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ORIGINAL ARTICLE

Allergy to goat and sheep cheese with tolerance to cow's milk and its derivatives

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Abstract
Objective: We present two adult and three paediatric patients who had allergic reactions after cheese ingestion and subsequently tolerated cow's milk derivatives. The objective of this study was to determine possible cross-reactivity between different types of cheese.
Methods: Skin tests were performed to cow's milk fractions, and prick–prick tests for goat, sheep and cow cheese. Specific IgE to the fractions of cow's milk and cow, sheep and goat cheese was analysed. The protein profile of cow, sheep and goat cheese extracts was determined by SDS-PAGE and the allergenic profile by immunoblot. Cross-reactivity was investigated by immunoblot inhibition.
Results: Skin tests were positive for casein in the patients. The prick–prick tests were positive for the three cheeses in patients 1 and 4, for goat and sheep cheese in patients 2 and 3, and for sheep cheese in patient 5. The specific IgE test was positive in patients 1, 3 and 4 for goat and sheep cheese, and negative in patients 2 and 5. Serum 3 and 4 clearly recognised goat and sheep cheese extracts. Goat casein was almost completely inhibited with sheep casein and partially inhibited with goat and sheep serum proteins, while there was no inhibition with cow cheese. Sheep casein was totally inhibited with sheep serum proteins. Sheep casein was inhibited with goat and cow caseins, suggesting cross-reactivity among the three types of cheese.
Conclusions: We showed sensitisation to goat and sheep cheese in two patients, and only to sheep cheese in another two of the studied patients.

Introduction

Allergy to cow's milk proteins is one of the most common food allergies in children and caseins are probably the primary allergens involved.[1] A high degree of

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cross-reactivity between the milk caseins of different mammals has previously been described.[2–5] Allergy to goat's milk showing cross-reactivity between sheep and goat casein, without reactivity with cow casein, was published in 1995 by Wüthrich and Johanson.[3] Since then, several cases have been reported of anaphylaxis and other allergic reactions related to goat's and sheep's milk and their derivatives, with good tolerance to cow's milk and its derivatives.[4–7]

Case reports

Patient 1: A 54-year-old male who presented anaphylaxis after the ingestion of cured cheese made from a mixture of goat's, sheep's and cow's milk. He subsequently tolerated cow's milk and its derivatives.

Patient 2: A 61-year-old woman who reported repeated episodes of angio-oedema of the lips, eyelids, tongue and glottis, which she occasionally related to eating cured cheese.

Patient 3: A nine-year-old boy diagnosed with allergy to nuts and seafood, who presented anaphylaxis after eating a portion of cheese. He subsequently tolerated cow's milk and its derivatives.

Patient 4: A six-year-old boy with a history of allergy to egg, which ceased at the age of five, who presented anaphylaxis after eating meat with Roquefort sauce. He subsequently tolerated cow's milk and its derivatives.

Patient 5: A nine-year-old boy allergic to nuts, egg, seafood and honey, who presented anaphylaxis after eating a mixture of cow's, sheep's and goat's cheese. He tolerated cow's milk without a problem.

Material and methods

Skin tests

Patients were skin prick tested with commercial casein, alpha-lactalbumin and beta-lactoglobulin extracts (Laboratorios Diater, Madrid, Spain). Prick-by-prick tests with goat, sheep and cow cheese were also performed.

Extracts manufacturing

Cow's, sheep's and goat's cheese was purchased at a local market. Serum proteins and casein of different cheeses were separated and extracts prepared. The protein content was measured by the Lowry-Biuret method (Sigma, St. Louis, Mo., USA).

Determination of total and specific IgE

Total and specific IgE, to cow's milk, cow's milk serum, casein alpha-lactalbumin, beta-lactoglobulin, sheep's milk and serum, goat's milk and serum, Cheddar cheese and a mixture of cheeses were quantitated by the ImmunoCAP® (Phadia, Uppsala, Sweden) technique.

