

Respiratory allergy to *Aspergillus*-derived enzymes in bakers' asthma

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*Baking and food industry workers are exposed to several powdered Aspergillus-derived enzymes with carbohydrate-cleaving activity that are commonly used to enhance baked products. We describe a retrospective study of sensitization to fungal α -amylase and cellulase on bakers. Five bakers in whom respiratory allergy symptoms developed when they were exposed to bread "improvers" that contained fungal α -amylase and cellulase were investigated by in vivo and in vitro tests. Type I hypersensitivity to these enzymes was demonstrated in the five patients by means of skin testing, histamine release test, positive reverse enzyme-immunoassay for specific IgE antibodies, and bronchial provocation test response to α -amylase or cellulase or both. Isolated immediate and dual responses to the bronchial challenge tests with these enzymes were observed. Immunoblot analysis with use of a pooled serum identified IgE-binding components in both enzymes. In the reverse-enzyme immunoassay-inhibition assays cross-reactivity between α -amylase and cellulase was not found, but some degree of cross-reactivity between α -amylase and *A. oryzae*, and between cellulase and *A. niger* was demonstrated. Four of the patients were also sensitized to cereal flour. Aspergillus-derived enzymes used as flour additives can elicit IgE-mediated respiratory allergy, and this fact has to be considered in the diagnosis and clinical management of bakers' asthma. (J ALLERGY CLIN IMMUNOL 1992;90:970-8.)*

Key words: α -amylase, *Aspergillus*, bakers' asthma, bronchial provocation test, cereal flour, cellulase, enzymes, occupational asthma

Enzymes of different origins are increasingly being used in the food, chemical, and pharmaceutical industries. These substances are high-molecular-weight (MW) proteins with a catalytic activity, that are suitable for a wide range of purposes. Several of these enzymes are considered potent inhalant allergens that sensitize a large number of exposed individuals, mainly at work.¹⁻⁹ Bakery workers are exposed to different enzymes, most of which are derived from genus *Aspergillus*, that is, α -amylase, hemicellulase, cellulase, and glucoamylase. These enzymes are commonly added to flour to enhance baked products.¹⁰

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Abbreviations used

BPT:	Bronchial provocation test
BSA:	Bovine serum albumin
FEV ₁ :	Forced expiratory volume in 1 second
FVC:	Forced vital capacity
MW:	Molecular weight
OD:	Optical density
PBS:	Phosphate-buffered saline
PC ₂₀ :	Provocative concentration of methacholine causing a 20% fall in FEV ₁
PEFR:	Peak expiratory flow rate
RAST:	Radioallergosorbent test
REIA:	Reverse enzyme-immunoassay
SDS-PAGE:	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

The first description of allergy to enzymes in bakers' asthma was given by Baur et al.¹¹ in 1986, who reported that 12 out of 35 (34%) symptomatic patients had immediate-type hypersensitivity to *Aspergillus* α -amylase, as demonstrated by in vivo and in vitro tests. Since then, only sporadic case reports of

TABLE I. Patients' characteristics

Patient	1	2	3	4	5
Age (yr)/sex	18/F	55/M	27/M	55/M	49/M
Occupation	Baker	Mill technician	Baker	Baker	Baker
Years employed	4	30	7	40	30
Occupational symptoms	R/A	RC/A	RC/A	R/A	RC/A
Duration of symptoms (years)	3	16	2	7	9
Smoking (pack years)	0	0	1.80	7.3	54.5
Own/family atopy	+ / +	+ / -	+ / -	- / +	+ / +
FEV ₁ (% predicted value)	95	85	101	80	89
Total IgE (KU/L)	270	>1,000	260	99	257
Skin test positivity to common allergens	Grass pollen	—	Dpt, cat dander	—	Dpt
Other allergies	AD, hay fever, food allergy to mustard				

R, Rhinitis; RC, rhinoconjunctivitis; A, asthma; AD, atopic dermatitis; Dpt, *Dermatophagoides pteronyssinus*.

bakers' asthma caused by fungal α -amylase have emerged.^{12, 13} In 1988 Baur et al.¹⁰ reported that the frequency of sensitization of symptomatic bakers to the *Aspergillus*-derived enzymes used in baking was the following: α -amylase 24%, hemicellulase/cellulase 8%, and glucoamylase 5%. These data justify that hypersensitivity to enzymes in bakers' asthma has aroused growing interest.

