Occupational Asthma and Food Allergy due to Soybean in a Bakery Worker

Narváez-Fernández E[1], Pose K[1], Caballero ML[1,2,3], Rodríguez-Pérez R[1,2,3], Quirce S[1,2,3]

[1]*Department of Allergy, Hospital Universitario La Paz, Madrid, Spain*

[2]*Hospital La Paz Institute for Health Research, IdiPAZ, Madrid, Spain*

[3]*CIBERES, CIBER of Respiratory Diseases, Madrid, Spain*

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Occupations involving food handling may result in exposure to aeroallergens, triggering allergic reactions, mainly in the respiratory tract. Occasionally, allergic symptoms manifest after consumption of previously well-tolerated food handled at work, a condition known as class 3 food allergy [1,2]. The soybean industry is expanding, and severe soybean allergies have been reported [3,4], rendering occupational exposure to soybean a significant clinical concern [5].

We present the case of a 33-year-old man (nonsmoker) who presented with respiratory symptoms and allergic reactions upon ingestion of several foods. He also had a history of persistent asthma since the age of 26 and had been treated with budesonide/formoterol 320/9 µg twice daily, montelukast, and daily cetirizine, along with multiple inhalations of salbutamol. No other allergic or medical history was reported. At the age of 18, he started working in a bakery improvers factory, where he was exposed to the mixture of oxidants, emulsifiers, and enzymes used to ensure the quality of baking dough. His tasks included unloading raw materials, handling soy products (lecithin, flour, and liquid soy), and mixing cereals and seeds with enzymes (α-amylase, G4-1100, V292). He did not use protective equipment. After 8 years of work, he began to experience rhinoconjunctivitis, wheezing, and dyspnea during the workday. The symptoms worsened progressively, and he attended the emergency department several times. Characteristically, his asthma improved and required less treatment during holidays.

For 3 months, the patient adhered to a soy-free diet owing to the sudden development of oral, tongue, and ear itching, along with episodes of abdominal pain and vomiting within minutes of consuming soy-containing products (biscuits, milk, and yoghurt), which he had previously tolerated. No other symptoms or associations with cofactors were observed. The patient gave his written informed consent for the allergology work-up and publication of this report.

Skin prick tests (SPTs) yielded positive results to soybean, wheat, rye, barley, and corn flour, as well as to *Cupressus arizonica* pollen and *Dermatophagoides farinae*. In the case of oats, rice, buckwheat, egg white, ovalbumin, ovomucoid, lysozyme, and storage mites (*Lepidoglyphus destructor*, *Acarus siro*, *Tyrophagus putrescentiae*), the SPT results were negative, as were those for soy lecithin and malt (provided by the patient). Serum total IgE was 113 kU/L, tryptase levels were normal (3.50 µg/L), and eosinophil cationic protein was 55.8 µg/L.

Specific IgE results (ImmunoCAP, Thermo Fisher Scientific) were positive to soybean (1.41 kU_A/L), nGly m 5 ß-conglycinin (1.13 kU_A/L), nGly m 6 glycinin (1.4 kU_A/L), wheat (0.61 kU_A/L), rye (1.11 kU_A/L), barley (0.51 kU_A/L), malt (0.41 kU_A/L), α-amylase (0.43 kU_A/L), and *C arizonica* pollen (23.5 kU_A/L). IgE determinations were negative (<0.35 kU/L) for rGly m 4, corn, sesame, rTri a 19 (ω-5 gliadin), gluten, *Dermatophagoides pteronyssinus*, *D farinae*, *L destructor*, and *Saccharomyces cerevisiae*. The allergen microarray assay (ISAC, Thermo Fisher Scientific) yielded positive results only for nCup a 1 (22 ISU) and nCry j 1 (4.4 ISU). Baseline spirometry was normal, and the bronchodilator test result was positive. Baseline fractional exhaled nitric oxide (FeNO) was 33 ppb. The methacholine inhalation test result was positive (concentration needed to produce a 20% reduction in FEV_1 [PC_{20}], 0.09 mg). A specific bronchial challenge with soy was suggested, although the patient declined. Consequently, a specific bronchial challenge with α-amylase was conducted to confirm the diagnosis of occupational asthma. An early asthmatic response was observed (PC_{20}, 48.78 mg/mL; concentration, 1:10 wt/vol), with subsequent spontaneous recovery and no late response. FeNO was 111 ppb 24 hours after challenge.

A soybean flour extract was prepared in phosphate-buffered saline 0.01 M pH 7.4 (Sigma-Aldrich) by shaking overnight at 4ºC. After centrifugation at 4000*g* for 30 minutes, the supernatant was dialyzed against distilled H_2O (cut-off point of 3.5 kDa) and freeze-dried. The protein concentration was determined using the Bradford assay (Bio-Rad). A CAP inhibition study was performed with the soybean flour extract and resulted in complete inhibition of specific IgE to nGly m 5 and nGly m 6. SDS-PAGE analysis and IgE-immunoblotting were performed with the soybean flour extract under both nonreducing and reducing conditions using dithiothreitol and on 12% acrylamide minigel under standard conditions. After electrophoresis, proteins were stained with Coomassie blue or electro-transferred onto a supported nitrocellulose membrane (0.45 µm [Bio-Rad]) and incubated overnight with the patient's serum (diluted 1:5). Specific IgE was detected by incubation for 2 hours at room temperature with a monoclonal mouse antihuman IgE antibody conjugated with horseradish peroxidase (HRP) (Southern Biotech) at a 1:10 000 dilution. The reaction was developed with the WesternBright ECL HRP substrate (Advansta) and visualized using chemiluminescence (Figure).

