

Occupational asthma and food allergy due to soybean in a bakery worker

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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 may manifest after consuming 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 male, a non-smoker, 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, being treated with budesonide/formoterol 320/9 µg twice daily, montelukast, and cetirizine daily, 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 (a mixture of oxidants, emulsifiers and enzymes that 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 (alpha-amylase, G4-1100, V292). Protective equipment was not used. After 8 years of work, he began to suffer symptoms of rhinoconjunctivitis, wheezing and dyspnoea during the workday. The symptoms became

progressively worsened and he attended the emergency department several times. Characteristically, his asthma improved and required less treatment during his holidays.

For three months, he adhered to a soy-free diet due 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, or yoghurt), which he had previously tolerated without issues. No other symptoms or associations with cofactors were observed. He gave written informed consent for the allergological work-up and publication of this report.

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

Specific IgE results (ImmunoCAP, ThermoFisher Scientific) were positive to soybean (1.41 kU/L), nGly m 5 beta-conglycinin (1.13 kU/L), nGly m 6 glycinin (1.4 kU/L), wheat (0.61 kU/L), rye (1.11 kU/L), barley (0.51 kU/L), malt (0.41 kU/L), alpha-amylase (0.43 kU/L) and *C. arizonica* pollen (23.5 kU/L). IgE determinations were negative (<0.35 kU/L) to rGly m 4, corn, sesame, rTri a 19 (omega-5 gliadin), gluten, *D. pteronyssinus*, *D. farinae*, *L. destructor* and *Saccharomyces cerevisiae*. The allergen microarray assay (ISAC, ThermoFisher Scientific) showed positive results only for nCup a 1 (22 ISU) and nCry j 1 (4.4 ISU). Baseline spirometry was normal and bronchodilator test was positive. Baseline fractional exhaled nitric oxide (FeNO) was 33 parts per billion (ppb). Methacholine inhalation test was positive (concentration needed to produce a 20% reduction in [FEV1] [PC20], 0.09 mg). A specific bronchial challenge with soy was suggested, but the patient declined. Consequently, a specific

bronchial challenge with alpha-amylase was conducted to confirm the diagnosis of occupational asthma. An early asthmatic response was observed (PC20=48.78 mg/ml; concentration 1:10 w/v), with subsequent spontaneous recovery and no late response. The FeNO 24 hours after challenge was 111 ppb.

A soybean flour extract was prepared in PBS 0.01 M pH 7.4 (Sigma-Aldrich) by shaking overnight at 4° C. After centrifugation 4,000 g for 30 min, supernatant was dialyzed against distilled H₂O (cut-off point of 3.5 kDa) and freeze-dried. Protein concentration was determined using a 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 non-reducing and reducing conditions using dithiothreitol (DTT), on 12% acrylamide minigel, under standard conditions. After electrophoresis, proteins were stained with Coomassie Blue or were 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 detection was performed by incubation 2 hours at room temperature with a monoclonal mouse anti-human IgE antibody conjugated with horseradish peroxidase (HRP) (Southern Biotech) at 1:10.000 dilution. The reaction was developed with the WesternBright ECL HRP substrate (Advansta) and visualized by chemiluminescence (figure 1).

IgE-immunoblotting (figure 1) performed with the serum of our patient revealed a protein with estimated molecular weight (MW) of 8 kDa, compatible with Gly m 2 [6]. A protein with estimated MW of 14 kDa, that could be Gly m 3, a proflin [3,7], and several proteins ranging (18-90 kDa), probably corresponding to isoallergens of Gly m 5 and Gly m 6. In the case of Gly m 5, it consists of 3 subunits (alpha, alpha' and beta), each of which is a potential allergen [3,7,8]. Therefore, our data with estimated MW between 40-90 kDa could correspond to these

3 subunits. In the case of Gly m 6, it consists of five subunits formed by basic (18-20 kDa) and acidic (31-45 kDa) polypeptide chains [3,7,8]. Since both are able to bind IgE, our findings (19-32 kDa) would correspond to several of those fractions which is concordant with the findings of CAP-inhibition.

Currently, eight soy allergens are described (WHO/IUIS Allergen Nomenclature Subcommittee) [3]. These allergens can cause different 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 sensitisations, including to Gly m 5 and Gly m 6 [5]. Furthermore, sensitisation to one of these two 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 (both from inhalation and from ingestion) due to occupational sensitisation to soy.

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Conflicts of interest

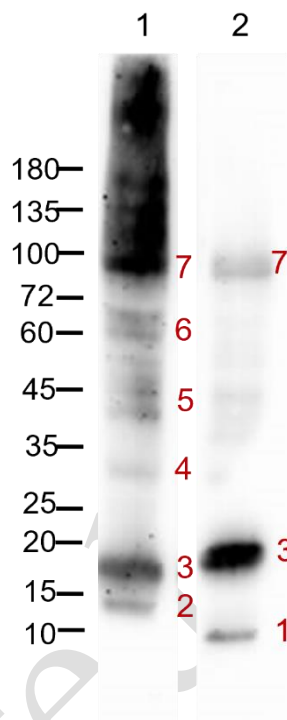
Dr. S. Quirce has no conflicts of interest to declare. Dr. R. Rodríguez-Pérez has not conflicts of interest to declare. Dr. M. Caballero has not conflicts of interest to declare. Dr. K. Pose has not conflicts of interest to declare. Dr. E. Narváez-Fernández has no conflicts of interest to declare.

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Figure 1. SDS-PAGE protein analysis and IgE-immunoblotting performed with the soybean flour extract performed with the serum of the studied patient. Lanes: 1) non-reducing conditions; 2) reducing conditions by treatment with dithiothreitol.



Legends: Compatible allergens from the list World Health Organisation and International Union of Immunological Societies (WHO/IUIS): Gly m 2 (8 kDa); Gly m3 (14 kDa); Gly m6 (fractions of 19-20 and 32 kDa) and Gly m5 (isoallergens Gly m5.0301 [42-53 kDa]; Gly m5.0101 [57-76 kDa] or Gly m5.0201 [57-83 kDa]/Gly m 5.0201 [57-83 kDa]).