



Letter

Microbial transglutaminase: a new and emerging occupational allergen

More than 40 years have lapsed since the first description of occupational allergic asthma caused by enzymes from *Bacillus subtilis*.^{1,2} Since then, several high-molecular-weight allergens have been identified, and allergic asthma is now recognized as the main cause of occupational airway disease, being responsible for approximately 25% to 30% of all incident causes.³

Transglutaminases (EC 2.3.2.13) are a family of enzymes found in mammals, fish, plants, and bacteria. In humans, transglutaminase 2 is involved in the immunopathogenesis of celiac disease. Transglutaminases catalyze posttranslational acyl-transfer reactions between the γ -carboxamide groups of glutamine residues and the ϵ -amino group of lysine, thereby causing the crosslinking of proteins.⁴ The low-cost mass production of a microbial form from *Streptomyces mobaraensis* by fermentation technology has boosted its application in food processing. Microbial transglutaminase displays a broad specificity toward food proteins and is Ca^{++} independent and remarkably stable over a wide range of temperatures and pH. Foods treated with microbial transglutaminases have enhanced texturization and resistance to proteolytic degradation, improved flavor and appearance, increased shelf-life, and even reduction of allergenicity.^{5,6}

We report a case of occupational sensitization to microbial transglutaminase in a worker from a company that commercializes ingredients for the food industry. In the plant, raw materials are weighed and mixed in various finished goods. The work environment is dusty, showing inhalable particulate concentrations between 3.2 and 9.0 mg/m^3 . In mid-2011, microbial transglutaminase was introduced in the productive cycle as dry powder preparations with different concentrations (60, 100, and 1,000 U/g). The material safety data sheet did not report any risk phrase but only the safety phrases S22 (“do not breathe dust”) and S24/25 (“avoid contact with skin/eyes”). Thus, use of personal protective equipment (gloves, glasses, and respiratory protections with particulate filters) was advised to workers handling microbial transglutaminase. Approximately 6 months after microbial transglutaminase introduction, a 40-year-old, male current smoker (15 pack-years), hired 6 years ago as a production superintendent, suddenly developed wheezing, cough, and shortness of breath during and after the shift. The symptoms rose acutely at work soon after the inhalation of microbial transglutaminase and continued outside. The worker confessed an inconsistent use of personal protective equipment during indirect exposure to microbial transglutaminase. Both medical and family histories were negative for atopy or allergies. At objective examination, there was diffuse wheezing at the end of expiration; at dynamic spirometry, the reduction of both the forced expiratory volume in 1 second (FEV_1) (2.43 L, 63% of the expected) and the maximal expiratory flow at 25% to 75% (1.48 L/s, 34% of the expected) was apparent. The saturation of peripheral oxygen was

