

Anaphylaxis after ingestion of wheat flour contaminated with mites

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Background: Anaphylaxis after ingestion of flours contaminated with mites has been recently reported.

Objective: The study was designed to determine whether flour-induced reactions in the absence of food sensitivity may result from mite contamination.

Methods: Patients with systemic reactions after ingestion of foods containing wheat flour and without food sensitivities were included in a prospective study. The flours were examined microscopically, and major *Dermatophagoides* allergens were quantified by monoclonal antibody techniques. Skin prick tests and IgE determinations to mites and flours were performed. Single-blind, placebo-controlled oral challenges were also performed.

Results: Sixteen patients were included in our protocol. They showed respiratory allergies to dust mites (100%) and intolerance to acetylsalicylic acid (87%). Microscopic examination of four flours implicated in allergic reactions revealed a high degree of mite contamination: *Dermatophagoides farinae* in one case and *Thyreophagus entomophagus* in three cases. Our patients' skin test and specific IgE responses to the flours implicated in the reactions were positive. A high level of Der 2 was found in the flour infested by *D. farinae*. Three of six food challenges with contaminated flours resulted in systemic reactions. Good tolerance to control flours was shown in our patients.

Conclusion: Ingestion of foods contaminated with mites may induce systemic anaphylactic reactions in patients with respiratory allergy to mites. (J Allergy Clin Immunol 1997;99:308-13.)

Key words: Anaphylaxis, acetylsalicylic acid intolerance, dust mites, food hypersensitivity, storage mites, wheat flour.

In 1993 Erben et al.¹ reported a case of anaphylaxis occurring after ingestion of beignets from New Orleans, Louisiana, contaminated with *Dermatophagoides farinae*, in a patient allergic to dust mites who had no food allergies. Recently, two other reports have dealt with anaphylaxis after ingestion of flours contaminated with mites: another case of anaphylaxis from beignets contaminated with *D. farinae*² and a study of 21 patients from Venezuela, with reactions to foods prepared with flours, who showed no food sensitivities.³

Abbreviations used

ASA:	Acetylsalicylic acid
SBPCOC:	Single-blind, placebo-controlled oral challenge
SPT:	Skin prick test

We had the opportunity to study several cases of anaphylaxis occurring after flour ingestion, between 1990 and 1992, in patients allergic to dust mites who had no food allergies. Therefore we designed a prospective study to test the hypothesis that flour-induced reactions in the absence of food sensitivity might be the result of mite contamination. Sixteen patients who had systemic reactions after the ingestion of different foods made of wheat flour and in whom no sensitization to any food could be found were included in our protocol. Our study included microscopic examination of flours, skin prick tests (SPTs), determination of IgE antibody against mites and flours, quantification of major mite allergens by monoclonal antibody techniques, and controlled oral challenges with flours. We have demonstrated, by both in vivo and in vitro studies, that contamination of food with different mite species was the cause of these reactions.

METHODS

Patients

A prospective protocol was designed to study subjects with a clinical history of immediate reactions to flour-based foods, suggestive of IgE-mediated hypersensitivity, and with no food allergies, who attended our outpatient clinic between 1992 and 1995. The following data were collected for all the patients: age, sex, medical history, and data related to the adverse reaction (food involved, elapsed time between ingestion and beginning of symptoms, clinical manifestations, and number of reactions experienced by each patient). Three control groups were also included: patients with allergy to dust mites ($n = 15$), patients with allergy to wheat flour ($n = 5$), and nonatopic acetylsalicylic acid (ASA)-intolerant subjects ($n = 5$). Written informed consent from the patients (or their parents, if patients were under 18 years of age) and control subjects was necessary to participate in the study, and the protocol was approved by the investigation and ethics committees of the hospital.