In this article we present the clinical and immunologic studies carried out on five bakery workers in whom allergic respiratory symptoms developed after occupational exposure to cereal flours and *Aspergillus*-derived enzymes used as baking additives.

CASE REPORTS

Five bakery workers were evaluated in our clinic because of their symptoms at work. Their ages ranged between 18 and 55 years (mean age, 40.8 years), and there were four men and one woman. They had been working in the baking industry between 4 and 40 years (22.2 ± 15.82 years). Patients' characteristics are presented in Table I.

Rhinorrhea, sneezing, itching and watery eyes, dyspnea, cough, and chest tightness developed in all five patients in a variable period after they started work in the baking industry (1 and 33 years). All of them improved on weekends, and at the beginning they were asymptomatic on vacations. However, as symptoms became progressively more severe, it took longer and longer to resolve when away from work. In patient 4 symptoms were chronic and severe, requiring hospital admission on several occasions. It is interesting to note that besides cereal flour exposure, these patients referred to the fact that they were also handling

other products that are currently used as flour additives, mainly *Aspergillus*-derived α -amylase and cellulase. Most of them established a causal relationship between handling of these powdered flour additives and the development within a few minutes of acute rhinoconjunctivitis, dyspnea, cough, and wheezing, often followed by a late aggravation of symptoms several hours after their work shift. Patient 3 had actually suffered very severe asthma attacks when opening fungal α -amylase sacks, and then he completely avoided this maneuver and contact with this enzyme.

On the other hand, patient 1 was not aware of exposure to these bread additives, and she had undergone repeated allergic investigations, including specific bronchial provocation tests (BPTs) with cereal extracts, with negative results, and the precise diagnosis could not be made until fungal enzymes were tested. None of the patients referred to cutaneous symptoms at work. All our patients ate bread and products containing cereal without any symptoms. After allergy diagnosis and consequent medical advice, only patients 1 and 3 were able to leave the workplace and had complete resolution of symptoms within a year. The other patients were encouraged to take hygienic measures at their workplace to ensure allergen avoidance as far as possible, and all of them are on permanent antiasthma therapy.

MATERIAL AND METHODS

Preparation of allergenic extracts

Alpha-amylase from *Aspergillus oryzae* and cellulase from *A. niger* were supplied by a pharmaceutical company (Europharma, Madrid, Spain). The purity of both enzymes

was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

The extracts of α -amylase, cellulase, and cereal flours (wheat and rye) were prepared at a concentration of 1:10 wt/vol by dissolving 2 gm of each substance in 20 ml of phosphate-buffered saline (PBS), pH 7.3, at room temperature. After they were stirred for 60 minutes and passed through filter paper, these solutions were dialyzed in PBS for 24 hours and then passed through a 0.22 μ m Millipore filter (Millipore Corp., Bedford, Mass.) for sterilization. Protein concentration of these extracts was estimated by the method of Lowry et al.¹⁴ using bovine serum albumin (BSA) as a standard, and the results were the following: 6 mg/ml in α -amylase extract, 3.2 mg/ml in cellulase extract, and 1 mg/ml in both wheat and rye extracts. Several dilutions in PBS of each extract were done for skin and inhalation tests.

Skin prick tests

Skin prick tests were done with the antigenic preparations described above. Histamine phosphate at 10 mg/ml and PBS were used as positive and negative controls, respectively. An immediate wheal with a mean diameter 3 mm or greater with erythema, occurring 15 minutes after testing, was defined as a positive reaction if the test with the diluent was negative.

Skin prick tests were also done with a battery of common inhalant allergens, including *Dermatophagoides pteronyssinus* and storage mites (*Acarus siro*, *Lepidoglyphus destructor*, and *Tyrophagus putrescentiae*), grass and olive tree pollen, and dog and cat dander (Abelló S.A., Madrid, Spain). *Aspergillus* spp., *Mucor racemosus*, *Cladosporium herbarum*, and *Alternaria tenuis* extracts (Hollister-Stier Laboratories, Spokane, Wash.) were also tested.

Fifteen atopic nonexposed subjects and 15 nonallergic patients from our clinic, who served as a control group, were tested with the maximum concentration of both α -amylase and cellulase extracts.

