

Diversity of allergens causing occupational asthma among cereal workers as demonstrated by exposure procedures

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Summary

Background Occupational asthma among cereal workers is frequently due to cereals but other allergens can also be responsible.

Objective We evaluated the allergens causing occupational asthma in the patients who had been diagnosed in our Department during the last 5 years. Specific bronchial reactivity to the implicated allergens was assessed and compared by standardized bronchial challenge.

Methods Twenty-one patients (12 bakers, three millers and six farmers) were studied. We carried out *in vivo* tests (skin and challenge tests) and *in vitro* tests (specific IgE measurement) with cereals, enzymes, soyabean, storage mites and egg. A definitive diagnosis was established by means of specific bronchial provocation tests (BPT), except in three patients in whom it could not be carried out due to the severity of their asthma. In these cases the causative agent had to be determined by means of conjunctival challenge.

Results Cereals were the main sensitizers among bakers (75%) and farmers (66%). Bakers were also sensitive to α -amylase (41%) and soyabean (25%), and farmers, to soyabean (33%) and storage mites (33%). Occupational asthma was due to cereals, soyabean and storage mites among millers.

Conclusions Besides cereals, other allergens such as enzymes, leguminous, egg and storage mites can be the causative agents of occupational asthma among cereal workers. Sensitization to different allergens in the different jobs is very likely due to differences in exposure.

Keywords: occupational asthma, α -amylase, soyabean, storage mites, bronchial provocation test

Clinical and Experimental Allergy, Vol. 26, pp. 147–153. Submitted 19 December 1994; revised 24 July 1995; accepted 25 August 1995.

Introduction

In 1713, Ramazzini first described that cereal dust exposure is harmful to the lungs. Since then, several clinical syndromes have been described related to this exposure: allergic asthma, non-allergic bronchial hyperreactivity syndrome, hypersensitivity pneumonitis and grain fever [1]. Bakery, milling and farming are the main jobs in which cereals are frequently manipulated. It has been estimated that the frequency of occupational asthma

among cereal workers ranges between 2 and 40% [1,2]. There may be different allergens implicated, as well as differences in the type of exposure and other environmental factors (humidity, temperature, allergen load, etc.).

Cereals are the main allergens which have been incriminated in the development of occupational asthma among these workers. There are other substances, however, which may be found in the workplace contaminating cereal dust (storage mites, moulds or pollens) or used as flour additives (enzymes, soyabean flour and egg), all of which have allergenic capacity and might be causative agents of occupational asthma [1].

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The aim of our work was to determine which were the allergens causing occupational asthma among the cereal workers who had been diagnosed in our Department during the last 5 years. Specific bronchial reactivity to the implicated allergens was assessed and compared by standardized bronchial challenge.

Subjects and methods

Subjects

We selected all the patients (12 bakers, three millers and six farmers) who had been diagnosed as suffering from occupational asthma in our Department during the last 5 years. The diagnosis of bronchial asthma was made following the recommendations of the American Thoracic Society [3]. Occupational asthma diagnosis was confirmed according to the algorithm proposed by Bernstein [4]. Several techniques (clinical data, skin-prick test [SPT], CAP, methacholine bronchial provocation test [BPT], specific BPT and PEF measurements) were performed on the subjects diagnosed before 1993. When these patients were studied again (1994), the algorithm of Bernstein [4] was followed. Three of the subjects had left their workplace shortly after they had been diagnosed. Patients' clinical data are shown in Table 1. All of them were diagnosed as suffering rhinitis and occupational asthma. The period between the onset of exposure and development of asthmatic symptoms (latency period) was very variable. None of the patients had any problem eating bakery products or egg. Atopy was defined by detection of specific IgE antibodies to one or more common inhalant allergens.

All the subjects underwent *in vivo* and *in vitro* tests as described.

Allergen extracts

We created extracts of cereals, egg and leguminous in our laboratory. Each raw material was extracted in 10% w/v

of phosphated buffered saline (PBS) (pH 7.3) in powder form. After stirring for 30 min, solutions were passed through a filter paper and dialysed overnight in PBS using Spectrapor dialysis membrane (molecular weight cut-off 10 kDa) (Spectrum Medical Industries INC, USA). After centrifugation at 3000 g for 15 min, supernatants were passed through a 0.22 µm Millipore filter (Millipore Group, Bedford, USA) for sterilization.

α-amylase, cellulase and papain were obtained from Sigma (St Louis, MO, USA) and solutions of each enzyme in PBS were made at 10 mg/mL.