Wheat flour extracts

Flours implicated in reactions (problem flours) and control flours (of the same brand and composition as problem flours but from unopened bags) were immediately frozen at -20°C to avoid mite proliferation. Soluble extracts were prepared from

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0091-6749/97 \$5.00 + 0 1/1/77354

problem and control wheat flour by agitating the flours with 0.5 mol/L NaCl (10% wt/vol) during 3 hours at 4° C, centrifuging the mixture at 10,000 g for 30 minutes, filtering the supernatant through a Millipore 0.22 µm filter, and aliquoting and storing the supernatants at -70° C. Problem and control wheat flour ethanol extracts or prolamin (major seed storage protein of wheat that is insoluble in aqueous solution but soluble in ethanol-water mixtures) preparations were also obtained by washing the residue with distilled water, further extracting with 70% (vol/vol) ethanol (10% wt/vol) for 1 hour at room temperature, centrifuging, filtering, aliquoting, and storing at -70° C. Extracts for SPTs were prepared by adding glycerine (50%, vol/vol) in sterile vials.

Isolation and identification of mites

Samples of control and problem flours were examined in a blinded manner. Mites were isolated by using a suspension method: 1.5 ml of 1% (vol/vol) Tween-20 was added to a Petri dish containing 15 to 150 mg of sieved flour. After incubation for 24 hours at room temperature, 2 ml of 70% (vol/vol) lactic acid and 1 drop of 1 mol/L KI were added. Floating mites were picked up manually under a binocular stereo microscope (magnification, 13.5 to 60), incubated at 60° C for 12 hours in 70% (vol/vol) lactic acid, and identified with a phase-contrast microscope. Mites were classified as described by Krantz.⁴

SPTs

SPTs were carried out in patients and control subjects with 19 commercial aeroallergen extracts (*D. pteronyssinus*, *D. farinae*, *Tyrophagus putrescentiae*, *Lepidoglyphus destructor*, *Aspergillus fumigatus*, *Cladosporium herbarum*, *Alternaria alternata*, *Penicillium notatum*, *Olea europaea*, *Lolium perenne*, *Cynodon dactylon*, *Parietaria judaica*, *Artemisia vulgaris*, *Plantago lanceolata*, dog dander, cat dander, rabbit epithelium, and *Blatella germanica*; Alergia e Inmunología Abelló S.A., Madrid, Spain), with a battery of 20 food and enzyme extracts (egg, milk, fish, fruits, nuts, legumes, mustard, seafood, flours, and α-amylase; Alergia e Inmunología Abelló S.A.), and with the flour extracts made in our laboratory. SPTs were performed by puncture with a Morrow-Brown needle (Bayer S.A., Madrid, Spain) on the volar side of the forearms. Histamine phosphate (10 mg/ml) was used as a positive control, and physiologic saline solution was used as a negative control. The results are expressed as the mean wheal diameter (in millimeters) after 15 minutes of puncture. A wheal diameter greater than 3 mm, compared with the saline control, was considered a positive response.

Total serum IgE

The total IgE concentration was determined by the DELFIA method (Pharmacia Diagnostics, Uppsala, Sweden) according to the manufacturer's instructions.

Specific serum IgE

Levels of specific IgE to dust mites (*D. pteronyssinus*, *D. farinae*, *L. destructor* and *T. putrescentiae*) and to wheat flour were assessed by the Pharmacia ImmunoCAP system (Pharmacia Diagnostics) according to the manufacturer's instructions. A level higher than 0.35 kU/L was considered a positive result.

Specific IgE levels to flours implicated in allergic reactions were measured as follows: paper disks were activated with CNBr and coupled with the different flour extracts as described by Ceska et al.⁵ RAST was performed by using the Phadebas RAST technique (Pharmacia), according to the manufacturer's recommendations. The standard curve of the manufacturer was

used as a reference for the interpretation of the test, and RAST values higher than 0.35 Phadebas RAST units (PRU)/ml were considered positive responses.

Quantification of mite allergens

The major *Dermatophagoides* allergens in wheat extracts were quantified by monoclonal antibody-based ELISA. Specific monoclonal antibodies against Der p 1, Der f 1, and Der 2 (specific for both Der p 2 and Der f 2) were used.^{6,7} After incubation of appropriate dilutions of the extracts, specific rabbit polyclonal antisera raised against purified group 1 or group 2 allergens were added, followed by peroxidase-conjugated goat anti-rabbit IgG-purified antibodies. The enzymatic activity was then determined by using ortho-phenylenediamine as substrate. Allergen concentrations were estimated from three independent experiments. The mean intraassay coefficient of variation was lower than 3%, whereas the interassay coefficient of variation from controls tested in all assays was lower than 15%. The detection limit of the assays was 1 ng/ml. The world Health Organization (WHO) international standard for *D. pteronyssinus* (82/518) was assayed, and a Der p 1 concentration of 12 ± 0.13 µg/ampoule was obtained.

