

Sheep Cheese Allergy in α -Gal Syndrome

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α -Gal Syndrome (AGS) is an allergy characterized by the development of hypersensitivity reactions after exposure to nonprimate mammalian meat and derived products (eg, lanolin, a product of the sebaceous glands of sheep), cetuximab infusion, and medical products (eg, gelatin, bioprosthetic heart valves, and antivenom). Cofactors such alcohol and exercise can affect reactivity. AGS is mainly associated with tick bites and is caused by sensitization to the oligosaccharide galactose- α -1,3-galactose (α -gal) [1]. However, mite species from other families, eg, Trombiculidae (chiggers), might also be a cause of sensitization to α -gal [2].

Mammalian milk contains α -gal proteins, although avoiding dairy products is not recommended, as 80%-90% of AGS patients do not react to milk or milk products [3].

We report on 2 patients. The first was a 54-year-old man who experienced 4 episodes of immediate urticaria after eating sheep cheese; alcohol was a cofactor in 2 episodes. These reactions resolved with oral antihistamines and parenteral corticosteroids. The patient had a personal history of anaphylaxis with no associated cofactors due to allergy to α -gal in relation to the intake of beef and pork. He is currently following a mammalian meat-free diet and tolerates cow and goat cheese.

The second patient was a 56-year-old man who experienced 2 episodes of immediate urticaria after eating sheep's cheese without cofactors. These reactions were resolved with oral antihistamines. He also had a personal history of anaphylaxis with no associated cofactors due to sensitization to α -gal in relation to the intake of beef. Nowadays, he follows a mammalian meat-free diet and tolerates cow's cheese and milk.

As both patients had AGS, the main component of sheep cheese is sheep milk, and cow milk proteins have been described as α -gal epitope carriers, we were interested to assess whether the presence of this epitope could be the cause of the sheep milk allergy. Subsequently, total IgE and specific IgE (sIgE) to α -gal, sheep milk, goat milk, cow milk, cow casein,

cow α -lactalbumin (ALA), cow β -lactoglobulin (BLG), and bovine serum albumin (BSA) were measured. Furthermore, CAP-inhibition was carried out with serum from patient 1 to evaluate cross-reactivity between α -gal determinant, sheep milk, veal, and pork using the α -gal carrier bovine thyroglobulin as an inhibitor. SDS-PAGE immunoblotting was carried out with 2-mercaptoethanol, as described by Laemli [4], with 2 dilutions of patient serum. SDS-PAGE immunoblotting-inhibition was performed with patient serum and sheep milk extract in the solid phase; as inhibitors, we used sheep, cow, and goat milk extracts and 2 concentrations of cow thyroglobulin and cetuximab as α -gal carriers (100 μ g/mL and 200 μ 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 and were processed following standard operational procedures with appropriate ethical approval.

For patient 1 and patient 2, respectively, the values recorded were as follows: IgE, 147 and 461 kU/L; α -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; and cow casein sIgE, 0.13 and <0.1. Values for BSA sIgE, cow ALA sIgE, and cow BLG sIgE were <0.1.

CAP-inhibition with serum from patient 1 and α -gal as the inhibitor revealed significant IgE inhibition (more than 70%) with α -gal (85.9%), sheep milk (79.7%), and veal (79.1%), as well as relevant inhibition with pork meat (66%).

SDS-PAGE immunoblotting showed a similar profile of IgE-reactive bands with both patient sera, namely, a main and highly intense band of approximately 75 kDa and a less intense band of approximately 58 kDa (Figure).

For the purpose of assessing whether the α -gal oligosaccharide was the IgE-reactive epitope in the sheep milk extracts, an immunoblotting-inhibition assay was performed with sheep milk extract in the solid phase and α -gal epitope carriers (thyroglobulin and cetuximab) and cow and goat milk extracts as inhibitors (Figure). Both dilutions of cow thyroglobulin and cetuximab produced total IgE-binding inhibition on sheep milk extract with the 2 patient sera. Furthermore, partial inhibition was observed with goat milk extract with serum from patient 1.

