

Occupational asthma caused by cellulase

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*Inhalation of enzyme dusts can cause occupational asthma, and the list of enzymes able to induce asthma is increasing. We report two patients with asthma induced by occupational exposure to cellulase powder derived from *Aspergillus niger*. A type I hypersensitivity to this enzyme was demonstrated by means of skin test reactivity, positive passive transfer test, positive reverse enzyme immunoassay for specific IgE, and immediate bronchial provocation test response to cellulase dust. Skin tests with an *A. niger* extract were positive. Cross-reactivity between cellulase dust and an entire *A. niger* extract was also demonstrated. (J ALLERGY CLIN IMMUNOL 77:635-9, 1986.)*

Occupational asthma caused by inhalation of a variety of enzyme dusts has been documented. Dusts of papain,¹⁻³ trypsin,⁴ α -amylase,⁵ bromelain,⁶ derivatives of *Bacillus subtilis*,^{7,8} flaviastase,⁹ pectinase,¹⁰ and pepsin¹¹ have been found to cause occupational asthma. This article concerns two cases of occupational asthma caused by cellulase powder in which a type I IgE-mediated mechanism was demonstrated.

To the best of our knowledge, these are the first reported cases of occupational asthma caused by this enzyme.

CASE REPORTS

Case 1

The patient was a 35-year-old woman, smoker of 10 cigarettes per day, who had worked in the packing department of a pharmaceutical firm for the last 15 years where cellulase powder was used in the manufacture of digestive aids. This department, as well as the rest of the factory, had no system to quantitate the level of dust exposure.

Four years after employment, she began to experience sneezing, nasal itching, rhinorrhea, cough, wheezing, and shortness of breath on exposure to cellulase dust.

There was significant improvement in her respiratory symptoms on weekends and vacations and when she was not exposed to this dust while she was working. When the patient avoided exposure to cellulase dust, the respiratory symptoms were markedly decreased until she remained

Abbreviations used

PBS: Phosphate-buffered saline
REIA: Reverse enzyme immunoassay
DSCG: Disodium cromoglycate
BPT: Bronchial provocation test

asymptomatic. She had no personal or family history of other allergies.

The patient was admitted for study in the Centro Ramon y Cajal after 2 weeks away from the job, and she was not receiving any medication at this time. Clinical examination and chest x-ray films were normal. White blood cell count was 7700 cells per cubic millimeter with 615 eosinophils per milliliter. Total IgE was 900 IU/ml. FEV₁ was 94% of predicted.

Case 2

The patient was a 20-year-old woman, nonsmoker, who started working 4 years previously in the same department of the same pharmaceutical company as patient 1. One year after employment, she began to experience ocular and nasal itching, nasal congestion, sneezing, rhinorrhea, occasional cough, wheezing, and shortness of breath when she was exposed to cellulase dust. On weekends and vacations she demonstrated significant improvement in her respiratory symptoms. The patient remained asymptomatic after avoidance of cellulase dust. She had no other history of allergies or atopy in her family.

As the first patient, she was admitted for study after a period of 2 weeks away from the job and was not receiving any medication at the time. Clinical examinations were normal. White blood cell count was 10,400 with 416 eosinophils per cubic millimeter. Total IgE was 100 IU/ml. Chest x-ray film was normal. A spirometry revealed a FEV₁ of 96% predicted.

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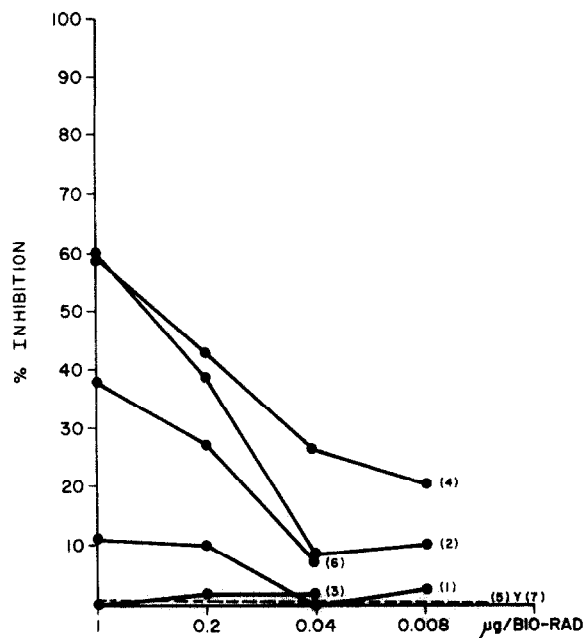


