

## Sheep cheese allergy in Alpha-gal Syndrome

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**Palabras clave:** Queso de oveja. Productos lácteos. Alfa-gal. Alergia alimentaria.

Alpha-gal Syndrome (AGS) is an allergy characterized by the development of hypersensitivity reactions after exposure to non-primate mammalian meat and derived products (e.g. lanolin, a product of the sebaceous glands of sheep), cetuximab infusion or medical products (e.g. gelatin, bioprosthetic heart valves, or anti-venom). Co-factors such as alcohol or exercise can influence reactivity. It is mainly associated with tick bites, and it is caused by sensitization to the oligosaccharide galactose- $\alpha$ -1,3-galactose (alpha-gal) [1]. However, other mite species, namely Trombiculidae (chiggers), might also be a cause of sensitization to it [2].

Besides, mammalian milk contains alpha-gal proteins, but avoiding dairy products is not recommended, as 80-90% of AGS patients do not react to milk or milk products [3].

We present two patients. A first one was a 54-year-old male who suffered four episodes of immediate urticaria after eating sheep cheese, two of them associated with alcohol as a cofactor. These reactions were resolved with oral antihistamines or parenteral corticosteroids. This patient had a personal history of anaphylaxis with no associated cofactors due to allergy to alpha-gal in relation to the intake of beef and pork. Currently, he is following a mammalian meat free diet and tolerates cow and goat cheese.

A second patient was a 56-year-old male who presented two episodes of immediate urticaria after eating sheep cheese without associating cofactors. These reactions were resolved with oral antihistamines. He also had a personal history of anaphylaxis with no associated cofactors due to sensitization to alpha-gal in relation to the intake of beef. Nowadays, he is on a mammalian meat free diet and tolerates cow cheese and milk.

As we were evaluating two patients with AGS, the main component of sheep cheese is sheep milk, and cow milk proteins have been describe as alpha-gal epitope carriers, we wanted to assess if the presence of this epitope could be the cause of the sheep milk allergy in these patients. Subsequently, total IgE and specific IgE (sIgE) to alpha-gal, sheep milk, goat milk, cow milk, cow casein, cow  $\alpha$ -lactalbumin (ALA), cow  $\beta$ -lactoglobulin (BLG) and bovine serum albumin (BSA) was measured. Furthermore, CAP-inhibition was carried out with patient 1 serum to evaluate cross-reactivity between alpha-gal determinant, sheep milk, veal meat and pork meat using as inhibitor bovine thyroglobulin as alpha-gal carrier. SDS-PAGE Immunoblotting was carried out with 2-mercaptoethanol as described by Laemmli [4] with two dilutions of patient sera. SDS-PAGE Immunoblotting – inhibition was performed with patient sera, sheep milk extract in solid phase, and as inhibitors we used sheep, cow, goat milk extracts and two concentrations of cow thyroglobulin and cetuximab as alpha-gal carriers (100  $\mu$ g/mL and 200  $\mu$ mL).

The study was approved by the OSI Bilbao Basurto Ethics Committee for Clinical Research. Written informed consent was obtained from both participants. Samples and data from patients were provided by the Basque Biobank [www.biobancovasco.org](http://www.biobancovasco.org) and were processed following standard operation procedures with appropriate ethical approval.

The results revealed a total IgE of 147 and 461 kU/l, and alpha-gal-sIgE >100 and 76.70, sheep milk-sIgE 10.18 and 5.18, goat milk-sIgE 0.21 and <0.1, cow milk-sIgE 3.76 and 0.75, cow casein-sIgE 0.13 and <0.1, and BSA-sIgE, cow ALA-sIgE and cow BLG-sIgE <0.1 for patient 1 and patient sera 2 respectively.

CAP-inhibition with patient 1 serum and alpha-gal as inhibitor showed a significant IgE inhibition (more than 70%) to alpha-gal (85.9%), to sheep milk (79.7%) and to veal meat (79.1%) and a relevant inhibition to pork meat (66%).