Specific IgE determination

The determination of specific IgE antibodies to α -amylase and cellulase was performed by use of a reverse enzyme-immunoassay (REIA) as previously described.^{7, 8, 15}

One milligram of periodate oxidized peroxidase was mixed with 150 μ l of α -amylase (3 mg/ml) or cellulase (3 mg/ml) and 100 μ l of carbonate-bicarbonate buffer (1 mol/l, pH 9.5) and kept at 4° C for 18 hours. Afterward, 2 mg of sodium borohydride was added, and the solution was dialyzed against PBS at 4° C for 24 hours. After dialysis, 1% BSA in PBS containing 25% glycerol was added to a final volume of 2 ml. This final solution was separated into aliquots and stored at 4° C.

Microplates (M 24 AR, Dynatech Laboratories, Inc. Alexandria, Va.) were coated with monospecific antihuman IgE (Tago Laboratories, Burlingame, Calif.) as previously described.⁷ Then 0.05 ml per well of patient's serum mixed

with 0.05 ml PBS-Tween was incubated for 18 hours at 4° C while being shaken.

After several washes, 200 μ l of a dilution of the allergen-enzyme conjugate made in PBS-Tween, 25% calf serum, containing 1 μ g of peroxidase, was added to each well and left shaking for 1 hour at room temperature. Then after various washes with PBS-Tween and tap water, the substrate (0.1 ml per well) was added and left for 30 minutes. The reaction was arrested with 4N H₂SO₄ (0.1 ml per well), and the plates were read on a Titertek-Multiskan (Flow Laboratories, Irvine, Scotland) at 492 nm. The substrate was composed of 1,2 phenylenediamine (2 mg/ml) citrate-phosphate buffer (0.1 mol/L, pH 5, 0.003% H₂O₂).

Specific IgE antibodies against cereal grains (wheat, rye) were measured by commercially available disks (Pharmacia Diagnostics, Uppsala, Sweden). Radioallergosorbent test (RAST) was performed according to the manufacturer's instructions with use of the commercially available anti-IgE labeled with iodine 125, and the results were expressed in Phadebas RAST units (PRU) per milliliter as derived from the Phadebas RAST reference system.

REIA-inhibition

This method was used to investigate the specificity of the α -amylase and cellulase REIA and the possible existence of cross-reactivity between both enzymes and between each enzyme with *A. oryzae* and *A. niger*. The sera of patients who gave positive results in the REIA assay to α -amylase ($n = 5$) or cellulase ($n = 4$) were pooled and incubated as described. Afterward, the same amount of the α -amylase or cellulase/peroxidase conjugate was mixed with increasing amounts of different allergens. The method previously described was followed.^{7, 8} Alpha-amylase, cellulase, *A. oryzae*, *A. niger*, wheat flour, and *Artemisia vulgaris* pollen extracts were used as allergens.

Histamine release test

This test was performed by use of an automated fluorometric method according to Siraganian,¹⁶ with the modifications previously described.¹⁷ Six consecutive fivefold dilutions of α -amylase, cellulase, wheat flour, and *Aspergillus* spp. 1:10 wt/vol extracts were used. Before allergen challenge, blood samples were tested with four dilutions of goat antihuman IgE (Tago). The total basophil histamine content was measured by adding to the sample blood perchloric acid at a concentration of 10% vol/vol. A result higher than 10% release was considered positive.

SDS-PAGE and immunoblotting

SDS-PAGE was performed according to Fling and Gregerson¹⁸ at an acrylamide gradient concentration of 8% to 25%. The following polypeptides were used as MW markers: phosphorylase b (92.5 kd), BSA (66.2 kd), ovalbumin (45 kd), carbonic anhydrase (31 kd), soybean trypsin inhibitor (21.5 kd), and lysozyme (14.4 kd). The electrophoresis was carried out in a vertical slab gel electrophoresis

unit (Hoefer Scientific Instruments, San Francisco, Calif.) and the protein bands were visualized with Coomassie brilliant blue.

Electrotransfer of proteins to nitrocellulose sheets was carried out according to Towbin et al.¹⁹ The sheets were then incubated with a pooled serum from the patients, washed, and finally incubated with Hamlet ¹²⁵I, a mouse antihuman IgE monoclonal antibody (Abelló S.A., Madrid, Spain). IgE binding proteins were visualized by autoradiography with appropriate exposure times.

Bronchial provocation tests

Methacholine inhalation test was performed according to Cockcroft et al.²⁰ with some modifications. The aerosolized particles were generated by a continuous pressurized nebulizer model DeVilbiss 646 (DeVilbiss Co., Somerset, Pa.) with an output of 0.28 ml/min.