Direct ELISA was used to determine specific IgE to the different cheese extracts in all patients.

Oral provocation tests

We performed oral provocation test with two different cheeses (cow's, sheep's and goat's mixture cheeses) only in patient 2 and they were all negative. In the rest of the patients we did not perform oral challenges with cheeses because they presented anaphylaxis with their ingestion and had positive skin prick tests. As all five patients tolerated cow's milk at home we considered that it was not necessary to perform oral provocation test with cow's milk.

SDS-PAGE and immunoblotting

A total of 40 μg of protein from each extract were loaded in a gel, run and stained with Biosafe Coomassie (Bio-Rad, Laboratories, Hercules, CA, USA.

Allergenic profile was studied by immunoblot. A total of 50 μg of protein from each extract were separated and electrotransferred to a PVDF and hybridised with the serum samples from different individuals (dilution 1/2).

Cross-reactivity studies

Immunoblot inhibition assays were performed to determine the possible cross-reactivity among different extracts. A serum pool from patients 3 and 4 was prepared (50%–50%). The goat and sheep cheese casein extracts were used in solid phase. The serum pool was inhibited with all the extracts (500 μg) used in the study.

Results

Skin tests

The skin prick tests with the commercial extracts were only positive to casein in the fourth patient. The prick-by-prick tests with the different types of cheese were positive for the three cheeses in patients 1 and 4, for goat and sheep cheese in patients 2 and 3, and for sheep cheese in patient 5.

Determination of total and specific IgE

Total and specific IgE values obtained by CAP are shown in Table 1. Specific IgE values obtained by direct ELISA are shown in Fig. 1. Patients 2 and 5 were negative for all extracts.

SDS-page

The protein profile of casein extracts showed two bands at 15 and 32 kDa approximately, being more prominent in sheep and cow casein extracts. In the whey protein extracts, the most abundant proteins corresponded to the 14 and 18 kDa bands The albumin band with 69.2 kDa was also observed in all cases (Fig. 2).

Table 1 IgE total values (U/l) and specific IgE values (kU/L). P.1: patient 1, P. 2: patient 2, P. 3: patient 3, P. 4: patient 4, P. 5: patient 5. Alpha: alpha-lactoalbumin, Beta: beta-lactoglobulin, SM: sheep's milk, SS: sheep's milk serum, GM: goat's milk, GS: goat's milk serum, CM: cow's milk, CS: cow's milk serum, Mix: Cheeses mix.

	Casein	Alpha	Beta	SM	SS	GM	GS	CM	CS	Cheddar	Mix	Total IgE
P. 1	<0.10	<0.10	<0.10	4.5	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	57.30
P. 2	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	<0.10	33.09
P. 3	<0.10	<0.10	<0.10	24.7	13.4	34.7	<0.10	0.13	1.10	<0.10	0.21	601.10
P. 4	2.78	<0.10	<0.10	43.1	34.3	53.3	<0.10	<0.10	<0.10	1.41	<0.10	227
P. 5	0.33	0.95	<0.10	0.67	<0.10	0.36	<0.10	0.53	<0.10	<0.10	<0.10	883.9

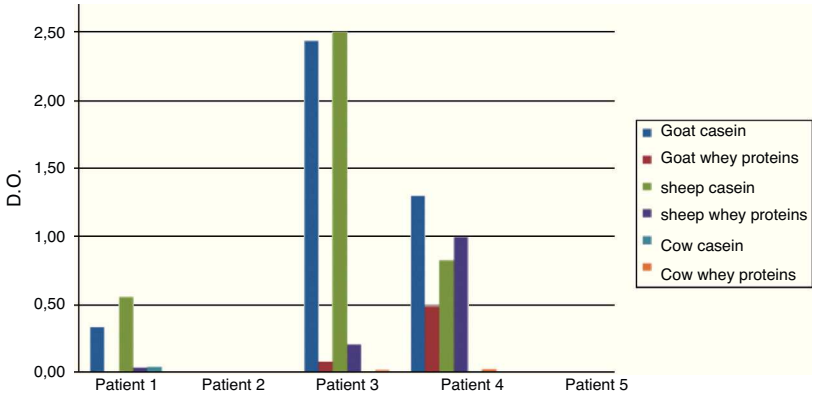

Figure 1 Specific IgE values by direct ELISA.