FIG. 1. REIA-inhibition performed coincubating labeled cellulase (1 µg per well) with (1) α-amylase, (2) cellulase (factory), (3) *M. racemosus*, (4) cellulase (Sigma Chemical Co.), (5) *C. herbarum*, (6) *A. niger*, and (7) *A. tenuis*.

MATERIAL AND METHODS

Two different forms of cellulase were used. The first form was factory obtained, and the second form was commercially obtained (S.E.P.I.M., Sees, France, lot No. C106525).

Two grams of each cellulase dust were dissolved in 20 ml of PBS, pH 7.3, at laboratory temperature. After stirring for 60 minutes and passing through filter paper, the solution was dialyzed in PBS for sterilization. This solution was considered a 10% w/v extract.

Skin prick tests

Skin prick tests were done with the antigenic preparations described above.

A battery of commercially available allergens including grass-pollens mix, dog dander, cat dander, *Dermatophagoides pteronyssinus* (Abelló Laboratory, Madrid, Spain), *A. fumigatus*, *A. niger*, *Mucor racemosus*, *Cladosporium herbarum*, and *Alternaria tenuis* (Hollister-Stier Laboratories, Spokane, Wash.) were also tested.

Fifteen exposed asymptomatic individuals, 15 atopic nonexposed subjects with seasonal allergic rhinitis and/or asthma, and 15 normal persons were also tested as control subjects.

Passive transfer test

A passive transfer test with factory cellulase and *A. niger* was done with the undiluted serum of patient 2 as previously described.¹² These tests were not done with the serum of patient 1 because she had a positive Australian antigen.

Precipitin tests

Patient sera and control sera were tested for precipitating antibodies against several dilutions of extracts of cellulase dusts, *A. niger* and *A. fumigatus*, by an agar gel diffusion method.¹³

Specific IgE determination

The determination of specific IgE antibodies was performed by use of a reverse immunoassay as previously described.^{12, 14}

One milligram of periodate oxidized peroxidase was mixed with 150 µl of both cellulases (3 mg/ml) and 100 µl of carbonate-bicarbonate buffer (1 mol/L, pH 9.5) and kept at 4°C for 18 hours. Thereafter, 2 mg of sodium borohydride was added, and the solution was dialyzed against PBS. After dialysis, 1% bovine serum albumin in PBS containing 25% glycerol was added to a final volume of 2 ml. This final solution was aliquoted and stored at 4°C.

REIA assay

Microplates (M 24 AR, Dynatech Laboratories, Inc., Alexandria, Va.) were coated with a monospecific antihuman IgE (Tago Laboratories, Burlingame, Calif.) as previously described.¹⁵ Serum, 0.05 ml, mixed with an equal volume of PBS-Tween, was incubated in each well at 4°C for 18 hours.

After several washes, 200 µl of a dilution of the allergen-enzyme conjugate made in PBS-Tween, 25% fetal calf serum, containing 1 µg of peroxidase, was added to each well and left shaking for 1 hour at room temperature. After 10 alternate washings 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 2 N H₂SO₄, 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, 0.03% H₂O₂).^{12, 14} Any result higher than the mean + 2 SD of eight exposed asymptomatic individuals was considered positive.