Specific BPTs were done with α -amylase or cellulase or both and with other allergens suspected of being responsible for occupational respiratory allergy on the basis of the skin tests and specific IgE results. The patients were first skin tested with 10-fold dilutions of the extracts to determine a safe starting dose for the challenge. Baseline pulmonary function measures of forced expiratory volume in 1 second (FEV₁) and forced vital capacity (FVC) were performed, and a control challenge with PBS was carried out before antigen provocation. The patients inhaled the aerosolized allergen using the nebulizer method mentioned above in progressive concentrations at tidal breathing for 2 minutes, and pulmonary function measures of FEV₁ and FVC were performed at 5, 10, 15, 20, 30, 60, and 120 minutes after challenge. A positive immediate response was defined as a greater than 20% fall in FEV₁. Afterward hourly peak expiratory flow rate (PEFR) measurements were performed (respecting sleeping periods) to evaluate the late response. This was considered a greater than 30% fall in PEFR from the baseline. The different doses of allergen were inhaled at 24-hour intervals.

The tests were undertaken when the patients were absent from work, asymptomatic, and without any medication that could affect the test results. A patient was considered suitable for the test if the basal FEV₁ value was greater than 80% of the predicted value. BPTs with the different allergens were carried out with at least a 2-week period between them.

Ten nonexposed asthmatic subjects who had similar clinical severity and antiasthma therapy requirements were also challenged with the maximum concentration of α -amylase and cellulase extracts (1:10 wt/vol).

RESULTS

Skin prick tests

After skin testing all the patients showed a positive response to α -amylase, and all of them except patient 3 reacted to cellulase as well. A positive response to wheat and rye flour extracts was observed in four of

TABLE II. Results of skin prick tests to occupational allergens

Patient	1	2	3	4	5
α -amylase 1:10 wt/vol	18	20	20	21	15
Cellulase 1:10 wt/vol	7	16	0	22	18
Wheat flour 1:10 wt/vol	2	9	7	14	11
Rye flour 1:10 wt/vol	0	20	9	9	12
<i>Aspergillus</i> spp.	0	5	0	2	6
<i>Alternaria tenuis</i>	7	3	0	0	0
Storage mites	0	0	0	0	0
Histamine phosphate (10 mg/ml)	6	5	6	7	6

Maximum wheal diameter in millimeters.

the patients, but not in patient 1. Skin tests results are shown in Table II.

The skin tests with the maximum concentration of α -amylase and cellulase were negative in the control group, indicating the nonexistence of irritating activity at the concentration tested. No adverse reaction was observed after skin testing.

Specific IgE determinations

The results of the REIA assay showed positive values for both α -amylase and cellulase in patients 1, 2, 4, and 5, and were positive only to α -amylase in patient 3, which is in agreement with the results of skin prick tests. In contrast, control patients had negative REIA values to both enzymes. The results of the REIA assay and RAST values for cereals are shown in Table III.

REIA inhibition

The existence of cross-reactivity between α -amylase and cellulase was not found. However, some degree of cross-reactivity seems to exist between α -amylase and *A. oryzae* and between cellulase and *A. niger*. The absence of inhibitory effect of *Artemisia vulgaris* pollen (control) together with the highest inhibitions obtained with either α -amylase or cellulase in the corresponding REIA assay, indicate that the observed effects were specific. The results of the REIA-inhibition assays are presented in Figs. 1 and 2.

Histamine release test

Leukocyte histamine release test results with the different allergens are shown in Table IV. These results

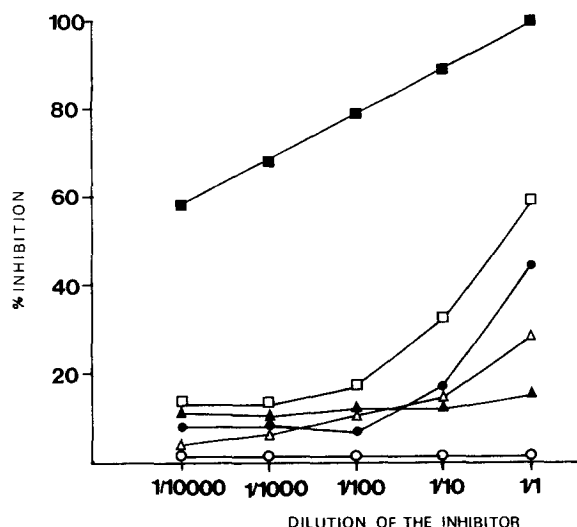


FIG. 1. REIA-inhibition performed coincubating labeled α -amylase (1 μ g per well) with (■) α -amylase, (▲) cellulase, (□) *A. oryzae*, (△) *A. niger*, (●) wheat flour, and (○) *Artemisia vulgaris* pollen.

