

Allergy to proteases in medical laboratory technicians: A new occupational disease?

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Occupational respiratory allergy caused by exposure of workers to proteolytic enzymes derived from *Bacillus subtilis* and used in the production of detergents was first described in 1969.^{1,2} Beside respiratory symptoms, cutaneous reactions after direct contact with solubilized enzymes have been reported.³ In addition to detergent enzymes, proteolytic enzymes used in medicine (trypsin and chymotrypsin), pharmaceutical manufacturing (pepsin and pancreatin), and food processing (papain and bromelain; cellulase and xylanase) have highly allergenic properties.^{4,5} Interestingly, allergy against pulverized or solubilized proteolytic enzymes used for immunohistochemical or molecular biologic procedures have not been reported until now.

We report 2 cases of respiratory symptoms (rhinitis and asthma bronchiale), conjunctivitis, and localized urticaria after contact with pulverized proteolytic enzymes in laboratory technicians.

CASE REPORTS

Case A

A 51-year-old white woman, working as a technician in a pathology laboratory over the last 20 years, reported rhinitis, conjunctivitis, and localized urticaria after skin contact with enzyme powder. The inhalational contact with pulverized, lyophilized proteinase resulted in immediate rhinorrhea and a burning sensation in both eyes within seconds after exposure, whereas handling of proteinase solubilized in buffers did not induce symptoms. Symptoms occurred at work and improved on weekends and holidays. After 2 years, rhinitis occurred when handling some detergents in pulverized form. Her medical history was significant only for an IgA deficiency. A sibling had hay fever. After contact with pulverized proteinases ceased, no recurrences were observed.

Case B

A 47-year-old female laboratory technician, working for many years in pathology laboratories, reported escalated symptoms of asthma

Abbreviation used

SPT: Skin prick test

ma during work in the laboratory. At that time, the symptoms were considered as an increase of intrinsic asthma, especially because an allergy work-up did not show any positive reactions in routine skin prick tests (SPTs), and the patient had postinfectious asthma since adolescence. Allergic reactions after exposure to her pet (a cat) or other animals were denied. Severe asthma symptoms had been claimed especially after exposure to the proteolytic enzyme Pronase E in its lyophilized pulverized form during immunohistochemical procedures, whereas no symptoms occurred when the enzyme was handled in a solubilized form. In 1994, after the accidental breakage of a bottle containing pulverized Pronase E, the patient experienced prolonged status asthmaticus, which led to the final diagnosis of an allergy against pulverized Pronase E. After this, she used only prediluted enzyme, and her asthma symptoms have stabilized.

METHODS

Skin prick tests (SPTs) were performed with commercial extracts of routine inhalants in glycerol (Stallergènes Laboratoires) on the volar side of the forearm. For the SPT with occupational-related allergens, solubilized enzymes Pronase E from *Streptomyces griseus* (EC 3.4.24.4, Merck Co) and proteinase from *B subtilis* (P-8038, Sigma Co; dilution medium: Tris buffer) were used. Glycerol diluent served as a negative control, and histamine (10 mg/mL) served as a positive control. A wheal greater than 3 mm but less than 5 mm in diameter in the presence of a negative response to the glycerol control was scored as positive (+), a wheal greater than 5 mm but less than 10 mm was scored as ++, and a wheal greater than 10 mm was scored as +++. Six control patients who underwent SPTs with diluted Pronase E and proteinase did not have allergic reactions.

Specific IgE antibodies were determined by ImmunoCAP fluoroenzyme immunoassay (Pharmacia & Upjohn Diagnostics), according to the manufacturer's recommendations. Pronase and proteinase, from the same source as that used for the SPTs, were coupled to ImmunoCAP by the Special Allergen Service provided by Pharmacia & Upjohn Diagnostics AB.

RESULTS

The results of the skin tests, CAP immunoassays, and pulmonary function tests are listed in Table I. Positive SPT responses with detergent and other proteolytic enzymes, as well as the detection of anti-Pronase E-specific IgE in the serum, demonstrated an isolated dilution-

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J Allergy Clin Immunol 1999;104:700-1.

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0091-6749/99 \$8.00 + 0 1/54/99407

dependent immediate-type allergic reaction against Pronase E in patient B. In contrast, patient A showed a broader reactivity against proteinase, as well as the detergent enzymes maxatase and alkalase. Patient A had no evidence for antecedent atopy on the basis of a negative SPT screening test response and a normal total serum IgE level. In contrast, patient B exhibited a markedly elevated total IgE level and had a positive Phadiatop result (Sx1 multi-RAST; in vitro atopy screening against inhalational allergens), reflecting the atopic background indicated by positive SPT responses to cat and dog allergens. Pulmonary function tests confirmed the obstructive lung disease of patient B (Table I).

