ₁₅ Bos d 2 (μg)	PD ₁₅ ALK (μg)	PD ₁₅ -histamine (mg)	ALK/Bos d 2 threshold ratio	Bos d 2-specific IgE (OD)	Bos d 2-specific IgG (OD)	RAST (IU/L)	Total IgE (IU/L)	SPT Bos d 2 concentration (μg)*	SPT ALK wheal size (mm)†
1	0.1	0.3	0.12	2.7	1.96	1.67	69.7	333	4	8.25
2	0.6	1.6	0.40	2.7	1.16	1.17	22.7	210	14	5
3	0.8	27.0	0.41	33.8	0.80	1.34	14.0	204	10	3.5
4	3.7	4.9	3.15	1.3	1.89	1.69	21.2	76	0.2	7.5
5	4.5	34.0	1.44	7.6	0.71	1.31	1.2	124	23	4
6	6.5	5.4	0.60	0.8	1.07	1.30	60.5	321	37	4
7	9.8	20.0	0.45	2.0	0.26	0.95	2.6	185	24	3.5
8	27.5	54.5	> 3.2	2.0	1.01	1.59	27.0	128	14	5.25
9	> 100	8.1	2.59	< 0.1	0.19	0.20	3.8	993	316	5.75

*Concentration calculated to induce a positive reaction of 3 mm weal diameter. †Mean diameter of the duplicate weals at a concentration of 1 : 100 w/v dilution PD₁₅, allergen/histamine dose provoking a 15% decrease in FEV₁ and/or PEF; ALK, standard commercial bovine dander allergen extract; OD, optical density; SPT, skin prick test.

Table 3. Spearman rank correlations between the bronchial threshold doses and other parameters of allergy

	PD ₁₅ -histamine		Bos d 2 IgE		RAST		Total IgE		SPT Bos d 2		SPT ALK	
	<i>r_s</i>	<i>P</i>	<i>r_s</i>	<i>P</i>	<i>r_s</i>	<i>P</i>	<i>r_s</i>	<i>P</i>	<i>r_s</i>	<i>P</i>	<i>r_s</i>	<i>P</i>
PD ₁₅ Bos d 2	0.73	0.03	−0.73	0.03	−0.37	0.33	−0.02	0.97	0.67	0.05	−0.16	0.68
PD ₁₅ ALK	0.55	0.13	−0.67	0.05	−0.52	0.15	−0.45	0.22	0.23	0.55	−0.53	0.14

ALK, standard commercial bovine dander allergen; *r_s*, Spearman correlation coefficient of determination.

Table 4. Multiple regression analysis on the relationship between the bronchial provocation results and other study parameters

Predictors	Variance explained (%)	Regression coefficients	Significance of the predictors (<i>p</i>)	Significance of the model (<i>p</i>)
Dependent variable: log PD ₁₅ Bos d 2	89.5			0.001
Log PD ₁₅ -histamine		1.28	0.0025	
Log Anti-Bos d 2 IgE		−1.47	0.006	
Constant		0.58	0.003	
+ Log SPT Bos d 2*	93.3			0.002
+ Log RAST	92.4			0.003
+ Log Total IgE	91.2			0.0045
Dependent variable: log PD ₁₅ ALK	86.9			0.002
Log PD ₁₅ -histamine		1.01	0.004	
Log SPT ALK		−3.82	0.003	
Constant		3.67	0.0006	
+ Log RAST*	87.8			0.010
+ Log Total IgE	87.7			0.010
+ Log PD ₁₅ Bos d 2	87.1			0.012
+ Log SPT Bos d 2	87.0			0.012

*Each of the variables marked with '+' was added individually as a third predictor in the analysis.

(*P* = 0.05). The correlation was similar to that observed between the allergen provocation results and PD₁₅-histamine: for Bos d 2, *r_s* = 0.73 (0.05 > *P* > 0.01); for the standard allergen, *r_s* = 0.55 (*P* = 0.13).

Multiple linear regression analysis was applied to assess the strength of the study parameters in predicting the results of the provocation tests. As shown in Table 4, the best combination of two predictors for the PD₁₅ of Bos d 2 was PD₁₅-histamine + Bos d 2 IgE antibodies. PD₁₅-histamine + SPT ALK was the best two-predictor combination for the standard allergen provocation. A prediction level of over 90% in the case of Bos d 2 and over 87% in the case of the standard allergen was achieved when a third variable, as shown in Table 4, was added to the two-predictor pairs.

Discussion

Quantitative provocation tests can be considered the gold standard for demonstrating a causal relationship between the exposure to an allergen and the clinical symptoms. Furthermore, they provide information that can be used to estimate the risks of working under exposure to aeroallergens. In our study, highly purified Bos d 2, the major allergen of cow epithelium, provoked a bronchial response in eight out of nine patients with clinically confirmed cattle asthma. This is in accordance with our previous results demonstrating that in

IgE immunoblotting 90% of all cow dander-reactive patients had serum reactivity against the 20-kDa major allergen Bos d 2 [25, 27]. A comparison of the threshold doses of Bos d 2 and the standard cow allergen in the provocation test revealed, however, that the proportional role of Bos d 2 can vary between very low or missing (patient 9, Table 2) to very high (patients 3 and 5).