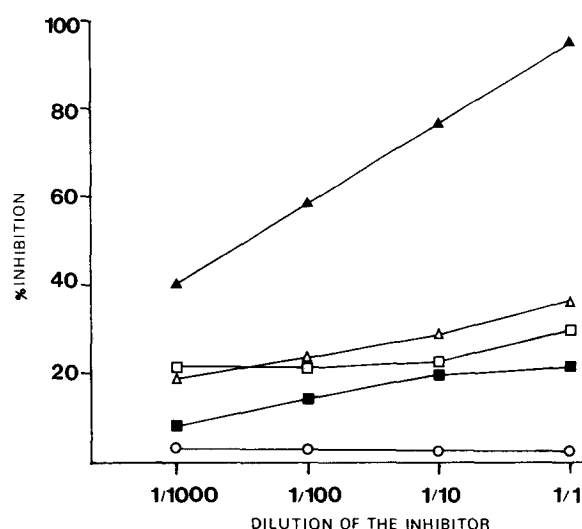


FIG. 2. REIA-inhibition performed coincubating labeled cellulase (1 μ g per well) with (▲) cellulase, (■) α -amylase, (△) *A. niger*, (□) *A. oryzae*, and (○) *Artemisia vulgaris* pollen.

TABLE III. Specific IgE values

Patient	1	2	3	4	5	Controls ($\bar{x} \pm SD$)
α -amylase	>4.000	3.782	2.165	3.086	3.740	0.140 $\pm$ 0.035 OD
Cellulase	2.286	3.158	0.135	3.128	1.724	0.120 $\pm$ 0.020 OD
Wheat flour	0.98	44	25	0.4	3.47	<0.35 PRU/ml
Rye flour	<0.35	ND	1.9	1.9	ND	<0.35 PRU/ml

ND, Not done.

TABLE IV. Histamine release test results (maximum % release)

Patient	1	2	3	4	5
α -amylase	19	55	30	NR	70
Cellulase	56	33	Neg	NR	49
Wheat flour	Neg	39	67	NR	24
<i>Aspergillus</i> spp.	Neg	18	Neg	NR	Neg

NR, Nonresponder; Neg, negative.

are given as the maximum percentage release obtained after each antigen challenge. Patient 4 was considered a nonresponder to this test, because no release was obtained after challenge with anti-IgE.

SDS-PAGE and immunoblotting

Molecular weight analysis of α -amylase and cellulase peptidic components as well as allergens is illustrated in Fig. 3. Lane A+ shows that α -amylase contains a unique peptide, of MW 52 kd, with capacity to bind specific human IgE from the serum pool. The remaining bands on lane A did not show binding ability even after a long exposure time. Cel-

lulase, however, demonstrated to have a peptidic allergen of MW 42 to 44 kd, a weak but clear allergen of approximately 85 kd, and a diffuse band of 62 to 75 kd, which probably consists of two allergenic peptides (Lane C+). All immunoblots with control sera were negative.

Bronchial challenge tests

BPTs with methacholine revealed severe or moderate bronchial hyperresponsiveness in all patients, with PC₂₀ values of 0.33, 0.5, 0.25, 0.085, and 2 mg/ml, respectively. It is interesting to note that PC₂₀ values notably improved in patients 1 (4 mg/ml) and

