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Letter

Allergy reaction mediated by Gal d 4 (lysozyme) after the induction of tolerance with egg

Lysozyme is a thermolabile enzyme present in egg white. It represents 3.5% of total egg protein and has a molecular weight of 14.3 kDa.[1] It is used in pharmaceutical products and in the cheese and wine industries.

As an allergen, lysozyme has been termed Gal d 4. Although up to 32% of individuals with egg allergy are sensitized to lysozyme,[2] hypersensitivity reactions have rarely been reported.[3,4]

We report the case of a 10-year-old girl with a history of allergy to cow's milk proteins that was resolved at 2 years of age and persistent hen's egg allergy. At 8 years of age, specific oral tolerance induction (SOTI) with hen's egg was performed. She was allergologically studied and a serum sample was collected. Skin prick tests to hen's egg (5 mg/mL; Laboratorios LETI, Madrid, Spain) and individual proteins, namely ovalbumin (1 mg/mL; LETI), ovomucoid (1 mg/mL; LETI), and lysozyme (1 mg/mL; ALK-Abéllo, Hørsholm, Denmark), were performed. Specific IgE (sIgE; CAP System, Thermo, Uppsala, Sweden) to egg white, yolk, ovomucoid, ovalbumin, conalbumin, and lysozyme was determined.

The SOTI consisted of gradually increasing the dose of powdered pasteurized egg, according to our usual protocol.[5] After 10 weeks, the patient experienced vomiting and facial erythema after the ingestion of 1 g of powdered pasteurized egg (one tenth of egg). The active substance was replaced with cooked egg (omelet) to continue with the protocol. After 14 weeks, she achieved desensitization, as shown after eating a 1-egg omelet.

Table 1
IgE, IgG4, and wheal size values before and after specific oral tolerance induction for the different egg extracts used in the study

	IgE (kUA/L)		IgG4 (mgA/L)		Wheal size (mm diameter)	
	Before	After	Before	After	Before	After
Egg white	2	1.32	0.16	3.19	10	8
Egg yolk	0.91	0.71			5	6
Ovomucoid	1.93	1.33	0.05	2.2	10	6
Ovalbumin	1.27	0.66	0.06	1.77	8	6
Conalbumin	N	N	0	0.65	ND	ND
Lysozyme	0.48	0.7	0.02	0.39	ND	12

Abbreviations: N, negative (IgE <0.35 kUA/L); ND, not determined.

Clinical follow-up was performed at 6 and 12 months after the end of desensitization. Eighteen months after achieving cooked-egg desensitization, an oral food challenge (OFC) with 10 mm (1,100 mg) of liquid pasteurized egg white was performed. Immediately after the OFC, the patient developed oral pruritus and vomited. According to these results and the mild symptoms observed, we considered the avoidance of small amounts of raw egg white unnecessary.

One month after the OFC, she experienced oral and pharyngeal pruritus, including difficulty swallowing, after ingesting a Lizipaina lozenge (Boehringer Ingelheim, Reims, France) containing 3 mg of

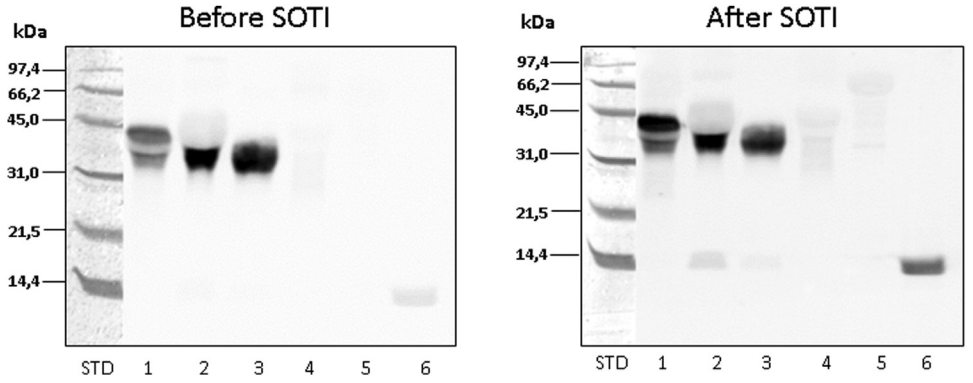


Figure 1. Immunoblot before and after specific oral tolerance induction (SOTI). Ten micrograms of protein from each extract or purified proteins was run on sodium dodecylsulfate polyacrylamide gel electrophoresis, transferred by electrotransfer to an Immobilon-P membrane (Millipore, Bedford, Massachusetts), and dried at room temperature. Thereafter, membranes were incubated overnight with the serum diluted 1:3 in phosphate buffered saline. After incubation with monoclonal anti-human IgE-PO (Ingenasa, Madrid, Spain), the reaction was developed with luminol (Bio-Rad, Hercules, California) and visualized by chemiluminescence. *Lane 1,* egg yolk; *lane 2,* egg white; *lane 3,* ovomucoid; *lane 4,* ovalbumin; *lane 5,* conalbumin; *lane 6,* lysozyme.

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bacitracin, 2 mg of papain, and 5 mg of lysozyme. She received oral treatment with dexchlorpheniramine, resulting in the complete relief of symptoms. A new serum sample was collected. Two samples (before SOTI and after the adverse reaction with Lizipaina) were analyzed. The sIgE and sIgG4 levels to egg white, yolk, ovomucoid, ovalbumin, conalbumin, and lysozyme were determined. The allergenic profile was investigated by immunoblot. Skin prick tests with hen's egg and individual proteins were performed, and the reactions remained positive (Table 1). The negativity of the skin prick tests and sIgE ruled out sensitization to papain. Allergy to bacitracin was ruled out because the patient had used ointments containing this antibiotic several times without experiencing problems.

