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CONTACT DERMATITIS

Lactase-induced occupational protein contact dermatitis and allergic rhinoconjunctivitis

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Enzymes are high-molecular-weight proteins and highly sensitizing occupational allergens used widely in industrial processes. Lactase has been described to cause work-related respiratory and conjunctival immunoglobulin (Ig)-E-mediated sensitizations in workers in the pharmaceutical industry. In these previous reports, allergic rhinoconjunctivitis or asthma was confirmed with prick tests but not by challenge tests. Lactase previously has not been described as a cause of immediate or delayed contact skin reaction. Furthermore, there are no previous reports of lactase-specific IgE. We report a case of protein contact dermatitis and allergic rhinoconjunctivitis from occupational exposure to lactase in a pharmaceutical worker. The patient exhibited strong positive responses to lactase in prick tests. In an open application test, lactase elicited whealing, and in patch testing, lactase elicited an eczematous reaction. Serum lactase-specific IgE antibodies were demonstrated in immunospot and radioallergosorbent test assays, and lactase-IgE-binding fractions and their specificities were examined in immunoblot and immunoblot inhibition assays. The chamber challenge test was performed to detect the association between lactase sensitization and rhinoconjunctival symptoms. Our results have confirmed the previous observations that lactase can induce occupational IgE-mediated respiratory and conjunctival sensitizations, but they show that contact skin reactions caused by lactase may also occur.

Key words: allergic rhinoconjunctivitis; enzyme allergy; immunospot; lactase allergy; occupational; protein contact dermatitis. © Blackwell Munksgaard, 2007.

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Background

Lactase is a disaccharidase enzyme that hydrolyzes non-absorbable milk sugar lactose into monosaccharides glucose and galactose that are readily absorbed by the intestine. People with lactose intolerance can consume dairy products if they take commercial supplemental lactase enzyme products at the same time. Industrial enzymes are produced by bacterial or fungal fermentation for several purposes, and they are used extensively in the chemical, food, and pharmaceutical industries (1, 2). Microbial enzymes are considered as potent allergens that can sensitize a large number of the exposed individuals (1–4). The greatest risk is associated with work where the enzyme is processed in a powdered form (3, 4). Powdered allergens may induce cutaneous or respiratory symptoms by both direct contact and through airborne exposure. Although immunoglobulin (Ig)-E-mediated occupational sensitization to microbial enzymes has been well documented, as far as we are aware, there are only 2 previous reports of occupational lactase sensitization. Based on positive prick test results, lactase has been reported to cause occupational IgE-mediated rhinitis, conjunctivitis, and asthma especially in atopic pharmaceutical workers formulating and packaging supplemental lactase enzyme products (5, 6). Furthermore, there is 1 case report of allergic symptoms after the ingestion of supplemental lactase enzyme pills (7). However, there are no previous reports on the presence of lactase-specific IgE antibodies or lactase-induced contact skin reactions. In this article, we report an occupational lactase-related protein contact dermatitis (PCD) and allergic rhinoconjunctivitis in a pharmaceutical worker.

Case Report

The patient is a 31-year-old female pharmaceutical worker, whose history was negative for atopic

diseases and lactose intolerance. She was working as a packer in a pharmaceutical plant, where supplementary lactase enzyme capsules were manufactured for 1- to 3-week periods a couple of times each year. The raw material was powdered lactase that had been derived from *Aspergillus oryzae*. The patient had been working for about 1 year when she began to experience rhinoconjunctivitis episodes, throat irritation, cough, and itching of the skin during her work. Later, urticarial wheals and eczematous eruptions also manifested on her hands, arms, and face. The patient connected her symptoms with exposure to the dust of powdered lactase released in her work environment. Her symptoms disappeared gradually when the lactase capsule production period ended. On vacations, she experienced no symptoms.

Methods

Skin tests

The prick testing was performed with lactase enzyme raw material at concentrations of 0.1% and 1% in an aqueous solution, with the contents of the supplemental lactase capsule as is, and with the additives of the capsule contents. Furthermore, prick test was also conducted with *Aspergillus fumigatus*, 13 common environmental airborne allergens including pollens, pets, and house dust mites, and 3 storage mites (ALK-Abelló A/S, Hørsholm, Denmark) as well as latex (Alyostal, Stallergenes SA, Antony Cedex, France). The prick tests were performed and interpreted according to the recommendations of the European Academy of Allergology and Clinical Immunology (8, 9). Histamine hydrochloride (10 mg/ml) and allergen diluent were used as positive and negative controls, respectively. A wheal diameter of at least 3 mm and 50% of the histamine control was considered positive when there was no reaction with the negative control.

Lactase enzyme raw material was patch tested at concentrations of 0.3% and 1% in petrolatum (pet.), and the contents of a lactase capsule were patch tested at a concentration of 30% in pet. Furthermore, the additives of the lactase capsule contents, standard and preservative series, and also protective gloves were tested. Patch testing was performed and scored according to the guidelines and criteria of the International Contact Dermatitis Research Group (9). The occlusion time was 2 days (D), and reading was performed when the patches were removed (diameter 10 mm; Finn Chambers® on Scanpor, Epitest Ltd Oy, Tuusula, Finland) and at D5. Patch test substances in standard and preservative series were purchased from Hermal (Reinbeck/Hamburg, Germany) and Chemotechnique Diagnostics AB (Malmö, Sweden).