Immunoblotting

Patients 3 and 4 recognised more bands with greater intensity, and in both cases they were more abundant in goat's and sheep's cheese and very weak in cow's cheese. Greater intensity was also found in the casein fraction than in the whey proteins. Patient 2 was negative for all the extracts. Patients 1 and 5 recognised very weak bands in the sheep's cheese casein and a 14 kDa band in the cow cheese casein (Fig. 3).

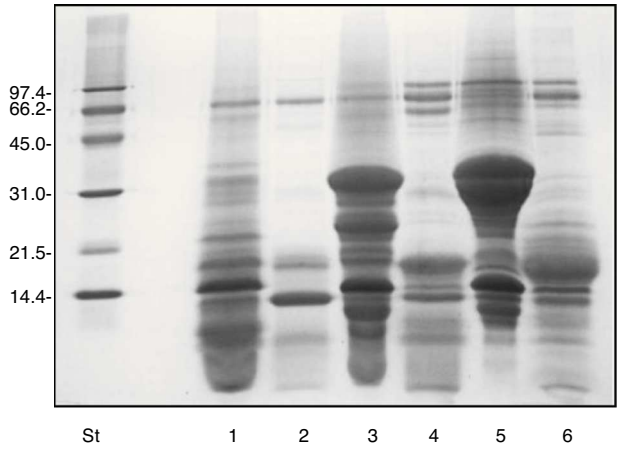

Figure 2 SDS-PAGE. 40 μg of protein by lane.
St- Molecular weight markers.
(1) Goat casein. (2) Goat whey proteins. (3) Sheep casein.
(4) Sheep whey proteins. (5) Cow casein. (6) Cow whey proteins.

Immunoblot inhibition

The goat casein (Fig. 4A) was almost completely inhibited with the sheep casein and partially inhibited with both goat and sheep whey proteins. There was no inhibition of either fraction with the cow cheese. Using the sheep casein in solid phase (Fig. 4B), there was total inhibition with the sheep whey proteins, and similar inhibition with the goat and cow fractions, with the 14 kDa band inhibited with both caseins (Fig. 4).

Discussion

Allergy to cow's milk is the most common cause of milk allergy in children and adults.[5,6] Most patients who are sensitised to cow's milk do not tolerate goat's or sheep's milk.[8,9]

The first paper showing cross-reactivity between sheep and goat casein, without reactivity with cow casein, was published in 1995 and used RAST inhibition.[3] Another paper was published in 1999 which included SDS-PAGE of goat, sheep and cow casein, and in which immunoblotting identified a number of IgE bands in goat and sheep casein, but not in cow casein.[5] Isolated cases of allergy to sheep's and goat's cheese with cow's milk tolerance were subsequently reported.[6,7] In 2004, Muñoz et al.[6] published the case of a boy who had allergic reactions when he ate sheep's cheese, showing selective recognition by ELISA inhibition and immunoblotting of epitopes specific to sheep and goat casein but not to cow casein. Moreover, in 2007, the Tavares group [7] identified a 14 kDa goat cheese protein that could be alpha-lactalbumin, as the allergen responsible for the