also slightly decreased (95%). The stop and start work test revealed a clear relationship between job and clinical picture. In the following months, the symptoms progressively worsened despite asthma therapy (beclomethasone dipropionate and formoterol fumarate dehydrate). The arterial blood gas analysis revealed a PO_2 of 91.2 mm Hg, whereas eosinophilia was apparent in the blood cell count (1,261/ μL ; 21.2% of the white blood cell count). High-resolution computed tomography of the thorax revealed broad centrilobular and particularly paraseptal emphysema, mainly in the upper lobes, thickness of bronchial walls, and bronchial mucous impaction in peripheral bronchial airways. After 2 months of absence from work on sick leave, the patient was admitted to a respiratory disease unit. α_1 -Antitrypsin deficiency was ruled out according to both genetic testing and serum α_1 -antitrypsin levels (101–159 mg/dL). The results of skin prick tests for common aeroallergens and foods, performed to assess the patient's overall allergic status, were negative, but there was a clear immediate positivity (wheal and flare of approximately 0.8×0.8 and 1×1.2 cm, respectively) to a diluted solution (1 U/mL in water) of microbial transglutaminase. The test result was negative in 2 controls (1 exposed and 1 unexposed to microbial transglutaminase). All the individuals undergoing the skin prick test gave their informed consent. Total serum IgE levels were approximately 7-fold the upper reference value (710 IU/mL), whereas, in agreement with skin prick test results, IgE levels specific for common foods and aeroallergens were within the reference ranges, as were IgG levels for *Micropolyspora faeni*, *Alternaria tenuis*, *Aspergillus fumigatus*, *Penicillium notatum*, and pigeon proteins. IgE levels specific for microbial transglutaminase (starting batch of 100 U/g) were measured in serum samples from 10 workers by enzyme-linked immunosorbent assay, as elsewhere described.⁷ The in-triplicate assay revealed clearly increased microbial transglutaminase specific IgE levels in the patient compared with other workers (mean [SD] optical density values of 1,163 [153] vs 221 [65], respectively). The IgE specificity was supported by an IgE immunoblot inhibition experiment using serum from the patient and nonallergic colleagues, preincubated with microbial transglutaminase or a control protein. Figure 1 shows that the immunoreactive band of approximately 40 kDa, consistent with microbial transglutaminase molecular weight (38 kDa),⁸ disappears when the patient's serum is preincubated with either commercial or standard microbial transglutaminase. Other laboratory examinations revealed a reversion to normality of eosinophil count, normal serum levels of eosinophil cationic protein, a slight elevation of erythrocyte sedimentation rate (28 mm; reference range, 2–25 mm), and antinuclear speckled and anti-smooth muscle antibodies (dilution of 1:80 in both cases), whereas all other tested autoantibodies, serum complement, and IgG, IgA, and IgM levels were within the respective reference ranges. The online measurement of exhaled nitric oxide (8.9 ppb) was within the reference range.⁹ At static spirometry, there was an

Disclosures: Authors have nothing to disclose.

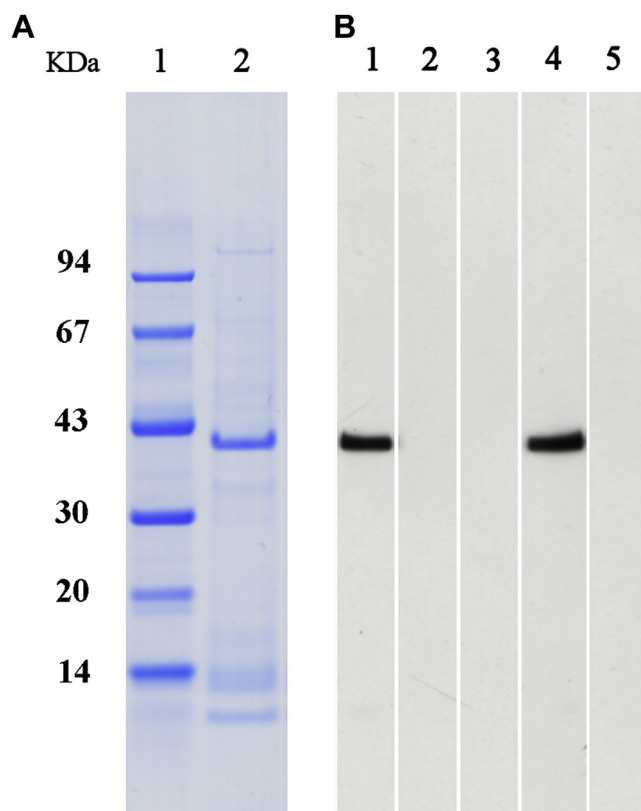


Figure 1. A, Coomassie blue–stained sodium dodecyl sulfate–polyacrylamide gel electrophoresis gel. Lane 1: molecular mass markers; lane 2: microbial transglutaminase (100 U/g). B, Inhibition of serum IgE reactivity to nitrocellulose-blotted microbial transglutaminase (100 U/g). Lanes 1 through 4: patient serum pre-adsorbed with dilution buffer (lane 1), microbial transglutaminase (100 U/g) (lane 2), standard microbial transglutaminase (lane 3), and unrelated control protein (bovine serum albumin; lane 4). Lane 5: nonallergic workers' pooled sera pre-adsorbed with dilution buffer.