REIA inhibition

The specificity of the cellulase REIA was tested by a REIA-inhibition assay in the following manner: The most positive serum was incubated as described. Afterward, the same amount of the conjugate was mixed with increasing amounts of different allergens, added to the wells, and left shaking for 1 hour at room temperature. The allergens tested were both cellulases, *A. niger*, *A. tenuis*, *C. herbarum*, *M. racemosus* and α-amylase (Sigma Chemical Co., St. Louis, Mo.). After this step, the microplates were washed, and the substrate was added.

Bronchial provocation tests

Inhalation tests with factory cellulase were done during asymptomatic periods. Patients were not receiving any medication at the time of the study. Capsules, containing either 100 mg of lactose or 99.90 mg of lactose mixed with 0.1

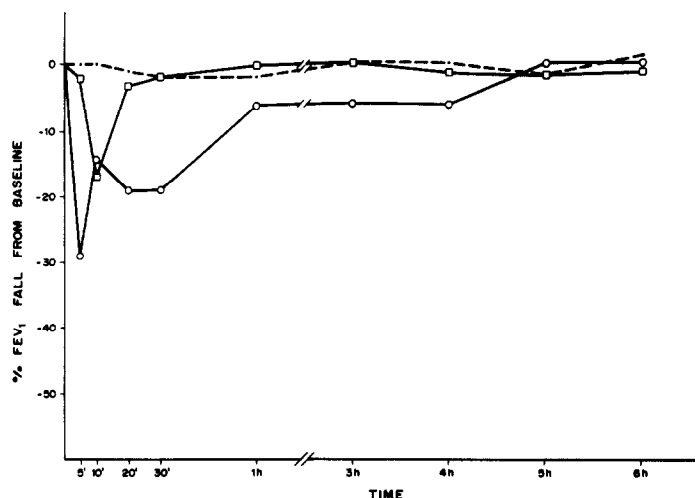


FIG. 2. Demonstration of an immediate asthmatic reaction after challenge with 0.5 mg of cellulase (patient 1). ○, cellulase, 0.5 mg; □, cellulase, 0.5 mg after 40 mg of cromolyn sodium inhalation; ● —, lactose.

mg of cellulase, and finally, 99.50 mg of lactose mixed with 0.5 mg of cellulase were prepared. Capsules were fitted into a hand-held patient-activated turboinhaler (Spinhaler, Fisons Corp., Bedford, Mass.). Challenges were started with 0.1 mg of cellulase, and if a negative response was obtained, challenges with 0.5 mg were done. FEV₁ was measured before antigen provocation, at intervals of 5 minutes after each inhalation for the first half hour, then at intervals of 1 hour to 8 hours, and again at 24 hours. Only one extract was used for provocation on any one day.

A positive response was defined as a decrease in FEV₁ $\geq 20\%$ after inhalation challenge. Challenges with cellulase were preceded by control inhalations with only 100 mg of lactose powder. Two unexposed patients with asthma, two normal subjects, and five exposed asymptomatic individuals were challenged with 0.50 mg of cellulase after obtaining informed consent.

The effect of DSCG on the inhalation reactions was also studied in the two patients. Forty milligrams of this medication was administered by inhalation by use of a Spinhaler 30 minutes before the provocation tests.

RESULTS

Skin tests

The patients exhibited an immediate positive reaction after skin prick testing with the factory cellulase and *A. niger* extracts. Control subjects did not have reactions to these antigens. This proves the nonexistence of unspecific reaction in spite of the concentration used. Neither the patients nor the control subjects had positive skin reactions to the remaining commercial common allergen battery, except for the atopic control subjects who had reactions to several grass pollens.

TABLE I. Specific cellulase IgE determination by REIA

Patient	Normal value
1	1.790
2	0.340
Eight exposed asymptomatic workers	0.112 $\pm$ 20
Blank	0.060 $\pm$ 10

Passive transfer test

The Prausnitz-Küstner test was positive, obtaining a 12 by 9 and 10 by 7 wheal with pseudopods response with cellulase and *A. niger*, respectively, in the recipient of the patient's serum. Control prick test in nonserum-injected site was negative.

Precipitin tests and REIA assay

Precipitins to different dilutions of cellulase and *Aspergillus* species were not formed in the patient's sera.