SDS-PAGE Immunoblotting showed a similar profile of IgE reactive bands with both patient sera, a main and high intense one of approximately 75 kDa and a one of low intensity of aprox. 58 kDa (Figure 1).

For the purpose to assess if the alpha-gal oligosaccharide was the IgE-reactive epitope in the sheep milk extracts, an immunoblotting-inhibition assay was performed with sheep milk extract in solid phase and alpha-gal epitope carries (thyroglobulin and cetuximab) and cow and goat milk extracts as inhibitors (Figure 1). Both dilutions of cow thyroglobulin and cetuximab produced a total IgE-binding inhibition on sheep milk extract with the two patient sera. Furthermore, a partial inhibition was observed with goat milk extract with patient 1 serum.

Hypersensitivity reactions to sheep milk have been mainly attributed to caseins such a  $\kappa$ -casein of 19 kDa [5] or  $\alpha$ -2-casein of 35 kDa [6] that present cross-reactivity with goat casein, but not with cow casein which explains the tolerance to cow milk in many patients [7]. The restricted specificity to  $\beta$ -casein in patients allergic to goat milk is mainly directed against the 49-79 domain, which differs from its bovine counterpart by only three amino acid substitutions [8]. Other allergens of sheep milk allergy have also been described as ALA of 15 kDa or BLG of 12 kDa [5].

Alpha-gal epitope from bovine  $\gamma$ -globulin (BGG), lactoferrin (LF) and lactoperoxidase (LPO) have been described to be recognized by sIgE from patients with AGS. Besides, LPO has been described as allergen. BCG proteins of 58 and 80 kDa, LF protein of 75 kDa and LPO protein of 70 kDa have been detected in SDS-PAGE immunoblot analysis with alpha-gal sensitized patient sera, without recognition of the major milk allergens described to genuine milk allergy as caseins, ALA, BLG or BSA [9]. In addition, it was speculated that LF sensitization might be a high risk marker of anaphylaxis in association with high levels of alpha-gal-sIgE in patients with AGS [10].

SDS-PAGE immunoblotting results suggest that proteins of 75 kDa and 58 kDa might be responsible for the sheep cheese allergy in these patients. The molecular mass of these IgE-reactive proteins led us to suppose they are LF and BCG respectively. Results from CAP-inhibition and immunoblotting-inhibition assay point to the alpha-gal as the epitope involved in the IgE recognition, as it is suggested by the total IgE-inhibition obtained with the two alpha-gal carriers. There do not seem to be other epitopes responsible for the sheep cheese allergic reaction.

We present the first two cases of allergy to sheep cheese in AGS. The patients were also sensitized to other dairy products with no clinical relevance. We believe it is likely that sheep proteins contain a greater number of alpha-gal epitopes in their structures than cow and goat milk ones. Further research on this will be needed in the future.

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### **Conflicts of Interest**

The authors declare that they have no conflicts of interest.

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Figure 1. **I) SDS-PAGE Immunoblotting.** Lane P1, P1': Patient serum 1. Two dilutions, Lane P2, P2': Patient serum 2. Two dilutions. Lane C: Control serum (pool of sera from non-atopic subjects) Lane M: Molecular mass standard. **II) SDS-PAGE Immunoblotting – inhibition.** Lane C: Control serum (pool of sera from non-atopic subjects) Lane 1-9: Patient serum pre-incubated with sheep milk extract (lane 1), with goat milk extract (lane 2), with cow milk extract (lane 3), with cow thyroglobulin (100 µg/ml) (lane 4), with cow thyroglobulin (200 µg/ml) (lane 5), with cetuximab (100 µg/ml) (lane 6), with cetuximab (200 µg/ml) (lane 7), with chicken ovalbumin (lane 8), with sunflower pollen extract (lane 9) Lane M: Molecular mass standard.

