

Allergy from cellulase and xylanase enzymes

K. TARVAINEN, L. KANERVA, O. TUPASELA, B. GRENQUIST-NORDÉN,
R. JOLANKI, T. ESTLANDER and H. KESKINEN

Institute of Occupational Health, Topeliuksenkatu 41 aA, Helsinki, Finland

Summary

Modern biotechnical methods have enabled production of many new types of potentially allergenic proteins. Enzymes have long been known to be respiratory allergens, but relatively few cases of skin allergy have been reported. Here we describe four patients who developed occupational allergic respiratory symptoms, three with bronchial asthma and one with allergic rhinitis, caused by cellulase and/or xylanase enzymes. Each patient also had urticarial symptoms after skin contact with these enzymes. In addition, one of the patients had allergic contact dermatitis from cellulase, and one from xylanase. Allergic contact dermatitis was verified by positive patch tests with the enzymes, and the immediate allergy was revealed by skin prick tests, specific IgE determinations (RAST) and RAST-inhibition tests. All patients had positive RASTs to both cellulase and xylanase. In the RAST inhibition test 20 µl of cellulase brought about a 94% inhibition, indicating the specificity of the RAST. Xylanase (20 µl, 5% w/v) gave an 92% inhibition of cellulase RAST, indicating cross-reactivity between cellulase and xylanase. Three patients have been able to continue at their previous places of work, but at different worksites. One of the patients requires continuous medication for asthma and had to change her job because of persistent symptoms.

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Introduction

Enzymes are high molecular weight proteins, minute quantities of which catalyse specific chemical reactions. Enzymes have long been used as catalysts in processes of the chemical and foods industries [1]. Modern biotechnical methods have recently led to production of new types of enzymes suitable for a wide range of purposes. Cellulases are a group of enzymes that catalyse the degradation of cellulose to glucose [2]. Xylanases can break down a hemicellulose, xylan, which after cellulose is the most abundant renewable polysaccharide that occurs naturally [3]. Materials released from wood during pulping and pulp processing contain high quantities of xylan, an ecologically harmful waste if deposited in streams and rivers. Agricultural residues also contain xylan. Bioconversion of cellulose and xylan by cellulases and xylanases is beneficial to paper and pulp industry both economically and ecologically [3]. The aim of the biotechnical industry to produce energy from a renewable source has also

increased use of and research into cellulases and xylanases [3,4].

Inhalation of enzyme dusts can lead to respiratory allergy [5–13]. Enzymes have also caused occupational and non-occupational allergic and irritant contact dermatitis [7,14–19]. Few reports on allergic reactions caused by cellulases have been published [12,20], although cellulase has a long history of use in digestive aids and in the foods industry [12]. Here we report the cases of four patients who developed allergic respiratory and skin symptoms after occupational exposure to cellulase or xylanase. To the best of our knowledge, skin allergy induced by these enzymes has not previously been reported.

Patients and methods

Prick tests were performed with 18 common inhalant allergens (Allergologisk Laboratorium A/S, Copenhagen, Denmark). Cellulase and xylanase of mould origin were used for prick testing with enzymes. Enzymes without preservatives were supplied by the factory employing patient 1. Enzyme solution (0.2 mg/ml) was prepared in Coca solution (0.5% sodium chloride, 0.3%

Correspondence: Dr K. Tarvainen, Kovelipolku 12 B, 00430 Helsinki, Finland.

sodium hydrogen carbonate, 0.4% phenol), and 2.5 ml of that solution was passed through a Millex-GV filter containing a 0.22 µm membrane (Millipore Ltd, U.S.A.) into a sterile vial containing 2.5 ml glycerol. Histamine hydrochloride (10 mg/ml) served as the positive control. The reactions were scored as described previously [21,22]. Reactions 3 mm or larger in diameter and equal to or greater than half of the histamine weal were defined as positive if the test with the diluent was negative. Twenty non-exposed control subjects were negative when tested with the enzymes.

Patch tests

Patch tests were performed using the Finn Chamber technique, the duration of occlusion on the upper back being 48 hr. The tests were read on removal and 24–48 hr after removal (three readings), and scored as described previously [23], 2+ to 4+ being considered allergic reactions. In addition to the European standard series (Chemotechnique Diagnostics A/S, Sweden), xylanase and cellulase were tested in a dilution series (3.3%, 1%, 0.33%) in petrolatum and aqua. As a control, 20 non-exposed subjects (10 atopics) were patch tested with a concentration of 3.3% w/v in petrolatum, with negative results.