Spectrophotometric determination of ASA

Spectrophotometric determinations of ASA in flour extracts were performed in a blinded manner with the method of Trinder,⁸ by using salicylate reagents (Sigma Diagnostics) and measuring the color intensity at 540 nm with a Beckman DU-640 spectrophotometer, before and after addition of ferric ions to the extracts. Duplicate samples of flour extracts with known quantities of ASA, ranging from 10 mg/dl to 100 mg/dl, were used as controls.

Single-blind, placebo-controlled oral challenge (SBPCOC)

Patients were included in a previously established SBPCOC protocol. Patients were first challenged with a control wheat flour and, if available, with their own problem wheat flour (the flour implicated in each patient's reaction). If the problem wheat flour was not available, SBPCOCs were performed with one or more of the other patients' problem flours. The same protocol was applied to control and problem flours, with 100 and 500 mg doses being administered on the first day, and 1 gm and 10 gm doses on the second day. Patients were also included in an SBPCOC protocol with ASA, with 50 mg and 100 mg doses being administered on the first day and 250 mg and 500 mg doses on the second day.⁹ All the foods and drugs, including lactose as placebo, were administered in opaque gelatin capsules (except the 10 gm flour doses that were prepared as omelets) and with a 180-minute interval between each dose.

A clinical history of a severe food or drug reaction contraindicated the SBPCOC. None of the patients had episodes of urticaria or angioedema in the week before the controlled oral challenge, and FEV₁ values were at least 80% of predicted values, with an absolute value greater than 1.5 L. Hourly measurements of peak expiratory flow with a mini-Wright peak flow meter (Clement Clarke International Ltd., London, England) during the day before challenge showed a variability less than 20% in peak expiratory flow values in all subjects. The following medications were stopped before the challenge: antihistamines (72 hours), cromolyn (12 hours), theophylline (12 hours), and inhaled sympathomimetic agents (12 hours). Each SBPCOC was carried out separately on different days, and written informed consent from the patients (or their parents if

TABLE I. Clinical features of the patients

Patient No.	Age (yr)/Sex	Atopy	ASA intolerance	Implicated food	Symptoms
1	19/F	Mite R/BA	AE	Legume*	Shock
2	26/F	Mite R/BA	AE/BA	Pancake	AE/BA
3	17/M	Mite R/BA	AE/BA	Sponge cake	U/AE/BA
4	38/F	Mite R/BA	AE	Fried bread	AE/BA
5	26/F	Mite RC	AE/BA	Pizza	AE/BA
6	33/F	Mite R	AE/BA	Pancake	AE
7	24/F	Mite R	AE	Squid*	U/AE/BA
8	17/F	Mite RC	AE	Sponge cake	AE/BA
9	17/M	Mite R/BA	AE/BA	Pizza	AE/BA
10	13/M	Mite R	Absent	Fish*	AE/BA
11	14/M	Mite R/BA	Absent	Sponge cake	AE/BA
12	13/F	Mite RC	AE	Sponge cake	AE/BA
13	33/F	Mite R	AE	Pancake	AE/BA
14	21/F	Mite R	AE	Sponge cake	RC/BA
15	29/F	Mite R/BA	AE/BA	Fish*	AE/BA
16	13/F	Mite R/BA	AE	Sponge cake	AE/BA

AE, Angioedema; BA, bronchial asthma; F, female; M, male; R, rhinitis; RC, rhinoconjunctivitis; U, urticaria.

*Foods fried in batter.

the patients were under 18 years of age) was necessary to perform each SBPCOC. After a positive SBPCOC, the next food or drug challenge was separated by at least 7 days.

The clinical characteristics recorded for each SBPCOC were symptoms, dose, and time elapsed. Clinical reactions during SBPCOC were classified as follows: (1) bronchial asthma, if there was a greater than 20% decrease in the FEV₁ values; (2) nasocular reaction; (3) gastrointestinal reaction (abdominal pain, nausea, vomiting, and diarrhea colic); (4) cutaneous reaction (urticaria or angioedema); or (5) systemic anaphylaxis. During the challenge procedure, blood pressure was monitored along with FEV₁ values (at 15 minutes, 30 minutes, and every hour after administration of each food or drug dose or at any time during which other symptoms were reported by the patient).

