

Identification of duck egg vitellogenin as responsible for duck egg selective anaphylaxis

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Allergy to duck egg is rare and it is usually associated with IgE-mediated hypersensitivity to other types of bird egg, mainly hen eggs [1,2]. But although infrequent, some cases of selective duck egg allergy have been described [3-5]. We present the case of an adult patient with anaphylaxis after ingestion of duck egg, with vitellogenin as the responsible allergen, and subsequent tolerance to hen egg,

A 71-year-old man with no personal history of interest, after eating flan made with duck and hen eggs, presented immediately oropharyngeal and palmo-plantar pruritus. Thirty minutes later, he associated abdominal pain with vomiting and generalized urticaria. Patient treated himself with antihistaminics with progressive remission of symptoms, but he did not request urgent medical attention. The patient lives in a rural area and has got chickens and ducks. One week later, he ingested again cooked duck egg and five minutes after the patient developed hives, abdominal pain and vomiting. On this occasion he requested medical attention, receiving emergency treatment with dexchlorpheniramine 5 mg, methylprednisolone 40 mg, adrenalin and fluid therapy with resolution of the symptoms. After both episodes the patient recognized tolerance to hen egg and chicken meat, and continues to avoid duck egg.

Skin prick tests were performed with commercial extracts (Roxall, Bilbao, Spain) of chicken egg and their fractions, most frequent aeroallergens in our area, food extract battery (cow milk, hen egg, shrimp, *Anisakis simplex*, gluten, cod, veal, peanut, soybean, wheat) and panallergens (nsLTP-Pru p 3, pollen profilin), all with negative results. Prick-by-prick test with raw duck egg, white and yolk, were performed with positive results: 10 mm (yolk) and 5 mm (white). Histamine was used as a positive

control (4 mm) and saline as a negative control. An analytical determination of basal tryptase (3.2 ug/ml), total immunoglobulin IgE (IgE) (363 IU/ml) were performed as well as specific IgE (ImmunoCAP -Thermo Fisher Scientific) to hen egg: 0.46 kU_A/L, hen egg white: 0.75 kU_A/L, hen egg yolk: 0.2 kU_A/L, ovomucoid: 0.35 kU_A/L, ovalbumin: 0.07 kU_A/L, conalbumin: 0.05 kU_A/L, lysozyme: 0.1 kU_A/L. Patient recognized tolerance to hen egg in different types of food (omelette, homemade ice cream and boiled egg). Since the patient had two anaphylaxis after ingestion of duck egg, an oral challenge test with duck egg was not performed due to ethical reasons and to avoid unnecessary risk. He also did not want to perform a tolerance study to other poultry eggs.

A sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) immunoblotting was performed with patient's serum under reducing electrophoresis conditions (with 2-mercaptoethanol) with extracts of egg yolk and egg white from duck, chicken, quail, guinea fowl and ostrich eggs. A main IgE binding band of approximately 33 kDa was detected in duck egg white and yolk with various samples of high dilution serum (1/100, 1/200, 1/500), a band with the same molecular mass was detected in egg white and yolk from guinea fowl and ostrich with a much lower serum dilution (1/4) (Figure 1). Bands of 66 kDa was detected in duck egg white with 1/100 serum, and with a much lower serum dilution (1/4) in hen egg white, in quails egg white and ostrich egg yolk. The high intensity of IgE binding detected in the 33 kDa-band in duck egg and the absence of this band in samples of the tolerated hen egg let us to suppose that the 33-kDa-IgE binding band is the possible protein responsible of the duck egg anaphylaxis.

The 33kDa-protein was identified by mass spectrometry, as previously reported [6], as well as by searching a nonredundant protein sequence database (NCBI) using the Mascot program (<http://www.matrixscience.com>) in the Proteomic Service of

Complutense University of Madrid, which is a member of the ProteoRed Network. Research conducted with protein databases identified the 33kDa-protein as duck vitellogenin. Written informed consent was obtained from the patient for all in vitro and in vivo studies.