Specific IgE determinations (RAST)

IgE specific to enzymes. Five grams of enzyme powder were dissolved in 100 ml of 0.01 M phosphate buffered (pH 7.4) saline (0.15 M NaCl). The powder solutions were allowed to stand overnight at room temperature, and were then centrifuged. The cellulase solution was diluted to 1:10 000 and the xylanase solution to 1:500 before coupling with cyanogen bromide-activated paper discs, using the method of Ceska *et al.* [24]. One hundred microlitres of diluted supernatant was used per disc. The other reagents for the specific IgE determinations (RAST) were obtained from the Phadezym RAST® kits (Pharmacia Diagnostics A/S, Sweden). The results, in PRU/ml, were based on the RAST reference serum of Pharmacia. RAST inhibition experiments were carried out as described by Yman *et al.* [25]. In order to measure the inhibitory capacity of the enzyme solutions, serial phosphate buffered saline dilutions (0.002, 0.02, 0.2, 2 and 20 µl) of the solutions were allowed to react with serum from patient 1 and patient 2 (20 µl of enzyme dilution + 50 µl of serum) overnight at + 4°C. These mixtures were then used for RAST determination. PBS (20 µl) in 50 µl of serum was used to obtain the inhibited results. The degree of reduction of the serum RAST values after absorption was expressed as per cent of inhibition.

Lung function and provocation tests

The spirometers were recorded with a Medikro 101 spirometer (Medikro Oy, Finland). Rimiterol hydrobromide 0.2 mg (three puffs) was used in the bronchodilation test. An increase of at least 15%, and 150 ml in peak expiratory flow rate (PEFR), was regarded as significant. Bronchial hyperreactivity was assessed with a histamine challenge test described by Laitinen [26], using an automatic dosimetric inhaler.

Case reports and results

The patients' main characteristics are shown in Table 1.

Patient 1

Patient 1 had handled amylase enzymes in a biotechnical laboratory for 8 years, with no symptoms. After 1 year of working in a factory producing xylanase and cellulase, she developed watery eyes, rhinorrhoea, hoarseness, cough and dyspnoea. The symptoms disappeared during holidays. Two years later she developed an asthmatic reaction, itching and redness on uncovered skin, mainly on her face and lower arms, while she weighed the xylanase. Investigations were carried out 5 months after the last acute episode. The prick test with xylanase was positive. Three hours after the prick test, the patient developed an acute attack of asthma. A chest radiograph was normal. She had moderate hyperreactivity in the histamine challenge test. Spirometry was normal, and the bronchodilation test was negative. Also the exercise test for exercise-induced asthma was negative. The diffusion capacity was normal. The PEFR of the control day ranged from 400 to 330 (l/min), and bronchial placebo provocation test with lactose powder gave a PEFR range of 395–350 (l/min). A bronchial provocation test with enzymes was considered contraindicated because of strong local and systemic reactions to enzyme in skin tests. A two-week PEFR follow-up at the place of work and at home was performed instead. During that period she had only two immediate PEFR falls of 48% and 50%, the first upon visiting a room where another person was weighing enzyme powder and the other when she entered a room where powdered enzyme had been handled the day before. Occupational bronchial asthma due to xylanase enzyme was considered as proved on the basis of the exposure data, the clinical picture, positive specific skin and RAST tests and the positive workplace provocation test combined with a negative placebo provocation test. The patient had to change jobs, and has been on permanent medication for asthma since then.