All the other allergen extracts used were biologically standardized extracts which were provided by a pharmaceutical laboratory (Abello SA, Madrid, Spain). Twofold dilutions were made for cutaneous and challenge tests.

Skin tests

Cutaneous tests were performed by means of a skin-prick test (SPT) as described [6]. We tested a standard battery of allergens including *Dermatophagoides pteronyssinus*, pollens (*Phleum pratense* and *Plantago lanceolata*), moulds (*Alternaria tenuis*, *Cladosporium herbarum* and *Aspergillus* spp.) animal dander (cat and dog) and foods (milk, peanut and egg). We also tested a battery of allergens that could be relevant at the workplace, such as cereals (wheat, rye and malt flour, and wheat, rye, barley, oat, rice and corn grain), leguminous (soyabean flour), enzymes (α-amylase, cellulase and papaine) and storage mites (*Lepidoglyphus destructor*, *Tyrophagus putrescentiae* and *Acarus siro*). In farmers, we also tested cow, goat, rabbit, sheep and horse dander extracts.

Histamine phosphate at 10 mg/mL and PBS were used as positive and negative controls, respectively. Lancets (Prick-Lancetter, Dome Hollister-Stier, Milles Laboratories Ltd, UK) were used to puncture the skin. The results of the skin tests were read at 15 min. Weal diameters equal or greater than 3 mm were considered as positive in the absence of a response to PBS.

Table 1. Clinical data of the patients

	Bakers	Millers	Farmers
Number	12	3	6
Sex	100% male	100% male	83% male
Age (years)	39 ± 17	34 ± 12	39 ± 14
Latency period (years)	13.2 ± 15.5	7.4 ± 4.7	12.8 ± 13
Duration of symptoms (years)	3.6 ± 4.17	3.7 ± 4.7	10.2 ± 13
Atopics	30%	33%	71%
Smokers	8%	33%	14%

Specific IgE measurement

Specific IgE to the allergens which had been positive in SPT was measured. When clinical data suggested sensitization to an allergen in spite of negative skin test, specific IgE antibodies were measured as well. IgE antibodies were measured by means of Pharmacia CAP system IgE fluoro-enzyme-immunosorbent assay (Pharmacia Diagnostics, Uppsala, Sweden). Tests results were expressed as CAP scores ranging from class 0 to 6, according to manufacturers instructions. A CAP score equal or higher than class 1 was considered as a positive test.

Bronchial provocation tests (BPT)

Methacholine inhalation test Non-specific bronchial responsiveness was assessed by means of abbreviated methacholine inhalation challenge test as described by Chatham [6]. Forced expiratory volume in one second (FEV₁) higher than 70% predicted normal was required to start (BPT). Tests were made after withholding inhaled short-acting β -adrenergic agonist and ipratropium bromide for 6 h, and long-acting β -adrenergic agonist and theophylline for 24 h [5]. Methacholine (Provocholine, Roche Lab., Nutley, NJ, USA) was administered with a nebulizer, model DeVilbiss 646 (DeVilbiss Co, Somerset, PA, USA) connected to a Rossental French dosimeter (Laboratory for Applied Immunology, Baltimore, MD, USA), powered by an oxygen tank with a pressure of 20 psi and calibrated to deliver 15 μ L per breath. The result of this test was expressed in terms of the provocation dose necessary to decrease FEV₁ by 20% (PD₂₀) given in cumulative breath units (cbu) (1 unit = one inhalation of aerosol from 1 mg/mL solution of methacholine). Test cut-off point was 125 cbu. Thus, according to the reproducibility of the test [6], subjects who showed PD₂₀ values equal or lower than 250 cbu were considered as hyperreactive. Bronchial responsiveness was classified as severe (PD₂₀ < 50 cbu), moderate (PD₂₀ = 50–125 cbu) or mild (PD₂₀ = 125–250 cbu).