RESULTS

Sixteen patients reporting anaphylactic food-induced reactions and without food hypersensitivities were included in our protocol. The clinical features of our patients are summarized in Table I. There were 12 women and 4 men, aged 22 ± 8 (mean $\pm$ SD) years, who shared the following three characteristics.

1. A food item made of wheat flour, either fried in batter or made into cakes, had been ingested from 5 minutes to 1 hour before the anaphylactic reaction.
2. All patients had a history of perennial rhinoconjunctivitis or bronchial asthma and had positive SPT responses and specific IgE responses to house dust mites or storage mites (Table II).
3. All but two patients (87%) had a clinical history of ASA intolerance that manifested as periorbital angioedema with or without accompanying dyspnea (Table I) and that developed from 1 to 3 hours after intake of ASA or other related nonsteroidal antiinflammatory drug (NSAID).⁹

Clinical manifestations of the flour-induced reactions ranged from isolated angioedema to anaphylactic shock with cardiorespiratory arrest (requiring aggressive resuscitation measures). The most common symptoms were periorbital angioedema with dyspnea and wheezing (Table I). All reactions were treated in the emergency department, and total improvement was achieved from 4 hours to 72 hours after the onset of symptoms. Three patients reported several (from two to five) flour-induced anaphylactic reactions over the past 5 years.

The clinical history indicated that most of the flours implicated in allergic reactions were special flours for pastry making. Four of our patients brought us the remainders of four wheat flours implicated in reactions (problem flours, corresponding to patients 1, 8, 9, and 16) in their original containers. Three of these problem flours were special flours for pastry making, originating from the same factory and consisting of wheat flour containing 2% (wt/wt) baking powder. The baking powder consisted of cornstarch, sodium chloride, calcium carbonate, sodium bicarbonate, and sodium acid pyrophosphate. The fourth problem flour (from patient 9) was a flour for pizza-making from another factory, and it consisted of a 1% (wt/wt) mix of wheat flour and baking yeast, containing α -amylase.

Microscopic examination of the four available wheat flours that had been implicated in reactions disclosed a high level of mite contamination: *D. farinae* in the flour provided by patient 1 and the storage mite *Thyreophagus entomophagus* in the other three flours. This was in contrast to the almost total absence of mites in the control flours (Table III).

SPT responses to soluble extracts from problem flour were positive in all our patients (Table II), as well as in several control patients with dust mite allergy, and in all

TABLE II. SPT and IgE responses

Patient No.	Total IgE	House dust mites				Storage mites				Problem flours							
		D.p.		D.f.		T		L		P1		P8		P9		P16	
		SPT*	IgE†	SPT	IgE†	SPT	IgE†	SPT	IgE†	SPT	IgE†	SPT	IgE†	SPT	IgE†	SPT	IgE†
1	181	5	95.5	10	21.9	4	1.1	0	0	14	2.2	4	2.1	0	0.5	0	0.7
2	685	6	21.0	10	7.5	8	2.0	5	3.0	4	2.1	5	4.6	4	2.8	4	1.5
3	155	5	8.4	4	5.9	4	3.9	0	2.0	4	0.9	4	3.5	5	4.1	4	1.7
4	137	0	8.3	0	8.5	0	2.7	0	2.7	0	1.6	5	3.1	4	0.4	6	0.8
5	484	7	21.4	6	3.8	6	2.8	4	3.8	6	4.6	6	3.9	5	2.8	5	3.1
6	78	6	29.8	11	29.7	5	9.1	10	0	5	0	4	0	4	0	0	0
7	226	6	8.8	4	4.0	5	1.4	4	0	7	0.8	0	2.3	0	0.4	0	0
8	343	11	3.0	6	1.5	9	0	0	0	4	0.5	7	6.8	5	4.3	5	2.5
9	265	19	92.5	7	48.5	7	13.7	0	4.2	5	6.8	8	7.5	7	9.4	6	5.3
10	128	8	4.5	11	4.0	10	3.3	4	3.1	4	0	4	2.0	5	1.2	4	1.7
11	2000	8	23.0	6	70.0	7	5.7	4	3.8	4	4.5	6	6.8	5	4.3	5	2.3
12	27	6	0.7	4	0	0	0.9	0	0	4	0	4	0.5	5	0.7	4	0
13	29	5	0.7	0	0	0	0	0	0	0	0	5	0	4	0	4	0
14	187	9	2.9	4	2.3	4	1.9	0	0.7	4	0	7	2.6	9	1.3	5	0.8
15	77	5	4.2	5	5.0	9	4.0	0	0	7	2.6	9	4.2	8	2.7	7	2.1
16	669	5	13.3	4	17.1	0	2.1	0	1.6	5	1.9	4	0.8	7	1.3	6	1.6