IgE-immunoblotting (Figure) performed with the patient's serum revealed a protein with an estimated molecular weight (MW) of 8 kDa, compatible with Gly m 2 [6]. It also revealed a protein with an estimated MW of 14 kDa, which could be Gly m 3, a profilin [3,7], and several proteins ranging from 18 to 90 kDa, probably corresponding to isoallergens of Gly m 5 and

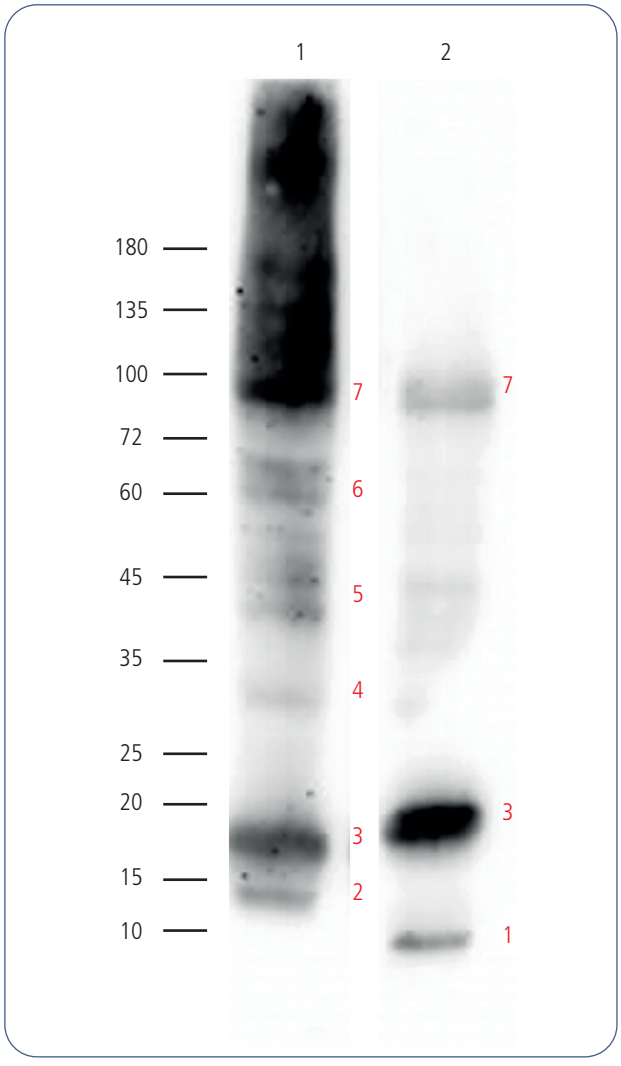

Figure. SDS-PAGE protein analysis and IgE-immunoblotting performed with the soybean flour extract and the patient's serum. Lane 1, Nonreducing conditions; Lane 2, Reducing conditions by treatment with dithiothreitol. Estimated MWs: 1=8 kDa; 2=14 kDa; 3=19-20 kDa; 4=32 kDa; 5=40-45 kDa; 6=60-68 kDa; 7=90, doublet 88-90 kDa. Compatible allergens from the list of the World Health Organization and International Union of Immunological Societies (WHO/IUIS): Gly m 2 (8 kDa); Gly m 3 (14 kDa); Gly m 6 (fractions of 19-20 and 32 kDa), and Gly m 5 (isoallergens Gly m 5.0301 [42-53 kDa]; Gly m 5.0101 [57-76 kDa] or Gly m 5.0201 [57-83 kDa]/Gly m 5.0201 [57-83 kDa]).

Gly m 6. In the case of Gly m 5, these consisted of 3 subunits (α, α', and ß), each of which is a potential allergen [3,7,8]. Therefore, our finding of an estimated MW of 40-90 kDa could correspond to these 3 subunits. In the case of Gly m 6, this consists of 5 subunits formed by basic polypeptide chains (18-20 kDa) and acidic polypeptide chains (31-45 kDa) [3,7,8]. Since both can bind IgE, our findings (19-32 kDa) would correspond to several of those fractions, concordant with the findings of CAP-inhibition.

Eight soy allergens have been described to date (WHO/IUIS Allergen Nomenclature Sub-Committee) [3]. These allergens can cause various clinical manifestations according to their MW. Epidemic asthma outbreaks were associated with low-MW proteins while occupational asthma due to exposure to soybean flour was associated with high-MW proteins [9]. Occupational handling of soybean has also been reported to be associated with new sensitizations, including to Gly m 5 and Gly m 6 [5]. Furthermore, sensitization to 1 of these 2 allergens has been postulated as a potential marker for severe allergic reactions [10].

To our knowledge, this is the first case of class 3 food allergy (resulting from both inhalation and ingestion) due to occupational sensitization to soy.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Emilio Narváez-Fernández
Servicio de Alergia
Hospital Universitario La Paz
28046 Madrid
Spain
E-mail: emilionarvaez2015@hotmail.com