Hypersensitivity reactions to sheep milk have been attributed mainly to caseins such a κ -casein (19 kDa) [5] and α -2-casein (35 kDa) [6], which cross-react with goat casein but not with cow casein, thus explaining how many patients tolerate cow milk [7]. The restricted specificity to β -casein in patients allergic to goat milk is directed mainly against the 49-79 domain, which differs from its bovine counterpart by only 3 amino acid substitutions [8]. Other reported allergens causing sheep milk allergy include ALA (15 kDa) and BLG (12 kDa) [5].

α -Gal epitopes from bovine γ -globulin (BGG), lactoferrin, and lactoperoxidase have been reported to be recognized by sIgE from patients with AGS. Besides, lactoperoxidase has been reported to be an allergen. BGG proteins of 58 and 80 kDa, lactoferrin protein of 75 kDa, and lactoperoxidase protein of 70 kDa have been detected in SDS-PAGE immunoblot analysis

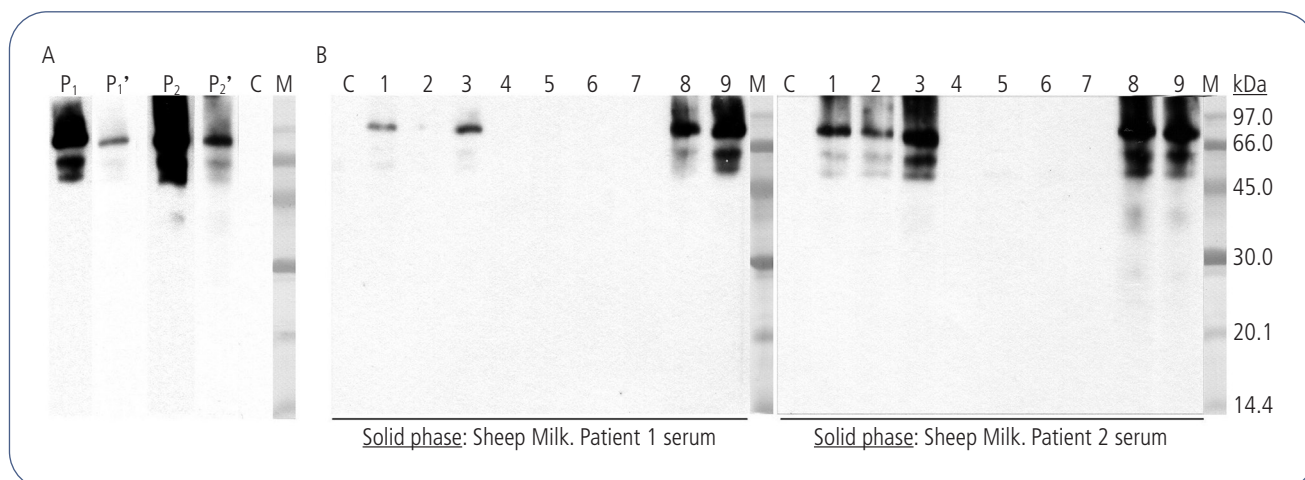


Figure. A, SDS-PAGE immunoblotting. Lane P₁, P₁', Patient serum 1. Two dilutions. Lane P₂, P₂', Patient serum 2. Two dilutions. Lane C, Control serum (pool of sera from nonatopic persons). Lane M, Molecular mass standard. B, SDS-PAGE immunoblotting-inhibition. Lane C, Control serum (pool of sera from nonatopic persons). Lanes 1-9, Patient serum preincubated with sheep milk extract (lane 1), goat milk extract (lane 2), cow milk extract (lane 3), cow thyroglobulin (100 µg/mL) (lane 4), cow thyroglobulin (200 µg/mL) (lane 5), cetuximab (100 µg/mL) (lane 6), cetuximab (200 µg/mL) (lane 7), chicken ovalbumin (lane 8), and sunflower pollen extract (lane 9). Lane M, Molecular mass standard.

using α -gal-sensitized patient sera, without recognition of the major milk allergens reported in genuine milk allergy, such as caseins, ALA, BLG, or BSA [9]. In addition, it was speculated that sensitization to lactoferrin might be a high-risk marker of anaphylaxis in association with high levels of α -gal sIgE in patients with AGS [10].