Table 1. Characteristics and test results of four patients allergic to enzymes

	1	2	3	4
Age (years)/sex	49/F	38/F	25/M	29/F
Occupation	Lab. Assistant	Lab. Assistant	Process Worker	Lab. Assistant
Enzymes exposed to	Xylanase Cellulase Amylase	Cellulase	Cellulase	Xylanase Cellulase
Duration of Exposure (years)	4	2	2	0.5
Causative enzyme	Xylanase	Cellulase	Cellulase	Xylanase
Symptoms	Asthma Rhinitis Urticaria	Rhinitis Urticaria Dermatitis	Asthma Urticaria	Asthma Urticaria Dermatitis
Prick Tests:				
cellulase	0.01% neg	0.001%; 3+	0.01%, 3+	0.001%; 2+
xylanase	0.01%; 3+	0.001%; 3+	n.d.	0.001%; 3+
Own/family atopy	-/+	+/+	+/+	-/+
Total IgE (kU/l)	72	4500	1000	110
Patch tests:				
cellulase	n.d.	3.3%; 3+ 1%; 3+ 0.33%; 3+	n.d.	Neg
xylanase	n.d.	Neg	n.d.	3.3%; 3+ 1%; 3+ 0.33%; 2+
standard series	Neg	Neg	Neg	Neg
Specific IgE (Normal below 0.35 PRU/ml):				
cellulase (PRU/ml)	6.2	1200	175	3.6
xylanase (PRU/ml)	6.5	490	68	1.4

n.d., not done; Neg, negative; PRU, Phadebas RAST Units.

Patient 2

Patient 2 had had atopic dermatitis as a child, and perennial rhinitis since 1971. Before her present job she had worked in a biotechnical factory manufacturing yeasts for 18 years. Since 1987 she has been exposed periodically to small (10 g) amounts of cellulase powder while weighing the powder probes in the same factory. Since 1988 she has handled enzymes only in liquid form. Soon after she began working with cellulase, she contracted a stuffy nose, and continuing rhinitis developed later. She also experienced itching on the face and the back of the hands during workdays. In 1989 she noticed that liquid enzyme cellulase caused weal-and-flare reactions if it came into contact with her skin. On examinations performed in 1989, subacute patchy eczema was observed on the dorsal sides of her hands after 1 month of

sick leave. Prick tests with cellulase and xylanase were positive. A patch test with cellulase was strongly positive both at the 48 hr and 96 hr reading. X-ray and the pulmonary function tests were normal. No significant fall in PEFR occurred during a workplace provocation test. She is now working in an office, and her rhinorrhoea is less severe.

Patient 3

Patient 3 had developed hives from contact with animal dander as a child, but had not had other atopic symptoms. He was employed in a factory producing enzymes, where he operated a closed enzyme fermentor. During the end process, enzyme mass passes over the drums for filtration, and fine enzyme dust is dispersed into the working

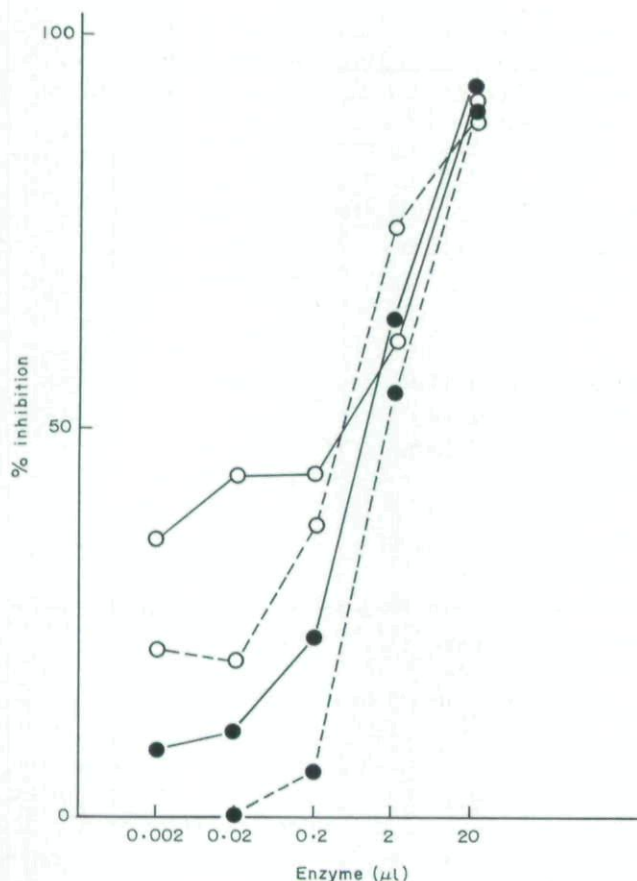


Fig. 1. Cellulase enzyme RAST-inhibition when tested with different enzyme concentrations. Patient 1 (—), patient 2 (—), inhibition when tested with cellulase (●), inhibition when tested with xylanase (○).