Specific bronchial provocation tests BPT were performed with the allergens to which specific IgE antibodies had been detected. Only one allergen challenge per day was done in each subject. Each methacholine or specific BPT was carried out at least 8 days apart. Drugs withholding criteria were the same used in methacholine test. Peak expiratory flow (PEF) and FEV₁ were measured. FEV₁ higher than 75% predicted normal was required to start BPT. The aerosolized particles were generated by a continuous pressurized DeVilbiss 646

nebulizer with a fitted output of 0.28 mL/min, and inhaled by tidal breathing for 2 min. SPT with twofold dilutions of allergen extract was performed to enable selection of a safe initial dose for BPT (concentration of allergen that produces a 3 $\times$ 3 mm weal). The threshold weal diameter of the allergen with skin testing and bronchial responsiveness to methacholine were used to set the starting dose of the BPT [7]. No response to PBS challenge was required to start allergen challenge. Allergen extract was inhaled every 10 min with a two-fold increasing allergen concentration at each step (FEV₁ was measured at 10 min after each inhalation), until the highest dose of the extract was attained or an early asthmatic reaction happened. This was defined as a fall in FEV₁ $\geq$ 20% from the post-diluent value. When the last dose of allergen was administered, FEV₁ measurements were performed at 20, 30, 60, and 90 min. To record any late asthmatic reaction, PEF measurements were done hourly until 10 h after challenge.

A late asthmatic reaction was considered as a fall of 25% or more from the basal value. Finally, a new spirometry was performed 24 h after BPT.

PEF measurements

Those subjects in whom cutaneous and serological tests with occupational allergens were negative but had a suggestive history of occupational asthma were asked to perform serial PEF monitoring. A Mini-Wright peak flow meter (Clement Clark International Ltd, London, UK) was used. PEF measurements were obtained every 2–3 h for 2 weeks at the workplace and for 2 weeks off work, respecting sleep hours. Visual inspection of the records was performed by three independent investigators as was suggested by Perrin *et al.* [8].

Conjunctival challenge tests

Conjunctival challenge tests were carried out as described by Möller [9], with the allergens to which specific IgE had been detected. They were made in those patients in whom FEV₁ values were repeatedly below 75% predicted normal in spite of optimal asthma treatment. Allergen extracts were applied in the lower conjunctival sac at increasing doses. Lecture was noted within 20 min. Criteria for positive test were congestion of the conjunctival mucosa and itching of the eyes.

Results

Skin tests and IgE measurements

Results of SPT and specific IgE measurement to the main

occupational allergens are shown in Table 2. Subject 8 was sensitive both to cereals and egg.

Since cutaneous test results as well as specific IgE values were very similar with the different cereals tested (wheat, barley and rye) in each patient, only data to wheat are shown. We found positive results with any α -amylase but not with cellulase or papaine. *Lepidoglyphus destructor* was the main storage mite found positive in our study.

Positive BPT with cereals was obtained in 77% and 71% of the patients who had positive SPT and specific IgE with these cereals, respectively.

Methacholine inhalation test

Patients' bronchial responsiveness to methacholine was severe in 50% of the patients, moderate in 22%, mild in 11% and 17% did not show bronchial hyperresponsiveness. Individual PD₂₀ values are shown in Table 2.

Specific bronchial challenge tests

Of the 12 bakers, 11 had specific IgE (SPT, CAP or both)

to wheat, seven out of nine who had BPT had a positive bronchial reaction to wheat; five had a positive skin reaction to α -amylase on skin testing, and four of them had positive bronchial reaction. None of the bakers had a positive skin test to storage mites. This is different for the six farmers in whom five out of six had positive IgE to wheat and only two out of four had a positive bronchial reaction to wheat. Four out of six farmers had positive IgE to storage mites, and in one out of two BPT a positive result to *L. destructor* was obtained. Individual results for each job category are shown in detail in Table 2. Detailed data about each specific BPT are shown in Table 3.

Subjects 15, 19 and 20 had been diagnosed of occupational asthma due to *Lepidoglyphus destructor*, soyabean and cereals, respectively, some years ago. They had not been occupationally exposed for 3.5–4.5 years. In these patients, although specific IgE to these allergens was demonstrated by cutaneous and serological tests, both specific BPT and methacholine inhalation test were negative.