The provocation dose of Bos d 2 was at the microgram level in highly sensitized persons but the amount of bovine allergens required to induce symptoms varied several hundredfold among patients. The amounts of allergen inhaled from a nebulizer are not directly convertible to airborne concentrations in the workplace [28], and the symptoms associated with the acute high-dose exposure do not reveal the effects of a prolonged low-dose exposure. An approximation can be made, however, of the practical significance of the challenge threshold. Louhelainen et al. determined airborne concentrations of cow dander and Bos d 2 in Finnish cowsheds [29]. The concentrations were measured by immunochemical quantification techniques [22, 30]. In the breathing zone of persons working in cubicle-type cowsheds (*n* = 21), the mean concentration of Bos d 2 (BDA₂₀) was 320 ng/m³ (range 60–700 ng/m³). Working in a cowshed at a power of 66 W corresponds to ventilation of 33 L/min [31]. Estimates can then be calculated of the length of time for working in a cowshed before the threshold amount of allergen is inhaled (Table 5). The values show that in a 'dirty' cowshed, a sensitive person inhales the

Table 5. Estimation of the working time in the cowshed before the threshold amount of allergen is inhaled

Patient	Bos d 2 concentration		
	Mean	Lowest	Highest
Most sensitive (min)	9	51	4
Least sensitive (days)	1.8	9.6	0.8
Mean (h)	11	56	5

Calculated from threshold values in Table 2. Bos d 2 concentrations in the breathing zone during feeding according to Louhelainen et al. [29]. Time for reaching the threshold amount of the allergen was calculated assuming working at a power of 66 W corresponding to ventilation of 33 L/min [31].

threshold amount in 4 min. At the other extreme, a minimally responsive patient (still having verified cattle asthma) could work in a 'clean' cowshed for days before the threshold dose would be reached. These estimations refer only to the amounts of allergen provoking symptoms in a sensitized person. A continued exposure to lower levels of airborne allergens is likely to be important in the pathogenesis of atopic asthma [32].

The data of our study show that the manifestation of clinically evident cow asthma occurs after a prolonged exposure (23.4 ± 12.4 years) at a relatively high age (38 ± 11 years). This is in striking contrast to the exposure time of 1–3 years during which the majority of the cases of respiratory allergy appear in laboratory animal workers [33–35]. Both groups are exposed to similar concentrations of lipocalin allergens [14, 22, 29]. One difference between the two groups is the age at the beginning of exposure. Exposure to cow dander from an early age associated with living in a dairy farm [36] might cause a stronger selective 'survivor' effect. On the other hand, indications of atopy in six out of nine patients argues against an earlier out-selection of persons becoming sensitized.

The absence of allergen does not explain the delay in the development of cow asthma in people living on dairy farms because cow dander concentrations in their whole living environment are fairly high [36]. On the contrary, it is possible that an early high-level exposure to dander allergens has a suppressive effect on the development of animal allergy [37–39]. Related to that is the discussion of the role of farming environment in the development of allergy in general. For reasons currently at the level of speculation, living on farms seems to protect children against the development of allergy [40–42]. In spite of this, farming was associated with the highest occupational risk of asthma in a multinational survey [43].

Allergic symptoms in immediate hypersensitivity of type I are provoked predominantly by allergen-induced cross-linking of specific IgE antibodies on the surface of mast cells and basophils in tissues and in the peripheral blood, respectively. This makes it reasonable to expect that the magnitude of a clinical allergic response was related to the concentration of specific IgE in the patients' serum. Our results (Table 3) and those of several earlier studies on other allergens and on different groups of patients show, however, that the usefulness of antibody measurements and SPT for predicting the outcome of a respiratory exposure is limited [2, 6–9, 44]. However, highly significant correlations between the threshold allergen dose provoking asthmatic symptoms and the amount of specific IgE or specific skin

reactivity have also been found [10]. Purified major allergens with known potency may reflect more the characteristics of patients and specific individual sensitization, whereas results with crude allergen extracts are determined predominantly by the characteristics of the preparation [2]. In accordance with this, we found that IgE antibody titre against Bos d 2 was better in predicting the results of the provocation tests than RAST or SPT with a standard cow dander preparation.

The patients in the present study were a selected small group, and this has to be taken into account when assessing the results. However, our results support the use of purified major allergens for associating work-related asthma with the exposure to a specific allergen source. Major allergens can be used both in the provocation tests and for measuring specific IgE antibodies. Provocation with pure major allergens also provides threshold values which can be related to ambient concentrations of the same allergens.

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