

Brief Report

Allergic Asthma Caused by Exposure to Bacterial Alpha-Amylase Termamyl®

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Background Airborne enzymes behave as potent respiratory allergens. Till date, allergic disorders caused by genetically engineered enzymes widely used in the industry, have not been reported.

Results and Conclusions We describe a worker employed in the detergent industry who developed asthma and rhinitis from IgE-mediated sensitization to the thermostable endo-alpha-amylase Termamyl® and to the protease Savinase®. This is the first report showing that Termamyl® elicits allergic respiratory disorders in humans. Am. J. Ind. Med. © 2012 Wiley Periodicals, Inc.

KEY WORDS: enzymes; Termamyl®; occupational asthma; specific IgE; rhinitis; lung function

INTRODUCTION

Enzymes are widely and increasingly employed as biological catalysts in a genetically engineered form in various industrial processes. Their production as well as application have been associated with the generation of airborne particulates or droplets that frequently elicit allergic reactions [Juniper et al., 1977; Cathcart et al., 1997; Nieuwenhuijsen et al., 1999; Schweigert et al., 2000; Vanhanen et al., 2000; Cullinan et al., 2003; Baur, 2005].

The gene of the thermostable endo-alpha-amylase Termamyl® originates from *Bacillus stearothermophilus* and is commercially biosynthesized in *Bacillus licheniformis* after changing four amino acids [Olempska-Beer, 2004]. This alpha-amylase hydrolyzes starch at high

temperatures, such as in the production of nutritive sweeteners from starch or glucose syrups and is used in alcohol processing and for the production of dishwashing and destarching detergents. Although Termamyl® is well-known for its allergic reactions in animal tests [Sarlo et al., 1997], we could not find a report about allergy in humans.

Savinase® [Gupta et al., 2002] belongs to the extracellular subtilisin serine proteases and is produced in the native form by *Bacillus amyloliquefaciens* for its main application as an alkaline washing detergent.

METHOD

Specific IgE-antibodies were measured by the fluorescence enzyme-labeled immunoassay (the specific IgE are expressed in kilounit per liter corrected with the WHO reference of human serum IgE; 1 KU = 2.4 ng/L) using recombinant Savinase® 8.0T or Termamyl® Ultra 300 L as antigen that was biotinylated and coupled to CAP cellulose sponges; the specific IgE antibodies were detected in patient serum using ImmunoCAP specific IgE fluorescence detector and seven point calibration curves ImmunoCAP is a registered trade name of Phadia, Uppsala, Sweden [Hamilton and Williams, 2010]. This method was developed in our lab for independent expert investigation of the

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case, after the worker had changed job to prevent further direct exposure to enzymes and had submitted a claim for compensation due to the disorder.

The patient gave consent to publish the case.

RESULTS AND DISCUSSION

A 60-year old male chemical worker, who had never smoked and was previously in good health, was engaged in the production and packaging of detergents for 32 years. During the last 10 years, the development of immediate-type rhinitis, conjunctivitis, and shortness of breath in the workplace arose after contact with various uncoated and coated detergents, including Termamyl[®] or Savinase[®]. No personal protective equipment was used and the exhaust systems were inadequate during the first years of employment—with dust visible in the air and on the floor—and were only improved later. The asthma became worse and, after repeated bouts of illness, a claim for acknowledgement of and compensation for an occupational respiratory disorder was made and the job was changed 6 years ago to that of a lorry driver in the same company.

Lung-function testing had shown a reversible obstructive ventilation pattern (FEV₁/FVC of 60% and 62% pre-bronchodilator, and 79%, and 78% respectively, post-bronchodilator; measured at a 20 month interval) during the last 2 years of employment as a chemical worker. The chest X-ray was normal. Specific IgE antibodies measured during this period in two years of follow-up were: 6.8 kU/L for Savinase[®] and 10.6 kU/L for Termamyl[®] in 2009, and 6.7 kU/L, and 21.6 kU/L, respectively, in 2011. Specific IgE antibodies against alkalase 2.5 L (*Bacillus subtilis*), cellulase (*Aspergillus niger*) and fungal alpha-amylase (*Aspergillus oryzae*) were not detectable and there was no response in the prick-test with common allergens. Total IgE was 131 kU/L (normal range: <100 kU/L).