Table 2. Individual results

Number	Job	NSBH	Wheat			α -amylase			Soyabean			<i>L. destructor</i>		
			SPT	CAP	BPT	SPT	CAP	BPT	SPT	CAP	BPT	SPT	CAP	BPT
1	B	52	7	4	+	–	0	ND	3	2	+	–	0	ND
2	B	158	–	3	–	6	1	+	–	ND	ND	–	ND	ND
3	B	20	3	3	+	7	1	+	–	ND	ND	–	ND	ND
4	B	20	4	2	+	–	ND	ND	3	0	ND	–	0	ND
5	B	30	–	0	ND	5	0	+	4	2	+	–	ND	ND
6	B	23	7	4	+	–	ND	ND	–	ND	ND	–	ND	ND
7	B	45	7	3	+	–	ND	ND	–	ND	ND	–	ND	ND
8	B	75	5	3	+	–	ND	ND	–	ND	ND	–	ND	ND
9	B	125	4	3	+	–	ND	ND	3	0	ND	–	ND	ND
10	B	60	4	3	–	12	2	+	–	ND	ND	–	ND	ND
11	B	ND	8	2	(+)*	–	ND	ND	–	ND	ND	–	ND	ND
12	B	ND	5	4	(+)*	7	1	(+)*	7	1	(+)*	–	ND	ND
13	M	15	–	0	–	–	ND	ND	–	ND	ND	–	0	ND
14	M	150	5	2	+	–	ND	ND	5	1	+	3	0	ND
15	M	–	3	0	ND	–	ND	ND	–	ND	ND	3	2	–
16	F	15	4	2	–	–	ND	ND	6	0	+	–	ND	ND
17	F	40	4	3	+	–	ND	ND	–	ND	ND	4	4	+
18	F	18	4	4	+	–	ND	ND	4	0	ND	3	1	–
19	F	–	–	ND	ND	–	ND	ND	7	2	–	–	ND	ND
20	F	–	4	3	–	–	ND	ND	–	ND	ND	3	0	ND
21	F	ND	13	3	(+)*	–	ND	ND	–	ND	ND	4	2	(+)*

L. destructor. *Lepidoglyphus destructor*. SPT. Skin-prick test (largest diameter expressed in mm). CAP (expressed in class units). NSBH. Non-specific bronchial hyperresponsiveness (PD₂₀). BPT. Bronchial provocation test. * Conjunctival challenge test. B. Baker. M. Miller. F. Farmer. – Negative test, + Positive test. ND. Not done.

Table 3. Individual results of BPTs

Number	Allergen	PC ₂₀	% FEV ₁ fall	% PEF fall
1	wheat	0.7	32%/immediate	—
	soyabean	5.0	34%/immediate	—
2	α -amylase	0.2	32%/immediate	—
	wheat	—	—	—
3	wheat	0.9	27%/immediate	—
	α -amylase	0.5	39%/immediate	30%/10 h
4	wheat	10.8	36%/immediate	40%/12 h
5	α -amylase	1.6	27%/immediate	—
	soyabean	2.8	24%/immediate	—
6	wheat	9.6	21%/immediate	—
7	wheat	5.4	26%/immediate	25%/10 h
8	wheat	1.0	30%/immediate	—
	egg	0.5	32%/immediate	—
9	wheat	8.1	23%/immediate	—
10	wheat	—	—	—
13	wheat	—	—	—
	<i>D. pteronyssinus</i>	518*	39%/immediate	30%/6 h
14	wheat	10.2	40%/immediate	—
	soyabean	4.5	21%/immediate	—
15	<i>L. destructor</i>	—	—	—
16	wheat	—	—	—
	soyabean	5.4	26%/immediate	—
17	wheat	9.4	24%/immediate	—
	<i>L. destructor</i>	7.6*	26%/immediate	—
18	wheat	0.5	38%/immediate	25%/11 h
	<i>L. destructor</i>	—	—	—
19	soyabean	—	—	—
20	wheat	—	—	—

D. pteronyssinus. *Dermatophagoides pteronyssinus*. *L. destructor*. *Lepidoglyphus destructor*. PC₂₀ expressed in mg/mL. *PC₂₀ expressed in BU/mL. h. hours.
— Negative.

PEF monitoring

All the occupational allergens tested on patient 13 were negative. As he exclusively worked with barley, a specific BPT was done with this cereal, but it was negative. PEF monitoring in this patient was suggestive of occupational asthma.

Conjunctival challenge tests

FEV₁ values $\geq 75\%$ predicted normal, were not achieved in patients 11, 12 and 21 at any time. Conjunctival tests in these patients were positive with the occupational allergens to which specific IgE had been detected. In the case this test was positive, the patient was diagnosed of occupational asthma.

Discussion

Cereals are the allergens classically incriminated in the development of occupational asthma among grain workers [10,11]. Nevertheless, there are other substances with immunogenic capacity at the workplace which contaminate cereal dust or are used as flour additives.