DISCUSSION

SPTs with solubilized enzymes derived from *S. griseus* (Pronase E) and *B. subtilis* (protease), respectively; the occurrence of respiratory symptoms during the skin test (in patient B); and the detection of serum IgE antibodies against Pronase E and proteinase prove an immediate-type allergy against these proteolytic enzymes. To our knowledge, serum IgE antibodies against these proteolytic enzymes have been demonstrated for the first time. The manifestations in our patients included respiratory symptoms (rhinitis and asthma) but also conjunctivitis and contact urticaria, as reported in other cases of allergy against proteolytic enzymes.¹⁻⁵ The remarkable delay between onset of symptoms and identification of the causative allergens may be due to the fact that only detergent enzymes are included in routine SPTs. Thus an allergy against other proteolytic enzymes may not be detected in cases without cross-reactivity toward detergent enzymes, as found in one of our patients (patient B). The allergic reaction with rhinitis also against detergents in pulverized forms, which was observed in patient A during the course of the disease, demonstrates an expansion of respiratory allergy against several proteolytic enzymes. This phenomenon may be due to cross-reactivity.

Because occupational allergies are known to occur at a higher incidence in distinct subgroups, especially in subjects with atopic diathesis and bronchial hyperreactivity, preoccupational tests to screen for predisposing factors can result in a lower incidence of occupationally induced allergic reactions. In the cases described here, only patient B had manifest asthma bronchiale before starting professional work, whereas patient A had a negative screening SPT response and normal values in functional lung tests. The incidence of allergic reactions against pulverized enzymes in laboratory technicians should be determined by larger studies, resulting in a rationale for whether screening in this occupational group is indicated. Besides this collective aspect, allergologists should be aware of the possibility of inhalational and cutaneous allergic reactions toward

TABLE I. Summary of the results of skin tests, IgE serum antibody measurements, and pulmonary function tests in 2 patients with allergy to proteolytic enzymes

	Patient A	Patient B
SPTs		
Routine inhalants (atopy screening)	Negative	Dog +, Cat +++
Detergent enzymes (maxatase)	++	Negative
Pronase E	Negative	+ (1:200,000) to +++ (1:2000)*
Proteinase	+ (1:200,000) to +++ (1:2000)	Negative
Serum IgE antibodies (CAP; kU/L)		
Total IgE	51.9	739
Sx1 (Phadiatop)	<0.35 (class 0)	13.3 (class 3)
Enzymes		
Proteinase	2.3 (class 2)	<0.35 (class 0)
Pronase E	<0.35 (class 0)	13.1 (class 3)
Maxatase	6.0 (class 3)	<0.35 (class 0)
Alkalase	6.3 (class 3)	<0.35 (class 0)
Savinase	0.43 (class 1)	<0.35 (class 0)
Other IgE		
Dog	ND	22.3 (class 4)
Cat	ND	0.37 (class 0)
Pax	<0.35 (class 0)	0.4 (class 1)
Formaldehyde	<0.35 (class 0)	0.49 (class 1)
Pulmonary function test		
FEV ₁ (mL)	3150 (normal, 2740)	1400 (normal, 2860)
FVC (mL)	3950 (normal, 3300)	2970 (normal, 3330)
FEV ₁ /FVC (%)	80	47 (normal, 86)
After salbutamol (%)	—	48
Bronchial provocation test (methacholine)	No hyperreactivity	ND

ND, Not done; FVC, forced vital capacity.

*During the skin tests, dyspnea and rhinorrhea occurred in patient B.

proteolytic enzymes in laboratory technicians. In addition, this allergy may represent a new occupational disease in technicians working in medical or research laboratories.

REFERENCES

1. Flindt MLH. Pulmonary disease due to inhalation of derivatives of *Bacillus subtilis* containing proteolytic enzyme. Lancet 1969;1:1177-81.
2. Wüthrich B, Ott F. Berufsasthma durch Proteasen in der Waschmittellindustrie. Schweiz Med Wochenschr 1969;99:1584-6.
3. Würsch TH, Wüthrich B. Anaphylaktische Reaktion nach Reibtest mit flüssiger Waschmittelpotease. Allergologie 1984;7:464-6.
4. Cartier A, Malo JL, Pineau L, Dolovich J. Occupational asthma due to pepsin. J Allergy Clin Immunol 1984;73:574-7.
5. Tarvainen K, Kanera L, Tupeasela O, Grenquist-Nordén B, Jolanki R, Estlandert T, et al. Allergy from cellulase and xylanase enzymes. Clin Exp Allergy 1991;21:609-15.