Case Report

Rye Flour Allergens: An Emerging Role in Baker's Asthma

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Background *Exposure to wheat flour is usually considered the most important cause of baker's asthma. However, other flours frequently used in bakeries may play an emerging role as relevant allergens causing occupational asthma.*
Aims of study *We report on two cases of baker's asthma mainly caused by exposure to rye flour. The profile of allergen sensitization to cereal flour was investigated.*
Methods *Two bakery workers suffering from rhinoconjunctivitis and asthma symptoms at work underwent an in vivo study (skin prick tests and bronchial allergen challenge) and in vitro study (total serum IgE, specific serum IgE and immunoblotting).*
Results *Specific inhalation challenge with wheat flour did not elicit an asthmatic reaction, however both patients showed an early asthmatic reaction with the rye flour challenge. Rye flour-immunoblotting showed IgE-binding bands around 12–15 kDa, that correspond to rye flour enzymatic inhibitors which were not present in the wheat flour immunoblot.*
Conclusions *Both bakers had developed occupational asthma to rye flour (confirmed by specific inhalation challenge test). Rye flour allergens (enzymatic inhibitors) are important allergens that should be considered in the diagnosis of baker's asthma.* Am. J. Ind. Med. 51:324–328, 2008. © *2008 Wiley-Liss, Inc*

KEY WORDS: alpha-amylase inhibitor; baker's asthma; occupational asthma; rye flour; specific inhalation challenge

INTRODUCTION

Baker's asthma is still one of the most common forms of occupational asthma. Its pathogenesis involves an IgE-mediated allergic response to a variety of cereal antigens, flour additives and storage mites [Houba et al., 1998; Quirce et al., 2006]. Of these causal agents, wheat flour is considered to be the most relevant allergen source. However, rye flour is being increasingly used by bakers and farmers [Ehrlich and Prescott, 2005].

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It has been reported that the major portion of the salt-soluble protein family (alpha-amylase and trypsin inhibitors) represents the best characterized allergens from cereal flour. These are salt-soluble endosperm proteins with molecular masses ranging from 12 to 16 kDa [Gómez et al., 1990; Fränken et al., 1994]. Most members of this family show IgE binding capacity in vitro and elicit positive responses in skin prick testing [García-Casado et al., 1995]. Little is known, however, about the allergenic role of rye flour enzymatic inhibitors in baker's asthma.

We describe two cases of baker's asthma mainly due to exposure to rye flour. The responsible cereal flour allergens were investigated.

MATERIALS AND METHODS

Patients

Case 1: A 35-year-old man. He had worked in a bakery since he was 12 manipulating cereal flours to make bread.

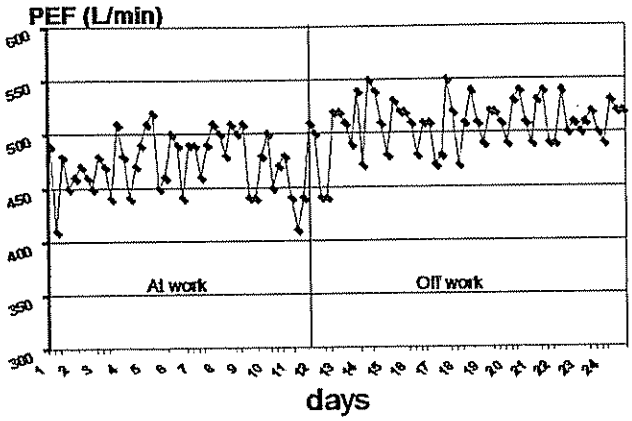

FIGURE 1. Peak expiratory flow (PEF) monitoring in patient 1 during days at work and away from work The results are consistent with occupational asthma (higher average PEF off work)

This patient had suffered from rhinoconjunctivitis and asthma symptoms (he needed bronchodilator drugs every night) at work for 7 years, and his symptoms worsened when he was exposed to different types of flour (more often when he used rye flour to make "special breads") His symptoms subsided on weekends and holidays.

Spirometry results were: FVC 4.77 L (82% predicted), FEV_1 3.63 L (81% predicted), FEV_1/FVC 76.1%. A bronchodilator test was positive (FEV_1 improved +12%). He was asked to perform serial PEF (peak expiratory flow) recordings, four times a day, during 15 days at work and 15 days away from work. A significant improvement in PEF records was observed during days off work (Fig. 1).