Several enzymes are employed in the bakery industry to improve the quality of bread and to decrease the costs of production. Their immunogenic capacity was first described by Flindt [12]. Baur *et al.* found a rate of 34% of α -amylase sensitization among bakers [2]. Sensitization to hemicellulase has also been proven in bakers, usually associated with α -amylase sensitivity [13]. Quirce *et al.* [14], found a rate of sensitivity to cellulase of 80% among bakers sensitized to α -amylase, but they found no crossreactivity among both enzymes. We believe, this

might explain our results in which, in spite of detecting a high rate of sensitivity to α -amylase, no positive results have been obtained with cellulase.

Sandiford *et al.* [15], found a high rate of sensitization to cereal α - and β -amylases among bakers and millers. They found a correlation between specific IgE values to cereals and to cereal amylases, but there was no cross-reactivity among cereal and fungal amylases [15]. It was not possible to include cereal amylases in our study, and this could explain the absence of sensitization to enzymes among millers.

Sensitization to soyabean flour through inhalation was first reported in a food processing plant worker [16]. Soyabean is mixed in animal feed formulas, and this may be the sensitization source to this legumine among farmers. Soyabean lecithin sensitivity among bakers was firstly described by Wuthrich and Baur [17] and recently by Lavaud [18]. Soyabean lecithin is also used as bread additive with the aim of improving its moisture. In spite of its low immunogenicity due to its lipidic composition, it contains about 2–4% of soyabean impurities which would determinate its allergenicity.

Egg is another substance usually used in bakery and confectionary industries. In spite of digestive allergy being the most frequent way of egg sensitivity, some cases of occupational asthma caused by inhalation of egg proteins have been reported [19]. We have demonstrated that egg protein inhalation caused an asthmatic response in patient 8.

Storage mites which contaminate cereal dust, may be causative allergens of occupational asthma among bakers [20,21]. Storage mites as a cause of occupational asthma among farmers was first proposed by Warren *et al.* [22]. Other studies illustrating storage mites sensitization among grain workers have been reported [23,24]. We have found storage mite sensitivity among millers and farmers, but not in bakers.

Specific BPT was negative in three patients who had been removed from occupational exposure and were symptom free. Several reports point out that the most frequent cause of false negative specific BPT in occupational asthma is a long time without exposure to the causative allergens [25,26]. Another cause of false negative specific BPT could be that patients were sensitized exclusively to cereal water insoluble allergenic fractions (gliadin and glutenin). Although most studies show that specific IgE responses are directed primarily to water soluble allergens, specific IgE responses to water insoluble fractions have also been demonstrated [27,28]. Extracts used in skin tests and specific BPTs were the same in our study. We cannot assert in a definitive way that negative BPTs in these patients were not false negative due to the insolubility of the allergen, but we think it is unlikely

because positive results in the same patients in SPT were obtained. Negative BPTs are more probably due to the removal of the patients from occupational exposure and to the disappearance of NBHR. It has been suggested that in the case of negative laboratory BPT, patients should return to their former workplace, and serial PEF measurements should be performed in order to assess occupational asthma. Nevertheless, it was not possible for our patients to return to their former workplace.

On the other hand, three of the subjects in whom exposure persisted had developed chronic asthma, in spite of the fact that anti-inflammatory medication was given. A parallelism between bronchial and conjunctival challenge tests has been suggested by Dreborg *et al.* [29]. Although we were not able to carry out BPT to these patients because of their basal spirometry values, they were diagnosed with occupational asthma due to the allergens which were positive in the conjunctival challenge.

Occupational asthma can lead to permanent disability, in spite of removal from exposure to the occupational sensitizer is achieved. Several factors such as age, duration of exposure, and duration of exposure after onset of symptoms have been identified as having prognostic significance [30]. Those patients who remain at their workplace, experience a worsening in their asthma symptoms and an increase in non-specific bronchial hyper-reactivity (NSBH) [30]. Early diagnosis and removal from exposure should be stressed in occupational asthma.

We conclude that other substances besides cereals can act as occupational allergens among cereal workers sensitizing exposed individuals through an IgE-mediated mechanism. Farmers were sensitized to storage mites and cereal but not to additives, while bakers were sensitized to cereals and additives but not to storage mites. Millers were more similar to farmers than bakers in the allergens that they were sensitized to. These results are very likely due to the different allergens they handled, types of exposure, and environmental factors (humidity, temperature, allergen load, etc.). We believe that not only cereals, but also several allergens such as enzymes, storage mites, soyabean and egg should always be considered as aetiological agents in the diagnosis of occupational asthma.

Acknowledgements

This study was supported in part by a research grant from the Departamento de Salud, Gobierno de Navarra, Fondo de Ayudas a Proyectos de Investigación.

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