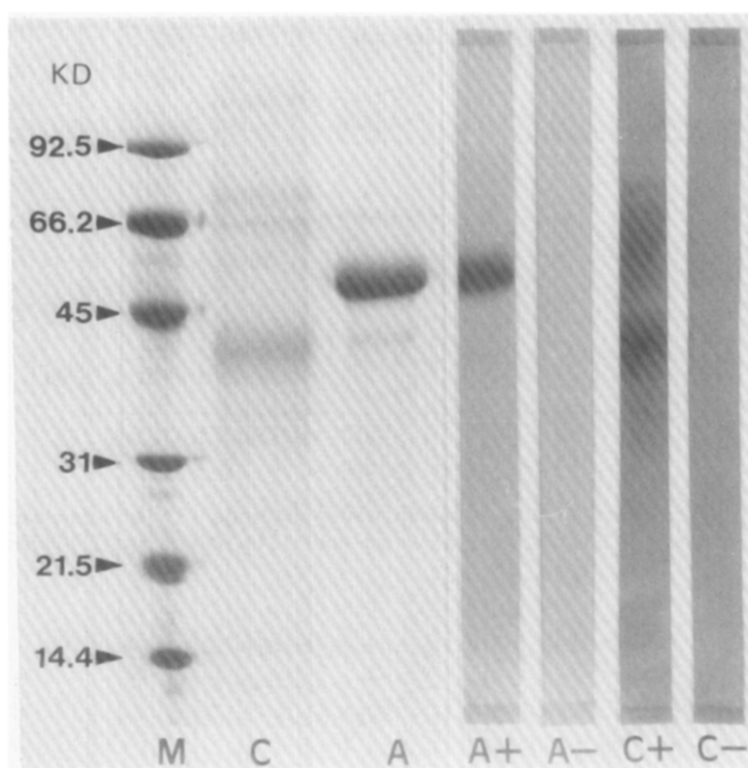


FIG. 3. SDS-PAGE of cellulase (C) and α -amylase (A) with molecular weight markers (M). Immunoblotting of allergen polypeptides in α -amylase (A+) and cellulase (C+) using a serum pool from the patients allergic to each enzyme. Negative control serum pool to α -amylase (A-) and cellulase (C-). 150 μ g of each enzyme was applied per gel and the radiostaining was achieved with 125,000 cpm per lane. The exposure duration was 72 hours.

3 (16 mg/ml) 1 year after they were removed from work.

After BPTs with α -amylase all patients gave a positive immediate response; this was followed by a late reaction in patient 1 (Fig. 4). After BPTs with cellulase, patients 1, 4, and 5 gave isolated immediate responses, and a dual reaction was observed in patient 2 (Fig. 5). BPTs with wheat flour resulted in immediate or dual responses in all patients; the exception was in patient 1 who showed no reaction (Fig. 4). Furthermore, BPTs with *Alternaria* and *Aspergillus* were carried out in patients 1 and 2, respectively, and no reaction was observed. The results of specific BPTs are summarized in Table V. In the control subjects PC₂₀ methacholine values were in a range of 0.15 to 3 mg/ml. However, in the specific BPTs with the maximum concentration (1:10 wt/vol) of both α -amylase and cellulase no falls higher than 10% in FEV₁ were observed.

DISCUSSION

Bakers' asthma is classically considered a form of allergic asthma mediated by IgE antibodies specific to cereal flour antigens.²¹ However, the list of caus-

ative agents of bakers' asthma has been expanded with the demonstration that flours from different sources (buckwheat,²² soybean¹⁰), and organic contaminants such as storage mites,²³ fungi,²⁴ and insects²⁵ are also capable of causing IgE-mediated occupational asthma. Furthermore, baking and food technology has notably progressed in the last decades, and a vast array of biologic and chemical additives are now commonly used. The *Aspergillus*-derived enzymes α -amylase and cellulase are some of the most important flour additives, which are extensively used to hasten and economize the breadmaking process.^{10, 11, 26} The actions of *Aspergillus*-derived enzymes used in breadmaking are summarized in Table VI (modified from Baur et al¹⁰).

In our patients a typical type I hypersensitivity reaction to fungal α -amylase and/or cellulase is strongly suggested by the results of skin testing, histamine release test, and specific IgE determination by REIA. Moreover, in the specific BPTs it was seen that controlled reexposure to α -amylase and/or cellulase induced a bronchospastic reaction with an immediate or dual response. The negativity of both the BPTs and the serologic tests with these enzymes in 10 nonex-