Twenty consecutive non-exposed subjects, 11 women and 9 men, aged 23–75 years, and examined for contact dermatitis, served as a control group for lactase prick test and patch testing. Among them, there were 9 atopic persons.

The open application test (OAT) was performed on the volar forearm of the patient with the contents of the lactase capsule as is. The spreading of the capsule contents was limited to a 5- × 5-cm area, and no occlusion was used. The test was read after 20 min.

In vitro tests

In addition to serum total IgE, laboratory tests included in-house immunospot and radioallergosorbent test (RAST) assays that were used to demonstrate serum IgE antibodies specific to the contents of a lactase capsule (10). In the immunospot assay, IgE antibodies were also evaluated to a mould skin test extract, *A. fumigatus* (1000 BU/ml; Allergopharma, Reinbek, Germany). The IgE-binding fractions of lactase extract and the specificity of the binding were evaluated in immunoblot and immunoblot inhibition studies as previously described (11). In immunoblotting, lactase proteins separated in reduced 10% and 15% sodium dodecyl sulfate–polyacrylamide gel electrophoresis were probed with the patient's serum (1:2 v/v). In immunoblot inhibition, the patient's serum was preincubated with a lactase extract and, as a control, with a mould (*A. fumigatus*) skin test extract. Autoradiography with iodine-125-labelled anti-human IgE antibody was used for detection.

Chamber challenge test

In a single-blinded chamber challenge test (CCT), the patient handled test powders in an air-conditioned chamber. On the D1, she was challenged with the negative control, which for powdered agents is lactose, and on D2 with lactase powder that had been mixed with placebo powder in the ratio of 1:10. Symptoms and signs were observed and recorded for the following 60 min. The interpretation of the challenge test was based on (i) clinical findings (nasal hypersecretion, nasal mucosal oedema, and conjunctival irritation) and symptoms (nasal obstruction, sneezing, and nasal and conjunctival itching) and (ii) minimum cross-sectional area changes in acoustic rhinometry. The test was considered positive for rhinitis if hypersecretion (weighed) and at least 1 of the other objective findings or symptoms were present (12). For a positive conjunctival reaction, at least

local redness of conjunctiva (score 1 in scale 0–3) was required.

Lung function tests

For assessment of lung function, flow volume spirometry was performed according to the criteria of the European Respiratory Society (13). In addition, a 2-week follow up of peak flow measurements was carried out at home but not at the workplace, as the patient had terminated her employment in the pharmaceutical plant.

Results

In the skin tests, prick tests both with tested lactase extracts and with the contents of supplemental lactase capsule were positive. The 0.1% and 1% lactase extracts resulted in positive wheals, sized 10 × 8 mm and 13 × 11 mm, respectively. The contents of the capsule resulted in a 8- × 7-mm wheal (Fig. 1). Histamine hydrochloride elicited a 5-mm wheal, whereas allergen diluent caused no wheal. Furthermore, prick tests with other tested allergens were negative. Patch testing both with lactase enzyme concentrations and with the contents of lactase capsule as well as with nickel sulfate led to a slight non-vesicular eczematous reaction (+). In OAT, the contents of the lactase capsule elicited whealing within 20 min (Fig. 2). In all the controls, the results of prick test and patch testing were negative.

Serum total IgE was elevated (155 kU/l, reference value below 110 kU/l). Lactase-specific IgE antibodies were demonstrated in RAST and immunospot assays. In the RAST assay, we noted a 20% binding of the total activity from the patient's serum as compared with 0% and 1% binding from the non-atopic and atopic control sera, respectively. The immunospot assay also showed a distinct IgE binding to the lactase extract but no binding to the mould extract (Fig. 3). In immunoblotting studies, IgE-binding bands were demonstrated with the lactase extract at approximately 20 000, 88 000, 110 000, and >206 000 MW, and these were inhibited by

Fig. 1. Prick tests with lactase extracts resulted in large positive wheals with pseudopodia.

Fig. 2. In open application test, the patient developed several wheals.

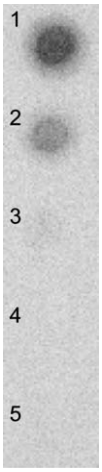

Fig. 3. Immunoglobulin E binding to lactase and a mould skin test preparation in immunospot. (1–4) lactase extract (1:5 w/v) and three 10-fold dilutions; (5) *Aspergillus fumigatus* skin test extract.

addition of the lactase extract but not with the control mould extract (Fig. 4).

In the CCT, placebo caused no reactions, whereas lactase powder induced within 10 min profuse rhinorrhoea and subjective and objective nasal obstruction, documented also in acoustic rhinometry. Eye reaction was visible as redness of the entire conjunctiva (score 2). These findings clearly fulfilled the criteria for positive reactions.