D.p., *Dermatophagoides pteronyssinus*; D.f., *Dermatophagoides farinae*; T, *Tyrophagus putrescentiae*; L, *Lepidoglyphus destructor*

P1, P8, P9, and P16: problem flours from patients 1, 8, 9, and 16 respectively

*SPT responses measured as mean wheal diameter (in millimeters).

†Specific IgE in kU/L as measured by ImmunoCAP.

‡Specific IgE in PRU/ml as measured by RAST.

TABLE III. Microscopic examination of wheat flour

Flour	Sample wt (gm)	No. of mites counted	No. of mites/gm	Mite species (% of total)		
				D.p.	D.f.	T.e.
P1	0.150	735	4,900	—	100	—
C1	0.378	20	53	75	25	—
P8	0.110	5,742	52,200	—	—	100
C8	0.450	15	33	40	60	—
P9	0.170	1,559	9,171	—	1	99
C9	0.250	0	0	—	—	—
P16	0.109	4,100	37,615	—	—	100
C16	0.825	0	0	—	—	—

D.p., *Dermatophagoides pteronyssinus*; D.f., *Dermatophagoides farinae*; T.e., *Thyreophagus entomophagus*

P1, P8, P9, and P16: problem flours from patients 1, 8, 9, and 16, respectively; C1, C8, C9, and C16, control flours for patients 1, 8, 9, and 16 respectively (same brand and composition as problem flours but from unopened bags).

control patients with flour allergy. SPT responses to α -amylase were negative. SPT responses to commercial flours and to control flour extracts were negative in all patients and control patients, except in the control group with flour allergy.

Levels of specific IgE to problem flours were positive in 14 of our patients (Table II), in 10 control patients with dust mite allergy (mean $\pm$ SD, 1.9 ± 0.5 PRU/ml), and in the five control patients with flour allergy (mean $\pm$ SD, 6.4 ± 2.3 PRU/ml). The specific IgE response to commercial flours and to control flours was negative in all our patients; positive results were present only in the control group with flour allergy. Specific IgE responses to commercial mites were positive in our patients (Table II) and in the control patients dust mite

allergy (mean $\pm$ SD for *D. pteronyssinus*, 39.2 ± 18.3 kU/L; for *D. farinae*, 19.4 ± 8.2 kU/L; for *L. destructor*, 1.9 ± 0.4 kU/L; and for *T. putrescentiae*, 3.0 ± 0.9 kU/L).

Quantification of the major *Dermatophagoides* allergens in problem and control samples was carried out (Table IV). Der 2 allergens were detected in all samples, with a high concentration in two of the samples, one that was contaminated with *D. farinae* and the other that had a high level of infestation with *T. entomophagus*. Whether a previous, undetected infestation of this flour with a *Dermatophagoides* species had occurred is not clear, because the ELISA method for Der 2 is specific for this genus of mites. Group I *Dermatophagoides* allergens were detected only in a sample from patient 1, although at a lower concentration than expected. The

TABLE IV. Quantification of major mite allergens

Wheat flour	Concentration of allergen ($\mu\text{g/gm}$)		
	Der p1	Der f 1	Der 2
P1	<0.05	14.6	35.90
C1	<0.05	<0.05	0.55
P8	<0.05	<0.05	22.70
C8	<0.05	<0.05	0.61
P9	<0.05	<0.05	0.80
C9	<0.05	<0.05	0.60
P16	<0.05	<0.05	0.70
C16	<0.05	<0.05	0.65

P1, P8, P9 and P16, problem flours from patients 1, 8, 9, and 16, respectively; C1, C8, C9 and C16, control flours from patients 1, 8, 9, and 16 respectively (same brand and composition as problem flour but from unopened bags).

absence or reduced levels of Group I allergens in cereal flour samples has been recently reported to be caused by a specific interaction between Group I allergens and cereal prolamin, an insoluble protein fraction of cereal seeds.¹⁰

Spectrophotometric determinations of ASA in the problem flour extracts were negative; however, ASA-containing control extracts were positive.