The two patients demonstrated positive cellulase-REIA values. In contrast, eight exposed asymptomatic workers had negative REIA values. Table I reflects the REIA results by use of peroxidase conjugated with the factory cellulase. The results of the REIA-inhibition assay are presented in Fig. 1.

Bronchial provocation tests

The results of challenge tests are presented in Figs. 2 and 3.

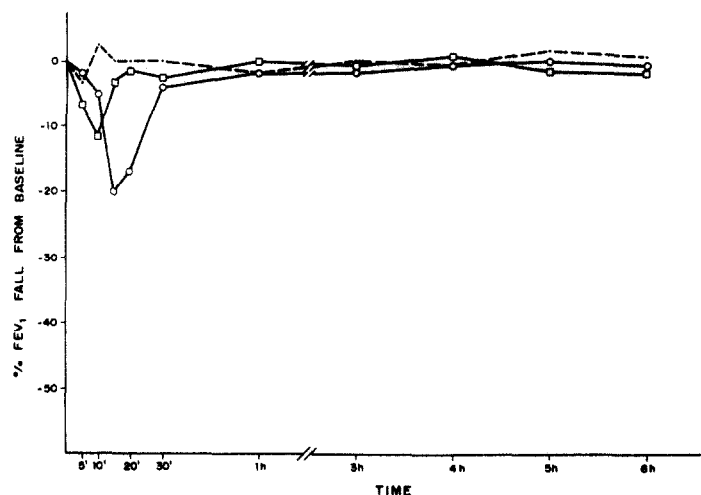


FIG. 3. Demonstration of an immediate response after provocation with 0.5 mg of cellulase (patient 2). ○, cellulase, 0.5 mg; □, cellulase, 0.5 mg preceded by the inhalation of cromolyn sodium, 40 mg; ● —, lactose.

No reaction was observed after challenges with 0.1 mg of factory cellulase. In contrast, challenges with 0.5 mg of factory cellulase caused an immediate response in both patients.

A drop in FEV₁ of 29% and 20% from baseline was observed in patients 1 and 2, respectively. No late reactions were observed. Premedication with DSCG partially modified the response in the patients. No reactions were observed in any of the control subjects.

DISCUSSION

Cellulase(s) is the trivial name used in reference to a group of enzymes that catalyze the degradation of cellulose to glucose. The systematic names are β -1,4-glucan glucanohydrolases, and they were obtained from a variety of molds, mainly *Trichoderma viride* and *A. niger* cultures.

Cellulase is used as an important member of combined digestive aids. Other current applications are related to the removal or softening of unwanted cellulase (e.g., in food preparations). They are useful in the isolation of soybean proteins, tea components, sweet potato or corn starch, agar-agar, citrus pulp, etc.

The clinical features of patients were highly suggestive of occupational asthma associated with exposure to cellulase dust. This relationship was suspected by the physician trained in allergy during the first visit because they elicited the history of improvement in symptoms when the patients were away from work.

A type I, IgE-mediated immunologic mechanism appeared to be operational in the patients' responses

to cellulase dust. Both positive skin tests and BPT performed on the two patients evinced an immediate response without late reaction. Furthermore, the presence of specific IgE in the patients' sera was demonstrated by Prausnitz-Küstner tests and REIA tests. In contrast, a control group of exposed asymptomatic individuals and atopic and normal persons did not respond to either the skin, REIA, or BPT tests. Moreover, the REIA-inhibition study confirmed the specificity of the assay for cellulase. Asthmatic reaction was provoked on BPT immediately and specifically by the inhalation of cellulase. The severity of the reaction was dose related and partially modified by pretreatment with DSCG. These findings are consistent with a classic IgE antibody mediated reaction.

The patients had positive immediate skin tests responses with *A. niger* extract. A cross-reactivity between the cellulase and *A. niger* extract was demonstrated in one patient by the REIA inhibition study. These findings strongly suggest that cellulases may be an important allergenic component of this mold. Further studies would be necessary to investigate the importance of mold-derived enzymes as antigens in mold allergy.