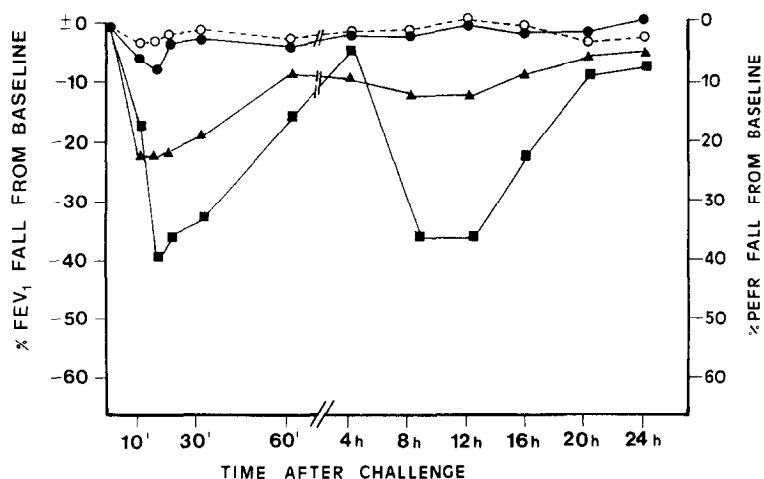


FIG. 4. Bronchial response patterns in patient 1 after challenge with (■) α -amylase 1:100,000 wt/vol, (▲) cellulase 1:1,000 wt/vol, (●) wheat flour 1:10 wt/vol, and (○) *Alternaria* 1:10 wt/vol.

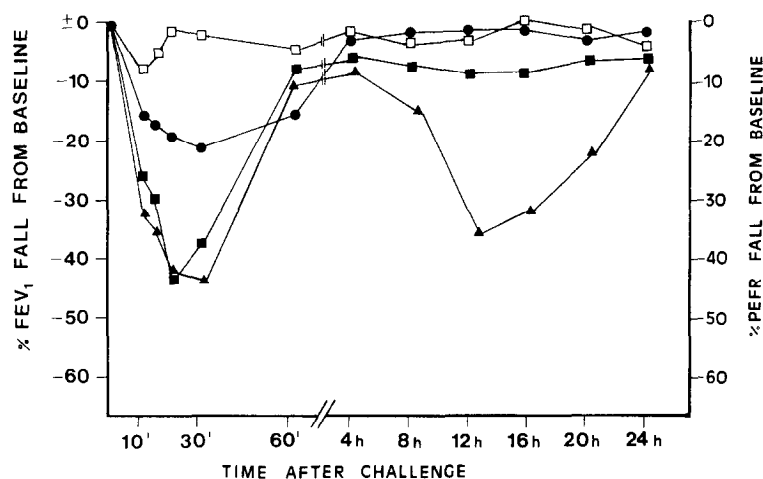


FIG. 5. Bronchial response patterns in patient 2 after challenge with (■) α -amylase 1:100,000 wt/vol, (▲) cellulase 1:400 wt/vol, (●) wheat flour 1:40 wt/vol, and (□) *Aspergillus* 1:10 wt/vol.

posed asthmatic patients support the specificity of these findings. Immunoblot analysis after SDS-PAGE by use of a serum pool from the patients allergic to each enzyme has further identified the IgE-binding components of α -amylase and cellulase. The radio-stained band of 52 kD observed in the α -amylase immunoblotting coincides with that described by Baur et al.¹¹ Alpha-amylase is composed of a group of ubiquitous endoglycosidases that hydrolyze 1,4-glucosidic linkages in polysaccharides containing three or more α -1,4-linked D-glucose units yielding a mixture of maltose and glucose. Discovery in 1884 by Takamine in the United States of the production of the α -amylase of *A. oryzae* by solid-state fermentation on wheat bran opened up the commercial use of en-

zymes in cereal technology, although major breakthrough in industrial enzymology took place first in the post-World War II period.²⁷ Alpha-amylase is added to flour to compensate for the low natural content of amylases of cereal flour and so enhance carbohydrate fermentation by yeast. The allergenic capacity of α -amylase was first documented by Flindt,²⁸ and since then other authors have pointed out its importance as an occupational inhalant allergen in pharmaceutical plant workers and in bakers' asthma.^{8, 10-13} It is interesting to note that it has also been shown that house dust mite-derived amylase is allergenic.²⁹

Cellulase refers to a group of enzymes that acting together hydrolyze cellulose to glucose. Although cel-

TABLE V. Results of specific bronchial challenge tests with concentrations (wt/vol) eliciting positive responses