Although rare, duck egg allergy is often associated with sensitization and symptoms to eggs of other birds [1]. However, cases of duck egg allergy with tolerance to chicken eggs have been reported [3-5]. These studies implicated ovalbumin [3,4] and lysozyme [5] as possible allergens involved in their selective reactions to duck egg, but proteins were not always identified and unlike in our case, vitellogenin was not involved. The reverse situation has also been described, with children with hen's egg allergy and tolerance to duck egg [7]. In our study, we identified the 33-kDa protein from duck egg which showed a high IgE-binding capacity, and it turned out to be the duck vitellogenin. The vitellogenin is a precursor of the lipoproteins and phosphoproteins in egg yolk. Its function is transporting lipids and proteins from the liver through the oocytes, and become part of the yolk. It can be found in all oviparous species including birds or fish. Vitellogenin from chicken eggs (Gal d 6, present in egg yolk) [8] and from some fish eggs [9,10] has been described as an allergen from these allergenic sources. In our determination, patient's serum showed very high levels of specific IgE against the 33 kDa protein, so we assume that even if there is a small amount of the protein in the egg white, it would be clearly detectable. We have identified vitellogenin (Gal d 6), previously described as the second allergen in chicken eggs [8], as the allergen responsible for the selective anaphylactic reaction to duck egg in our patient. Gal d 6 is the yolk glycoprotein 42 (YGP42), a fragment of the vitellogenin-1 (VTG1). It is a heat-resistant protein, but digestible by pepsin allergen. It forms together with VTG-2 the

main components of the yolk. Its cleavage produces apolipoproteins and phospholipids, components of the granular yolk lipoproteins [8].

In summary, we present a rare case of debut of duck egg allergy in an adult patient with no history of atopy and with subsequent tolerance to chicken eggs. The vitellogenin appeared to be the allergen involved in the anaphylactic reaction. This case suggests that duck egg allergy can be independent of hen egg allergy.

Abbreviations: Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), Enzyme Allergo Sorbent Test (EAST), IgE

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Conflicts of interest

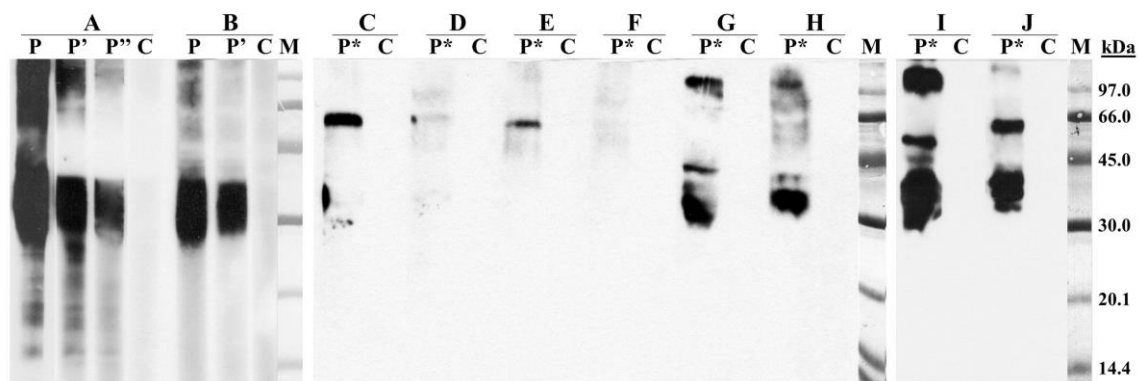
The authors declare that they have no conflicts of interest.

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FIGURE LEGENDS

Figure. SDS-PAGE Immunoblotting under reducing electrophoresis conditions (with 2-mercaptoethanol) with extracts of egg yolk and egg white from duck, chicken, quail, guinea fowl and ostrich eggs.



A) Duck egg white B) Duck egg yolk C) Hen egg white D) Hen egg yolk E) Quail egg white F) Quail egg yolk G) Guinea fowl egg white H) Guinea fowl egg yolk I) Ostrich egg white J) Ostrich egg yolk. Lane P, P', P'', P*: patient serum. Dilutions 1/100, 1/200, 1/500 and 1/4 respectively, Lane C: control serum (pool of sera from non-atopic subjects), Lane M: Molecular mass standard.