environment. After 2 years of working there, he noticed that itching hives appeared on the face, neck and upper arms during the cellulase enzyme filtration process. Six months later he also developed a cough, and he finally got bronchial asthma as well as generalized urticaria after exposure to cellulase dust. On examination he had no skin symptoms. A prick test with cellulase was positive. He had normal spirometric values, and the histamine challenge test was negative. The PEFR of the control day ranged from 450 to 490 (l/min), being 550–525 (l/min) after lactose placebo provocation. During a 2-week PEFR follow-up at work and at home, the PEFR ranged from 620 to 590 (l/min) except once when liquid cellulase enzyme was being processed. He then developed a cough and a 17% fall (615–510 l/min) in PEFR occurred. An occupational asthmatic reaction due to cellulase was considered proved on the basis of the exposure data, the patient's symptoms, his specific IgE to cellulase in skin and RAST tests and the negative placebo and positive

workplace provocation tests. He is still working in the same factory, but is granted sick leave on days when cellulase is processed. He is now symptomless.

Patient 4

Patient 4 was from the same workplace as patient 1. She handled cellulase and xylanase powders when testing the activities of these enzymes. She also pipetted liquid enzymes. Several laboratory assistants handled enzymes in the same small laboratory and enzyme dust was found on the working tables. After 6 months of working in this laboratory, she developed a cough and hoarseness as well as itching eyes. The symptoms usually developed 3–4 hr after exposure. She had no symptoms during holidays. An itching dermatitis first developed on areas of the hand that came into contact with the pipette, and developed later on the right cheek and upper eyelids as well. On examination, eczema was found on the dorsum of the right hand, between the thumb and the forefinger. Prick tests with cellulase and xylanase were positive. Three hours after prick tests a 9% fall in PEFR (400–365 l/min) was recorded. At that time she also had a frontal headache, a cough and hoarseness. A patch test with xylanase was positive both at the 48 hr and the 96 hr reading. The patch test reactions were slightly stronger in petrolatum than in aqua, and slightly urticarial at 48 hr when the occlusion was removed. She had normal spirometric values, and mild bronchial hyperreactivity was found in the histamine challenge test. The PEFR of control day varied 390–350 (l/min). After lactose placebo provocation a range of 405–365 (l/min) was recorded. Workplace provocation was performed twice after the work had been reorganized and the laboratories cleaned; the results were negative. She is now working in a different laboratory of the same factory, where she is no longer exposed to enzymes, and is symptomless.

Immunologic tests (Table 1)

All patients had positive RASTs to both cellulase and xylanase. In the RAST inhibition test, 20 μl of cellulase brought a 94% inhibition. Xylanase (20 μl, 5% w/v) gave a 92% inhibition of cellulase RAST for patient no. 2, indicating cross-reactivity between cellulase and xylanase (Fig. 1).

Discussion

Enzyme-induced skin reactions are mostly of irritant type [7,14–17,27]. A few case reports of skin allergy have been published. Workers handling papain, a proteolytic enzyme, have developed contact urticaria [9]. Contact

lens cleansing solution containing papain has caused contact urticaria of the lids [19,28]. Our patients no. 2 and 4 had both immediate (type I) and delayed (type IV) allergy to cellulase and xylanase enzymes, verified by skin prick and patch tests. Although it is clinically impossible to differentiate protein contact dermatitis caused by type I allergy reaction from delayed type IV allergy reaction, the positive patch test reactions confirmed the delayed type allergy in patients 2 and 4. Enzymes can cause different types of allergy (5–12, 18–20). Type I, type III and type IV allergy caused by alpha-amylase used as a dough additive has been described in a baker [13]. None of our patients had signs of allergic alveolitis (type III allergy). Commercial enzymes are crude products, which may contain remnants from the growth material, the micro-organism used for fermentation and preservatives in addition to the main enzyme [1]. For skin testing we used enzymes without preservatives. It is not yet known whether one or more allergens are responsible for the different types of allergy caused by cellulase and xylanase. There is increasing evidence that proteins may cause type IV allergic reactions [22].