Case 2: A 32-year-old man. He was born in Cuba (where he worked in a bakery without any symptoms for 8 years) Since he moved to Spain 3 years ago, he had worked in a bakery making bread He presented with rhinoconjunctivitis and asthma symptoms immediately after exposure to cereal flour for the last 5 months. He used inhaled bronchodilator drugs on daily basis and he had had severe asthma attacks requiring treatment in the emergency room on several occasions. His symptoms were more frequent and severe when he was exposed to rye flour in the bakery. He showed a marked improvement on days off work. His basal spirometry results were: FVC 5.12 L (98% predicted), FEV_1 3.84 L (88% predicted), FEV_1/FVC 75%. The bronchodilator test was negative (FEV_1 +9%). He was not able to record PEF measurements

Skin Prick Tests

Skin prick tests (SPT) were performed with wheat, rye, corn, barley, soy, and oat flour extracts (1% w/v) and fungal alpha-amylase (1 mg/ml) supplied by Bial-Aristegui (Bilbao, Spain) and ALK-Abelló (Madrid, Spain), mites (including storage mites), and *Aspergillus fumigatus* from ALK-Abelló (Spain).

Total Serum IgE and Specific IgE

We measured total serum IgE (Immunolite® DPC 1000, Dipesa, Los Angeles, CA) and specific serum IgE (Immuno-CAP 250®, Phadia, Uppsala, Sweden) to different cereal flours, soy flour, fungal alpha-amylase, mites, and *A. fumigatus*.

Methacholine Inhalation Test

Methacholine inhalation test was done in both patients following the five-breath dosimeter method [Chai et al., 1975], and it was repeated 24 hr after the specific bronchial allergen challenge if this challenge was negative. PC_{20} methacholine was calculated according to American Thoracic Society guidelines [American Thoracic Society, 2000]. Spirometry was performed with a Spira Analyzer ST-75 spirometer (Fukuda Sangyo, Nagareyama, Japan). Predicted normal values of spirometric parameters were based on the reference values from European Respiratory Society guidelines for adult patients [Quanjer et al., 1993].

Specific Inhalation Challenge Test

Specific inhalation challenge (SIC) test was performed using an electronic dosimeter Spire Elektro (Respiratory Care Center, Hameelinna, Finland) with an output of 0.45 µl and a nebulization time of 0.6 s. The patient inhaled the aerosolized allergen (wheat or rye flour) using a five-breath dosimeter protocol in progressive concentrations [Chai et al., 1975]. A control challenge with isotonic saline solution was carried out before antigen provocation. Increasing concentrations of allergen were given by inhalation starting with a concentration that induced a 2-mm wheal on skin prick test [Quirce et al., 2006] The dose was increased in fivefold increments at intervals of 10 min and FEV_1 was measured at 5 and 10 min after inhalation of each concentration. Inhalation challenge test was discontinued when there was a fall in FEV_1 of $\geq$20% from the lowest post-saline value, or when the highest concentration (1% w/v) had been given. At the end of the inhalation test, spirometry was performed at 20, 30, 40, 60, and 120 min after challenge. From that moment, PEF measurements were performed hourly with a portable peak flowmeter (for 24 hr after challenge, respecting sleeping time). A fall in FEV_1 of $\geq$20% from the lowest post-saline within 60 min of challenge was considered a positive early asthmatic reaction, and a similar fall in PEF between 2 and 24 hr after challenge was considered a late asthmatic reaction if no change was observed during the control day. Patients were monitored in the laboratory for at least 6 hr after the challenge. Written instructions were given to the patients

to treat a late asthmatic reaction with inhaled beta-2 agonists and oral prednisone (0.5 mg/kg) in the event this type of reaction developed after they had left the hospital.

A written informed consent that was approved by our hospital ethics committee was obtained from both patients before inhalation challenge tests

Crude Extract Preparations

Flours from wheat (*Triticum aestivum* L., cv. Chinese Spring) and rye (*Secale cereale* INIA C/171M) were defatted with cold acetone (2 × 1:5 [w/v] for 1 hr at 4°C). After drying, they were extracted with 0.5mol/L NaCl (2 × 1:5 [w/v] for 1 hr at 4°C). The salt extract was dialyzed (cut-off point, 3.5 kDa) against 0.1mol/L ammonium acetate and lyophilized. The flour was re-extracted with 70% (v/v) ethanol (2 × 1:10[w/v] for 1 hr at 4°C). Protein concentration was quantified according to Bradford's [1976] method.

Immunodetection Assays

Samples (20 μg) were fractionated by means of SDS–PAGE on Bio-RAD (Miniprotean II system gels; 15% acrylamide) according to the Laemmli method [Laemmli, 1970] Fractionated proteins were electrotransferred to polyvinylidene difluoride membranes, as previously described [Díaz-Perales et al., 1999]

After washing and blocking, membranes were incubated with individual sera from two patients with baker's asthma (1:5 dilution). After incubation with alkaline phosphatase-conjugated monoclonal anti-human IgE (clon GE-1, 1:500 dilution; Sigma, St. Louis, MO), it was revealed by adding a 5-bromo-4-chloro-3-indolyl phophatase/nitro blue tetrazolium solution (Sigma).