During SBPCOC, all patients who underwent oral challenges showed good tolerance to ingestion of control flour (Table V). Ten SBPCOCs were performed with ASA, of which nine were positive, with periorbital angioedema reactions occurring from 1 to 3 hours after ASA intake. Furthermore, six of our patients gave their consent to undergo SBPCOCs with problem flours. Three of these challenges resulted in systemic reactions, corresponding to patients 8, 9, and 16 (who were challenged with their own problem flours), with FEV₁ decreases of 28%, 43%, and 23%, respectively, accompanied by gastrointestinal symptoms in patient 8 and by periorbital angioedema in patient 16. Symptoms developed from 30 minutes to 2 hours after the ingestion of 1 gm of the corresponding problem flour. In all cases, symptoms were relieved 10 to 60 minutes after treatment. The other three patients, who were challenged with the other patient's problem flours, showed no reaction.

DISCUSSION

This study was designed to investigate the clinical observation of a cluster of food-induced anaphylactic reactions in patients with respiratory allergies to dust mites. Some of these patients had been formerly diagnosed with idiopathic anaphylaxis because of the absence of food allergies. Common clinical features among these patients included the implication in the reactions of different foods made of wheat flour and the coexistence of respiratory allergy to dust mites with intolerance to ASA.

Possible contamination of wheat flours with ASA was discarded because spectrophotometric determinations

TABLE V. Results of controlled oral challenge

Patient no.	ASA	Control flour	Problem flour*
1	ND	—	ND
2	AE	—	P1 (—)
3	ND	ND	ND
4	ND	—	ND
5	ND	ND	ND
6	AE	—	ND
7	AE	—	ND
8	AE	—	GR/BA
9	AE	—	BA
10	AE	—	P1 (—); P8 (—)
11	ND	ND	ND
12	ND	—	ND
13	AE	ND	ND
14	AE	—	ND
15	AE/BA	—	P9 (—)
16	—	—	AE/BA

ND, Not done; —, negative result; AE, angioedema; BA, bronchial asthma; GR, gastrointestinal reaction.

P1, P8, and P9: problem flours from patients 1, 8, and 9, respectively.

*The results correspond to SBPCOC with each patient's problem flour, when available. When flour was not available, SBPCOCs were performed with one or more of the other patients' problem flours.

for ASA in the problem flours gave negative results; also, visits to the flour factories and careful examination of the manufacturing process ruled out accidental ASA contamination. The special subset of ASA-intolerant patients, young patients with atopy who have isolated periorbital angioedema reactions to intake of nonsteroidal antiinflammatory drugs, has been recently described.⁹ However, we have estimated that the prevalence of ASA intolerance among our patients with allergy to dust mites is approximately 2%. The fact that 14 of our 16 patients (87%) showed ASA intolerance is highly significant (Fisher's exact test, $p < 0.001$). The reason for the clinical association between ASA intolerance and anaphylaxis after mite ingestion is unknown but deserving of further investigations.

Our hypothesis, that of contamination of flours with dust mites, was confirmed by microscopic examination of problem flours, revealing a high level of contamination with mites, and by controlled oral challenges with infested flours in three patients. The other three patients who did not show positive results after challenges with problem flours were in fact not challenged with the flours implicated in their reactions, suggesting that other mite species could have caused their reactions. Investigations are needed to identify the allergens responsible for mite ingestion reactions. The negative results after skin testing with the problem wheat flour ethanol extracts suggest that Group I allergens (selectively bound to prolamins in wheat flour)¹⁰ are not the cause of these reactions. The responsible mite allergens must be stable to heat, to extremes of pH, and to digestion by proteases, conditions that Group II allergens fulfill.^{11, 12, 13}