Before SOTI, the patient was mildly sensitized to lysozyme while presenting high levels of sIgE to yolk, egg white, ovomucoid, and ovalbumin. A decrease in sIgE levels was observed after SOTI for yolk, egg white, ovomucoid, and ovalbumin. The sIgE to lysozyme increased after treatment (Table 1). The sIgG4 levels increased for all proteins after SOTI (Table 1). The allergenic profile showed clear recognition of egg white, yolk, and ovomucoid, whereas ovalbumin and lysozyme were poorly recognized. After SOTI, the recognition of lysozyme increased, in correlation with sIgE levels (Fig 1).

To summarize, we report the case of an IgE-mediated hypersensitivity reaction to lysozyme in a patient who achieved desensitization to cooked hen's egg after SOTI. SOTI is currently recognized as a promising food allergy treatment for children who have not achieved spontaneous tolerance. Different protocols are currently used; we started a protocol with powdered pasteurized egg, but, unfortunately, we had to replace it with cooked egg because of adverse reactions.

In previously published studies, we demonstrated that by following this protocol, more than 90% of patients achieved desensitization.[5] Children who obtained desensitization to cooked eggs did not necessarily reach complete desensitization to raw egg white.[6] However, according to our experience, 54% of children who completed oral desensitization treatment with cooked egg had good tolerance to raw egg white. These children can have a normal diet devoid of any egg-related restrictions.[5] However, for 46% of children, a suitable tolerance threshold should be established. Our patient was initially sensitized to hen's egg and its proteins. At the end of SOTI, a general decrease in sIgE was observed, except for lysozyme. From an immunologic point of view, the patient responded to the SOTI with a significant increase in sIgG4 levels for all proteins.

The adverse reaction observed after the ingestion of 1 g of powdered pasteurized egg was not repeated when the SOTI was continued with cooked egg. Our hypothesis is that lysozyme levels in powdered pasteurized egg (5 times lower than in raw egg white; eFig 1) were not sufficient to induce an allergic response. Conversely, the proteins were denatured after cooking and the allergenicity of lysozyme could have been modified. This could be the reason the patient remained asymptomatic with cooked egg.

In the OFC (18 months after the SOTI), we administered 10 mL of liquid pasteurized egg white containing approximately 38.5 mg of lysozyme. The mild adverse reactions observed were, in all probability, mediated by the sensitization of lysozyme. The reason the patient responded positively to all the allergens, except to lysozyme, remains unclear and could be explained by the immunologic profile of the patient. Our final recommendation was to avoid ingestion of raw egg, even in small amounts.

Because lysozyme is used as a food additive (E-1105) and in the composition of drugs,[7] this should be considered in egg-sensitized patients and in those who did not achieve raw egg tolerance after SOTI.

In conclusion, although SOTI has been described as an efficient treatment for a large percentage of the population, a detailed clinical and immunologic study with individual proteins should be performed before recommending the ingestion of the offending allergens. This case shows the importance of knowing the involved proteins and of warning about the possible presence of egg proteins in manufactured food or drugs.

Supplementary Data

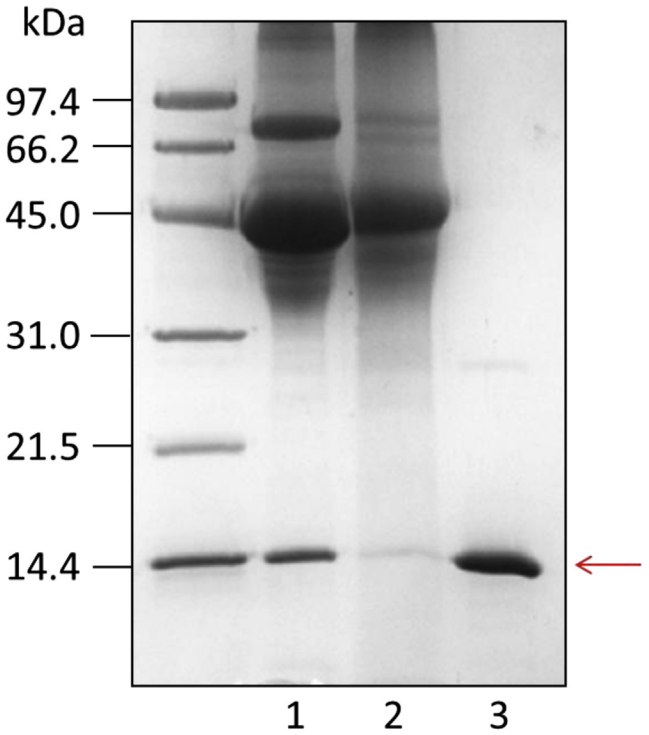
Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.anai.2014.07.022.

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eFigure 1. Sodium dodecylsulfate polyacrylamide gel electrophoresis, under reducing conditions and stained with Coomassie blue, of 20 μg of protein from egg white (*line 1*), 20 μg of protein from powdered pasteurized egg (*line 2*), and 5 μg of Lizipaina (1/1,000 of a lozenge; *line 3*). The band corresponding to lysozyme is marked with an *arrow*.