Since the worker suffered from work-related immediate type rhinoconjunctivitis and shortness of breath, and a reversible obstructive ventilation pattern as well as specific IgE antibodies against occupational allergens were demonstrated, the worker was considered to have occupational asthma [Bernstein et al., 2006]. The lung function values considered a contraindication for a challenge test during the period of employment in the production and packaging of detergents. Later, the worker had changed his duties, and no PEF monitoring at or off work could be achieved.

Interestingly, although subjectively aware of only mild symptoms since becoming a lorry driver in the same company, the patients antibody concentration has risen for Termamyl[®] and remained nearly unchanged for Savinase[®]. This may reflect a continuing intermittent low exposure to the enzymes within the company.

Although not all of the enzymes used in industry have been reported to cause allergic reactions in humans, there is evidence from extensive in-vitro and animal studies that all are allergenic without a major difference in relative allergenicity among enzymes [Martel et al., 2010].

Our patient is the first reported case of allergic occupational asthma caused by the genetically engineered bacterial alpha-amylase Termamyl[®]. Vanhanen et al. [2001] reported on an individual who was sensitized to alpha-amylase from another *Bacillus* species, *Bacillus amyloliquefaciens*. Several authors have described respiratory allergies caused by alpha-amylase from the fungus *Aspergillus oryzae* [Baur et al., 1986; Houba et al., 1996; Nieuwenhuijsen et al., 1999; Vanhanen et al., 2001]. IgE-mediated sensitization to the glucoamylase of another *Aspergillus* strain (*Aspergillus niger*) was shown in 11% of workers in the enzyme-producing industry [Johnsen et al., 1997], and in 5–8% of bakers [Baur et al., 1989; Johnsen et al., 1997; Sander et al., 1998]. In one case, the source of allergenic amylase was not identified [Brant et al., 2006], and in none of these studies was the source of the enzymes identified as either native or genetically engineered.

The absence of immunological cross-reactivity in our IgE antibody analyses and also in tests with polyclonal antibodies [Elms et al., 2006] is not surprising since the different enzymes, especially those from different microorganisms, differ significantly in their primary, secondary, and tertiary structures, although their substrate-specificity may be the same. This is of clinical relevance since it signifies that a specific IgE test, e.g., for fungal amylase might not pick up IgE antibodies against bacterial amylases and vice versa.

The Soap and Detergent Industry Association (SOIA) and the AMFEP [Association of Manufacturers of Fermentation Enzyme Products, 1994] have recognized the high sensitizing potential of airborne enzymes and issued guidelines on the safe handling of enzymes, for use by their member companies as well as customers [Gilson et al., 1976; Sarlo et al., 1997; Schweigert et al., 2000].

The ACGIH[®] [2011] proposed an upper threshold of 60 ng/m³ for the 100% pure, active form of the enzyme alkalase from *Bacillus subtilis* (Subtilisin). In the UK, the occupational exposure limit was set at a lower 40 ng/m³ [Health and Safety Executive (HSE, 2007)]. There is evidence, however, that there is a risk of sensitization and allergic reactions even in the low ng range [Juniper et al., 1977; Heederik et al., 2002; Cullinan et al., 2003]. Cullinan et al. [2000], Cathcart et al. [1997], Flood et al. [1985], and Brant et al. [2009] found in follow-up studies that there is a significant number of sensitized workers and as upper and lower respiratory diseases in the detergent industry, with some dose-dependency appearing from 2.3 ng/m³ upwards. This corresponds with findings in the

baking industry where the fungal α -amylase from *Aspergillus oryzae* is widely used [Houba et al., 1996; Nieuwenhuijsen et al., 1999]. These findings reinforce the necessity to evaluate working conditions, especially in small and medium-sized enterprises in order to prevent occupational diseases. Further, re-evaluation of health-based exposure standards in the light of latest clinical findings in exposed workers is necessary.

Another problematical issue is the detection of enzyme allergies as few standard tests are available and developing specific new tests as part of the clinical routine is not possible. The international cooperation of occupational physicians and allergologists would help to meet this shortcoming.

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