Patient	1	2	3	4	5
α -amylase	Dual (1:100,000)	Immediate (1:100,000)	Immediate (1:2,000)	Immediate (1:40,000)	Immediate (1:1,000)
Cellulase	Immediate (1:1,000)	Dual (1:400)	ND	Immediate (1:10,000)	Immediate (1:1,000,000)
Wheat flour	Neg	Immediate (1:40)	Dual (1:10)	Immediate (1:10,000)	Immediate (1:10)
<i>Aspergillus</i>	ND	Neg	ND	ND	ND
<i>Alternaria</i>	Neg	ND	ND	ND	ND

lulases are distributed throughout the biosphere, they are most manifest in fungi and microbial sources. During the last few years it has become permissible in most countries to add fungal cellulase to white bread dough to break up roughage,²⁶ although cellulase has a long history of use in digestive aids and in the food industry.⁷ The first case of occupational asthma caused by cellulase was reported by Ransom and Schuster³⁰ in 1981 in a pathologist working in plant cloning experiments, and the first demonstration of occupational asthma caused by fungal cellulase confirmed by BPT was done by our group in two pharmaceutical workers in 1986.⁷ Recently Tarvainen et al.²⁶ have reported on four workers in biotechnical laboratories in whom allergic respiratory symptoms and contact urticaria caused by cellulase and/or xylanase (a type of hemicellulase) developed, but specific BPTs were not carried out. In all the aforementioned patients specific IgE antibodies to cellulase antigens were demonstrated by skin prick testing and serologic techniques. In the baking industry, Baur et al.¹⁰ have reported that 8% of symptomatic bakers have IgE sensitization to partially purified hemicellulase, which also had cellulase activity. Differentiation of primary sensitization to cellulase or hemicellulase can be difficult because a high degree of cross-reactivity may exist between both enzymes, as has been described for mold cellulase and xylanase by RAST-inhibition studies.²⁶

It is worth mentioning the coexistence of relevant sensitization to both α -amylase and cellulase in all our patients except one. This cannot be attributed to a cross-reaction between these enzymes, because no cross-reactivity has been found between α -amylase and cellulase in the REIA-inhibition assays. On the other hand, unlike in our previous report,⁸ we found in the present work that some degree of cross-reactivity exists between α -amylase and *A. oryzae*, suggesting that this enzyme or some common epitopes may be present in *A. oryzae* extract, and even in *A. niger* extract. The discrepancies observed between

TABLE VI. *Aspergillus*-derived enzymes used in the baking industry

α -amylase (<i>A. oryzae</i>)	Provides fermentable sugars for yeast, improves loaf volume and texture
Cellulase (<i>A. niger</i>)	Digests cellulose and breaks up roughage
Hemicellulase (<i>A. niger</i>)	Removes pentosans from bread, improves volume, controls dough rheology
Glucoamylase (<i>A. niger</i>)	Provides fermentable sugars for yeast

the present results and our previous work may be due to the great variability of fungal extracts and to the lack of standardized extracts. It also seems difficult to explain why wheat flour extract weakly inhibited the α -amylase REIA, unless the natural wheat amylases or fungal contaminants may have interacted with *Aspergillus* α -amylase specific IgE antibodies. The partial inhibition of the cellulase REIA by *A. niger* extract is in agreement with our previous report,⁷ and supports the idea that cellulase may be an allergenic component of this mold.

It should be noted that four of our patients were also sensitized to wheat flour, as were the patients reported by Baur et al.¹¹ However, patient 1 was not allergic to cereal flours and gave no response to the BPTs with cereals. The low RAST levels to wheat flour observed in this patient might well be due to her concomitant sensitization to grass pollen.

Atopy seems to play an important role in the development of IgE-sensitization to enzymes,^{3, 11} as is supported by the important allergy background and/or increased total IgE serum levels observed in our patients. The common existence of polysensitization found in our patients might also be due to the fact that the initial IgE-sensitization, either to cereal antigens or enzymes, could have facilitated further

atopic sensitizations to other allergens found in the work environment, as a consequence of the ongoing immunoinflammatory reaction in the airways. So a burst of respiratory allergies may occur in atopic sensitized bakers, complicating both the diagnosis and the prognosis of their occupational disease.

Because of the use of products containing enzymes in powder form, baking and food industry workers are at high risk of becoming allergic to them, and it is expected that as food technology progresses, new sources of sensitization will be identified. The general population may be exposed to these allergenic additives through other routes such as the ingestion of bread and bakery products. Although all of our patients tolerated the ingestion of bread without any symptoms, we have reported on a patient in whom systemic anaphylaxis after oral challenge with α -amylase developed.⁸

We conclude that α -amylase and cellulase behave as potential occupational allergens capable of sensitizing exposed bakers and giving rise to respiratory symptoms by an IgE-mediated mechanism. Consequently, we think that these enzymes must be routinely tested in the evaluation of symptomatic bakers, and it should be stressed that if immunotherapy with cereal allergens is considered,³¹ allergy to enzymes and other potential allergens in the baking industry has to be ruled out.

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