RESULTS

Table I shows the results of the skin prick test and IgE determinations. Both patients were sensitized to all cereal flours tested as well as to fungal alpha-amylase. Total serum IgE was 884 and 1,151 kU/L in patients 1 and 2, respectively.

Both patients showed a positive methacholine inhalation test at baseline, with PC_{20} methacholine values of 3.8 mg/ml in patient 1 and 6.4 mg/ml in patient 2.

In patient 1, SIC to wheat flour was negative (PC_{20} methacholine 24 hr post-allergen inhalation challenge was 3.1 mg/ml), whereas SIC to rye flour with the 1:1,000 dilution elicited an isolated early asthmatic reaction (20% fall in FEV_1, Fig. 2).

In patient 2, SIC to wheat flour was also negative (PC_{20} methacholine 24 hr post-allergen inhalation challenge was 8 mg/ml). SIC to rye flour with the 1:500 dilution elicited an isolated early asthmatic reaction (28% fall in FEV_1, Fig. 3). He recovered with inhaled salbutamol.

TABLE I. Results of Skin Prick Tests (Orthogonal Wheal Diameters) and IgE Determinations

	Skin prick tests (mm)		Specific IgE (kU/L)	
	Case 1	Case 2	Case 1	Case 2
Wheat flour	3 × 3	4 × 4	519	93.8
Rye flour	3 × 3	6 × 5	54.9	300
Barley flour	4 × 4	7 × 6	51.3	78.3
Corn flour	0	0	11.6	<0.35
Oat flour	0	5 × 4	24.2	21.1
Soya flour	0	0	6.46	<0.35
Fungal alpha-amylase	8 × 9	12 × 6	14.3	7.11
Mites	0	0	<0.35	<0.35
A. fumigatus	0	0	<0.35	<0.35

Immunodetection Assays

Salt and ethanol extracts from wheat and rye were fractionated by SDS–PAGE showing components from 6 to 70 kDa (Fig. 4). To analyze the in vitro IgE-binding capacity, these extracts were blotted and then tested with individual sera from the two patients. A different IgE-binding profile was obtained depending on the serum and the extract used (Fig. 4). Interestingly, the IgE-binding intensity of the rye flour extracts was significantly stronger than that of the wheat extracts in both patients' sera. The most reactive components recognized by serum from patient 2 correspond to low molecular weight proteins (under 17 kDa) from salt and ethanol rye extracts.

Serum from patient 1 also recognized a band in the rye salt extract with the same molecular mass of that recognized by serum from patient 2 but with lower reactivity.

However, patients 1 and 2 sera did not recognize low molecular mass proteins from wheat extracts.

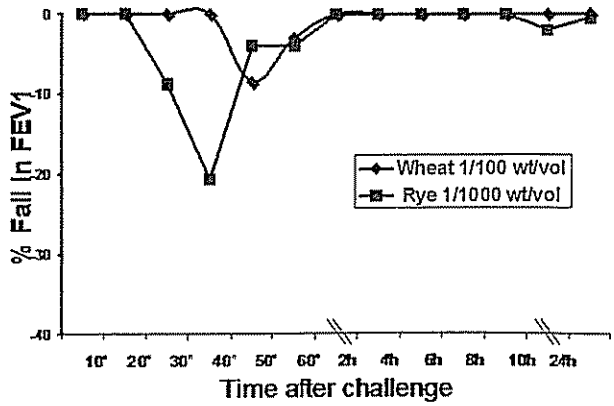

FIGURE 2. Specific inhalation challenge tests with rye and wheat flour extracts in patient 1. An early asthmatic reaction is observed with the rye flour challenge.

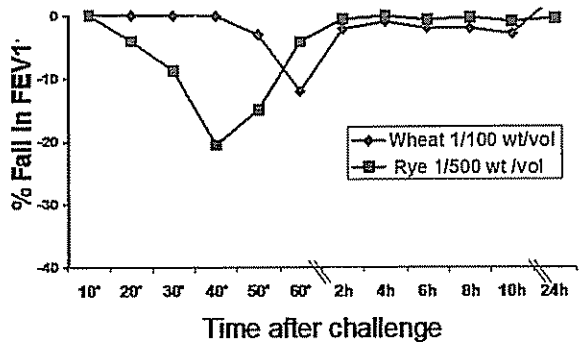

FIGURE 3. Specific inhalation challenge tests with rye and wheat flour extracts in patient 2. An early asthmatic reaction is observed with the rye flour challenge.