We conclude that cellulase, as an antigen-provoking asthma, is a health hazard to exposed workers even in nonatopic persons.

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REFERENCES

1. Ousgoog H: Atopic sensitivity to caroid (papain). *J ALLERGY* 16:245, 1945
2. Beecher W: Hyperesthetic rhinitis and asthma due to digestive ferments. *IMJ* 59:343, 1951
3. Milne J, Brand S: Occupational asthma after inhalation of dust of the proteolytic enzyme papain. *Br J Ind Med* 32:302, 1975
4. Zweiman B, Green G, Mayock RL, Hildreth EA: Inhalation sensitization to trypsin. *J ALLERGY* 39:11, 1966
5. Flindt MLH: Allergy to alpha-amylase and papain. *Lancet* 1:1407, 1979
6. Gallegillos F, Rodriguez JC: Asthma caused by bromelin inhalation. *Clin Allergy* 8:21, 1978
7. Flindt MLH: Pulmonary disease due to inhalation of derivatives of *Bacillus subtilis* containing proteolytic enzyme. *Lancet* 1:1177, 1969
8. Pepys J, Hargreave FE, Longbottom JL, Faux J: Allergic reaction of the lungs to enzymes of *Bacillus subtilis*. *Lancet* 1:1181, 1969
9. Pauwels R, Devos M, Callen L, Van der Straeten M: Respiratory hazards from proteolytic enzymes. *Lancet* 1:669, 1978
10. Hartman AL, Walter H, Wutrich B: Allergisches berufsasthma auf pektinase, ein pektolytisches enzym. *Schweiz Med Wochenschr* 113:265, 1983
11. Cartier A, Malo JL, Pineau RT, Dolovich J: Occupational asthma due to pepsin. *J ALLERGY CLIN IMMUNOL* 73:574, 1984
12. Hinojosa M, Moneo I, Dominguez J, Delgado E, Losada E, Alcover R: Asthma caused by African maple (*Triplochiton scleroxylon*) wood dust. *J ALLERGY CLIN IMMUNOL* 74:782, 1984
13. Ouchterlony O: Antigen antibody reactions in gels: types of reactions in coordinated system of diffusion. *Acta Pathol Microbiol Immunol Scand* 32:231, 1953
14. Dominguez J, Cuevas M, Moneo I, Ureña V, Ferreira A, Bootello A: Egg hypersensitivity as measured by RAST and a reverse enzyme immunoassay. *Allergy* 39:529, 1984
15. Moneo I, Cuevas M, Ureña V, Alcover R, Bootello I: Reverse immunoassay for the determination of *Dermatophagoides pteronyssinus* IgE antibodies. *Int Arch Appl Immunol* 71:285, 1983

Occupational asthma caused by eastern white cedar (*Thuja occidentalis*) with demonstration that plicatic acid is present in this wood dust and is the causal agent

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A worker developed symptoms of work-related asthma a few weeks after starting to work in a sawmill where eastern white cedar (Thuja occidentalis) was transformed into shingles. The diagnosis of occupational asthma was confirmed by (1) monitoring of peak expiratory flow rates and bronchial responsiveness to histamine off work and at work, and (2) specific inhalation challenges in the laboratory that demonstrated an isolated late asthmatic reaction after exposure for 4 hours to the wood dust. Specific inhalation challenges with western red cedar (Thuja plicata) for 2 hours and plicatic acid (PA) for 7 minutes also caused an isolated late asthmatic reaction. Elevated specific IgE levels to PA were present. Antiserum was produced in rabbits that were immunized with PA conjugated to human serum albumin. With this antiserum in inhibition experiments, cross-reactivity between western red cedar and eastern white cedar, both belonging to the family of arborvitae, was found. It is estimated that eastern white cedar contains approximately half the amount of PA present in western red cedar. (J ALLERGY CLIN IMMUNOL 77:639-45, 1986.)

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