SDS-PAGE immunoblotting results suggest that proteins of 75 kDa and 58 kDa might be responsible for sheep cheese allergy in the patients we assessed. The molecular mass of these IgE-reactive proteins led us to suppose that they are lactoferrin and BGG, respectively. Results from the CAP inhibition and immunoblotting-inhibition assays point to α -gal as the epitope involved in IgE recognition, as suggested by the inhibition of total IgE obtained with the 2 α -gal carriers. No other epitopes seem to be responsible for the sheep cheese allergic reaction.

We present the first 2 cases of allergy to sheep cheese in AGS. The patients were also sensitized to other dairy products, although sensitization was not clinically relevant. We believe it is likely that sheep proteins contain a greater number of α -gal epitopes in their structures than cow and goat milk proteins. Further research should be performed in this area.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

1. Commins SP. Diagnosis & management of alpha-gal syndrome: lessons from 2,500 patients. *Expert Rev Clin Immunol.* 2020 Jul;16(7):667-77.
2. Stoltz LP, Cristiano LM, Dowling APG, Wilson JM, Platts-Mills TAE, Traister RS. Could chiggers be contributing to the prevalence of galactose-alpha-1,3-galactose sensitization and mammalian meat allergy? *J Allergy Clin Immunol Pract.* 2019 Feb;7(2):664-6.
3. Román-Carrasco P, Hemmer W, Cabezas-Cruz A, Hodžić A, de la Fuente J, Swoboda I. The α -Gal Syndrome and Potential Mechanisms. *Front Allergy.* 2021 Dec 16;2:783279.
4. Laemmli U. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature.* 1970;227(5259):680-5.
5. Hirsiger JR, Heijnen IA, Hartmann K, Berger CT. Anaphylaxis to goat's and sheep's milk in an adult tolerant to cow milk: A sensitization profile study. *J Investig Allergol Clin Immunol.* 2022;32(2):154-6.
6. Suzuki K, Futamura K, Hasegawa E, Aoki Y, Nakamura M, Matsunaga K, et al. Adult-Onset Sheep's Milk Allergy in a Patient Without Cow's Milk Allergy. *J Investig Allergol Clin Immunol.* 2021 Jun 22;31(3):273-4.
7. Martins P, Borrego LM, Pires G, Pinto PL, Afonso AR, Rosado-Pinto J. Sheep and goat's milk allergy--a case study. *Allergy.* 2005 Jan;60(1):129-30.
8. Bernard H, Ah-Leung S, Tilleul S, Drumare MF, Paty E, Bidat E, et al. Specificity of IgE antibodies from patients allergic to goat's milk and tolerant to cow's milk determined with plasmin-derived peptides of bovine and caprine β -caseins. *Mol Nutr Food Res.* 2012 Oct;56(10):1532-40.

9. Perusko M, Apostolovic D, Kiewiet MBG, Grundström J, Hamsten C, Starkhammar M, et al. Bovine γ -globulin, lactoferrin, and lactoperoxidase are relevant bovine milk allergens in patients with α -Gal syndrome. *Allergy*. 2021 Dec;76(12):3766-75.
10. Kiewiet MBG, Grundström J, Apostolovic D, Andersson M, Borres MP, Hamsten C, et al. Elucidating the α -Gal syndrome at the molecular allergen level. *Allergy*. 2021 May;76(5):1576-8.

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