DISCUSSION

In this article we presented two cases of baker's asthma primarily due to exposure to rye flour. Patient 1 had been working for 7 years in a bakery with exposure to cereal flour (mostly wheat) with no symptoms. He first developed rhinoconjunctivitis and subsequently asthma symptoms shortly after he initiated the manipulation of rye flour. The clinical relevance of sensitization to rye flour was confirmed by the allergen inhalation challenge results. These patients were also sensitized to fungal alpha-amylase used as a dough additive, which is another important allergen causing baker's asthma [Houba et al., 1998]. Co-sensitization to fungal alpha-amylase and cereal flour among bakers with asthma is frequently observed, especially in Spain and other European countries [Houba et al., 1998; Quirce et al., 2006]. The possible existence of allergenic cross-reactivity between cereal amylase and fungal amylase appears to be very low.

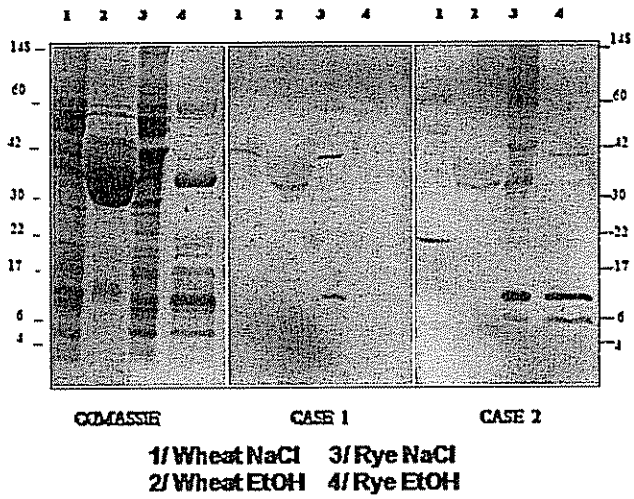

FIGURE 4. SDS–PAGE of wheat and rye flour salt and ethanol extracts. The most reactive components recognized by both patients' serum correspond to low molecular weight proteins (under 17 kDa) from salt and ethanol rye flour extracts.

Previous RAST inhibition studies have shown minimal cross-reactivity between cereal alpha or beta-amylase and fungal alpha-amylase [Sandiford et al., 1994].

The second patient had been exposed to wheat flour for 8 years and he was symptom-free, but shortly after he began to work with rye flour, he developed rhinoconjunctivitis and asthma symptoms. This is also in keeping with specific inhalation challenge results.

In the last years it has been pointed out that rye flour may play an important role in baker's asthma due to its recent extensive use (it is a usual component of flours to make "special breads"). In a study from Germany it was reported that in a group of 104 bakers with asthma symptoms at work, 52% were sensitized to rye flour [Baur et al., 1998]. In another report on 10 patients diagnosed with baker's asthma, the authors concluded that these patients needed exposure to less rye flour amounts as compared to wheat flour to provoke a positive bronchial response [Bensefa et al., 2004].

A recent publication reports on a 38-year-old baker who suffered from rhinitis, asthma and contact urticaria upon exposure to rye flour in the bakery. Using skin tests and specific IgE determinations the authors demonstrated sensitization to cereal flour (rye and wheat) in this patient, and afterwards, by simulating exposure to cereal flour at work, they demonstrated a stronger bronchial response to rye flour compared to wheat flour. The authors speculate that this response might be due to the fact that rye could generate more dust, or even because of its smaller particulate size [Ehrlich and Prescott, 2005].

García-Casado et al. [1995] described the only known major allergen from rye (termed Sec c 1) and its homologue from barley (BDP-1). They observed that these allergens had a high structural similarity with cereal α-amylase and trypsin inhibitors [García-Casado et al., 1995]. In 1996 they observed in 21 rye flour-sensitized patients that Sec c 1 and rye dimeric α-amylase inhibitor-1 (RDA-1) and rye α-amylase inhibitor-3 (RAI-3) elicited a positive skin prick test in more than 50% of the patients [García Casado et al., 1996].

We think that our patients' symptoms are mainly caused by exposure to rye flour. Both patients had symptoms related to exposure to this flour, positive skin and IgE tests, and a positive SIC test to rye flour. Immunoblotting results with whole flour extracts showed that rye flour enzymatic inhibitors may have a major allergenic role in these two patients. These data point out that rye flour allergens should be considered as possible causative allergens involved in bakers' asthma.

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