FEV₁ of 3.62 L (92% of the expected) and a forced expiratory flow at 25% to 75% of 2.5 L/s (56% of the expected), both partially reversible (+7% and +28%, respectively) after bronchodilator inhalation. The bronchial provocation test with methacholine revealed a light to moderate bronchial reactivity (provocation dose causing a 20% decrease in FEV₁, 299.05 µg). The patient denied consent for a specific bronchial challenge with microbial transglutaminase. He was discharged with a diagnosis of bronchial asthma.

To the best of our knowledge, this is the first report that microbial transglutaminase may act as a new and emerging inhalant high-molecular-weight allergen. This finding indicates that more

caution must be exercised when the potential allergenicity of commercial enzymes is evaluated.¹⁰ A strict control of exposure must be applied in workplaces where microbial transglutaminase is used to avoid exposure and sensitization in the occupational setting.

Giuseppe De Palma, MD, PhD*

Pietro Apostoli, MD*

Gianni Mistrello, BSc†

Stefania Zanotta, BSc†

Giuseppina Bertorelli, MD‡

*Department of Medical and Surgical Specialties, Radiological Sciences, and Public Health, Section of Public Health and Human Sciences

University of Brescia

Brescia, Italy

†Lofarma Research Department

Lofarma SpA

Milan, Italy

‡Department of Clinical and Experimental Medicine

University of Parma

Parma, Italy

depalma@med.unibs.it

References

- [1] Flindt ML. Pulmonary disease due to inhalation of derivatives of *Bacillus subtilis* containing proteolytic enzyme. *Lancet*. 1969;1:1177–1181.
- [2] Pepys J, Longbottom JL, Hargreave FE, Faux J. Allergic reactions of the lungs to enzymes of *Bacillus subtilis*. *Lancet*. 1969;1:1181–1184.
- [3] Meyer JD, Holt DL, Chen Y, Cherry NM, McDonald JC. SWORD '99: surveillance of work-related and occupational respiratory disease in the UK. *Occup Med (Lond)*. 2001;51:204–208.
- [4] Cozzolino A, Di Pierro P, Mariniello L, Sorrentino A, Masi P, Porta R. Incorporation of whey proteins into cheese curd by using transglutaminase. *Biotechnol Appl Biochem*. 2003;38:289–295.
- [5] Zhu Y, Tramper J. Novel applications for microbial transglutaminase beyond food processing. *Trends Biotechnol*. 2008;26:559–565.
- [6] van Esch BC, Gros-van Hest M, Westerbeek H, Garssen J. Sensitizing capacity and allergenicity of enzymatically cross-linked sodium caseinate in comparison to sodium caseinate in a mouse model for cow's milk allergy. *Toxicol Lett*. 2013;218:50–55.
- [7] Asero R, Amato S, Alfieri B, Folloni S, Mistrello G. Rice: another potential cause of food allergy in patients sensitized to lipid transfer protein. *Int Arch Allergy Immunol*. 2007;143:69–74.
- [8] Pasternack R, Dorsch S, Otterbach JT, Robenek IR, Wolf S, Fuchsbaue HL. Bacterial pro-transglutaminase from *Streptovorticillium mobaraense*: purification, characterisation and sequence of the zymogen. *Eur J Biochem*. 1998;257:570–576.
- [9] Olivieri M, Talamini G, Corradi M, et al. Reference values for exhaled nitric oxide (Reveno) study. *Respir Res*. 2006;7:94.
- [10] Pedersen MH, Hansen TK, Sten E, et al. Evaluation of the potential allergenicity of the enzyme microbial transglutaminase using the 2001 FAO/WHO Decision Tree. *Mol Nutr Food Res*. 2004;48:434–440.