All patients had noticed urticarial skin symptoms when in close contact with enzymes. The skin rash of patients 1 and 3 was triggered by airborne contact with enzymes, and patients 2 and 4 developed urticarial hives on their hands when handling enzyme powder or liquid. All the patients had positive immediate skin allergy tests. Airborne irritant dermatitis from proteolytic enzyme has previously been reported to occur in the enzyme detergent industry [7]. It is suggested that cellulase and xylanase be added to the list of the airborne skin contact allergens [29]. Cellulase has been classified as a mild skin irritant [30]. All skin prick and patch tests made on 20 non-exposed controls were negative. The control group included 10 atopics with atopic eczema or rhinitis.

The RAST inhibition test indicated cross-reaction between cellulase and xylanase. This may be a real cross-reaction of the type previously described between papain and pineapple derived bromelain [8]. Three patients (1, 2 and 4) showed positive immediate skin test reactions to both enzymes. Patient 2 had never handled xylanase. Theoretically patient 2 could have become hypersensitive to xylanase through the small traces of xylanase present in cellulase powders [3], but her positive reaction to xylanase was probably caused by a cross-reaction between cellulase and xylanase.

Three patients (1, 3 and 4) had occupational bronchial asthma. The diagnosis was supported by the patients' symptoms, bronchial hyperreactivity in the histamine challenge test [26] and a fall in PEFV-values during exposure at the workplace. Specific bronchial provocation tests with enzymes were not performed because the

high values of specific IgE determined by RAST and RAST inhibition tests were considered sufficient for the diagnosis of occupational disease needed for entitlement to compensation.

Skin prick tests are usually safe, and generalized reactions are infrequent [22]. Prick tests have long been used to diagnose type I allergy to enzymes [31]. However, a generalized reaction after testing with a cellulase enzyme has been described [20]. This is in accordance with our observations: patient 1 developed an acute asthma attack 3 hr after the prick test with xylanase and cellulase. Patient 4 had a 9% (400–365 l/min) fall in FEFR 3 hr after a prick test with the same enzymes, in addition to general symptoms. Prick tests with enzymes have frequently caused a prolonged dual-phase reaction lasting for 24–48 hr or even longer [31]. None of our patients had corresponding reactions.

Persons occupationally exposed during manufacture and individuals who use products containing enzymes in powder form might be expected to be potentially at risk of allergy to enzymes [27,32]. Enzymes are usually found in small amounts in the final product, e.g. the enzyme concentration in detergents is only from 0.1 to 1% [33], but sensitized individuals may develop symptoms even from minute traces of enzyme. Patient 1 got an asthmatic attack when talking with a colleague who worked with cellulase and whose clothes and hair may have been contaminated with enzyme. Anaphylaxis has followed the ingestion of papain [34], the use of chymotrypsin for treatment of epididymitis [35], and the use of chymopain for treatment of herniated lumbar discs [36]. Papain and bromelain, proteolytic enzymes, have provoked hypersensitivity reactions both when ingested and when inhaled [37].

Only some of the people who have allergic contact dermatitis develop reactions after ingesting the allergen [38]. Therefore patients with allergic contact dermatitis may be able to eat products containing the allergen. Since 1989 it has been permissible in Finland to add cellulase to white bread dough, to break up roughage. All our patients had eaten white bread without any symptoms. They had not taken any digestive tablets containing cellulase [12].

Cellulase and xylanase are new agents to be remembered in skin allergology. They are potential hazards in the new enzyme biotechnology industry and workers should be informed of the potential risks and how to avoid them, before starting to handle enzymes. Atopics probably have a greater risk of developing allergy to enzymes [39]. The safe handling procedures for microbial preparations proposed by the Association of Microbial Enzyme Preparation (AMFEP) [40] should include technically closed systems or fume hoods, minimization of

exposure by means of protective utensils and careful handling that avoids direct skin contact. Personal cleanliness is of the utmost importance. As most enzymes are soluble in water, first aid should include washing with abundant amounts of water [7,40]. Patients should be followed carefully and for some time after accidental exposure, because respiratory symptoms may develop up to 3–6 hr after exposure [31].

Part of the observations of the present report has been presented in a short communication [41] and a conference proceeding [42].

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