Both baseline flow volume spirometry and the 2-week follow up of home peak flow measurements provided normal results. At baseline, forced vital capacity was 4.21 l (99% predicted), and forced expiratory flow in 1 second was 3.30 l (90% predicted).

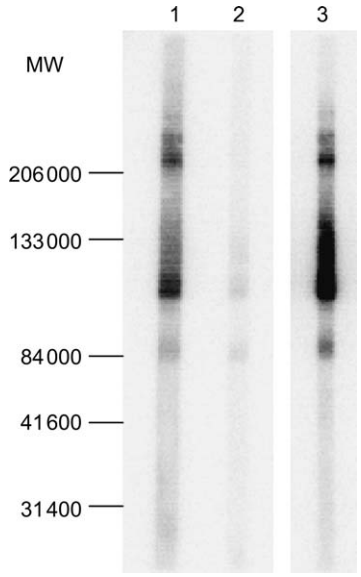

Fig. 4. Immunoglobulin E from the patient's serum binding to lactase proteins in immunoblotting after reduced sodium dodecyl sulfate–polyacrylamide gel electrophoresis (lane 1). The binding was inhibited by preincubation of the serum with lactase extract (lane 2) but not with the mould skin test extract *(Aspergillus fumigatus*) (lane 3). Molecular weight markers appear on the left.

Discussion

Occupational protein-containing allergens are by far the most important causes of PCD, which presents as typical eczema and is often accompanied by flares of urticarial or vesicular eruptions shortly after contact with the causative material (14). PCD occurs in both atopic and non-atopic individuals, but an atopic constitution does seem to be a predisposing factor (14). However, the pathogenesis of PCD is not yet completely understood. PCD has been claimed to represent an immediate (type 1) hypersensitivity reaction or a mixed situation including both immediate and delayed (type 4) hypersensitivity reactions to allergens (14, 15). In most cases, patch tests are negative, but prick tests with the responsible allergens are positive, and occasionally, specific IgE antibodies can be detected in the blood (14). It is also possible that immediate allergy may lead to an eczematous patch test reaction in which the cell-mediated delayed type 4 allergy is not involved (16, 17). In some cases, eczema may have an irritant origin or can result from a delayed-type allergic reaction caused by additives, e.g. preservatives in the test material (14, 18).

The case described here provides the first clear evidence that lactase dust can induce also contact skin reactions in addition to provoking respiratory and conjunctival symptoms. Our patient presented with eczema and whealing not only in the hands but also in the uncovered skin areas on the face and forearms, which fits well with airborne contact. The skin symptoms can be classified as PCD from lactase, as all the criteria for that diagnosis were fulfilled. IgE-mediated immediate hypersensitivity to lactase was confirmed by demonstrating lactase-specific IgE in *in vitro* tests as well as a positive prick test and a whealing reaction in OAT.

Patch testing elicited a weak eczematous reaction with each lactase-containing test material. These reactions were interpreted as being allergic on the basis of their clinical appearance and because they were still evident on D5. The distinction of an irritant patch test reaction from allergic reaction can be very difficult, particularly with less than ++ reactions. In order to limit the misinterpretation of possible false-positive reaction, 20 control subjects were patch tested with the same test material. In these individuals, the results were absolutely negative, proving that lactase powder is not a potent irritator. This reinforced our view that the patient's

patch test reactions to lactase were allergic in their nature rather than irritant. On the grounds of the test results and clinical skin symptoms caused by proteinaceous material, we prefer the diagnosis to be PCD rather than coexistent contact urticaria and allergic contact dermatitis.

The occupational rhinoconjunctivitis caused by lactase was confirmed by a positive challenge test.

The CCT simulates the working conditions. This test is a safe and straightforward method for clinical practice in cases where occupational rhinitis or rhinoconjunctivitis related to biological exposure is suspected and no commercial allergen solutions for local nasal and conjunctival provocation tests are available. The placebo-controlled and single-blinded test has been proved to be reliable, and reactions can be documented with clinical and rhinometric monitoring and weighing of nasal secretion (19).

Inhalation and contacts with enzyme preparations may cause respiratory and skin irritations or allergic reactions. As previously reported, lactase behaves as a potential occupational allergen which is capable of sensitizing exposed workers (5, 6). To minimize the risk, it is important to prevent inhalation and direct contact by provision of protective equipment, to prevent creation of dust, to ventilate the area, and to keep the working area and equipment clean. Preventive hygienic measures should be taken to ensure allergen avoidance. If an occupational disease is diagnosed, there is a legal requirement that there should be a reduction in the workplace exposure. This may be achieved by different measures that improve the working environment and conditions.

In conclusion, this is the first report of lactase-induced PCD and IgE-mediated allergic rhinoconjunctivitis, where immediate allergy to lactase was shown not only by prick test but also by positive OAT and CCT as well as by demonstrating lactase-specific IgE in *in vitro* tests. Furthermore, patch testing showed eczematous reactions with lactase. There was a good correlation between the lactase exposure, the clinical symptoms and the results of the diagnostic tests.

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