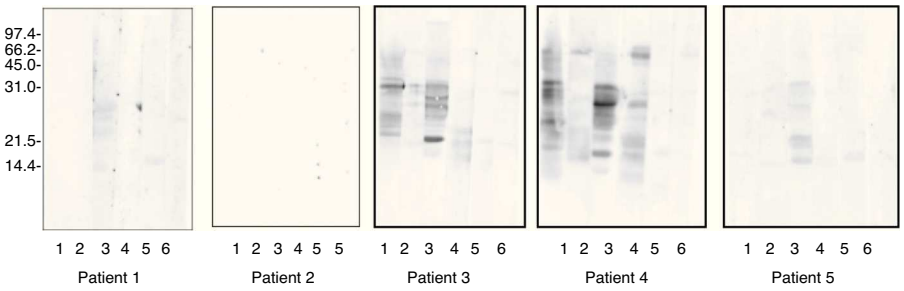

Figure 3 Immunoblotting. Dilution serum 1/2. Solid phase: 50 μg of protein of either extrac/lane. Lane 1: Goat casein. Lane 2: Goat whey proteins. Lane 3: Sheep casein. Lane 4: Sheep whey proteins. Lane 5: Cow casein. Lane 6: Cow whey proteins.

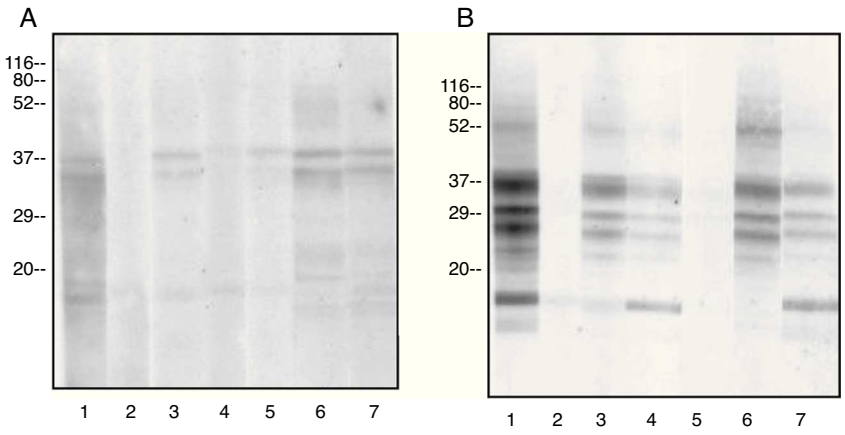

Figure 4 Inmmunoblot inhibition. Dilution serum 1/2. Solid phase: 50 μg protein/lane. Inhibition: 500 μg protein.
A: Solid phase: goat casein B: Solid phase: sheep casein
Lane 1: No inhibition Lane 1: No inhibition
Lane 2: Inhibition with goat casein Lane 2: Inhibition with sheep casein
Lane 3: Inhibition with goat whey proteins Lane 3: Inhibition with goat casein
Lane 4: Inhibition with sheep casein Lane 4: Inhibition with goat whey proteins
Lane 5: Inhibition with sheep whey proteins Lane 5: Inhibition with sheep whey proteins
Lane 6: Inhibition with cow casein Lane 6: Inhibition with cow casein
Lane 7: Inhibitio with cow whey proteins Lane 7: Inhibition with cow whey proteins
B: Solid phase: sheep casein
Lane 1: No inhibition Lane 1: No inhibition
Lane 2: Inhibition with goat casein Lane 2: Inhibition with sheep casein
Lane 3: Inhibition with goat whey proteins Lane 3: Inhibition with goat casein
Lane 4: Inhibition with sheep casein Lane 4: Inhibition with goat whey proteins
Lane 5: Inhibition with sheep whey proteins Lane 5: Inhibition with sheep whey proteins
Lane 6: Inhibition with cow casein Lane 6: Inhibition with cow casein
Lane 7: Inhibitio with cow whey proteins Lane 7: Inhibition with cow whey proteins.

sensitisation of a 27-year-old female patient who presented urticaria in relation to goat cheese consumption and tolerated cow's milk and sheep cheese.

We present a series of five cases, two of them with anaphylaxis, after the ingestion of cheese. Direct ELISA and immunoblotting suggest that sheep casein shows a high degree of cross-reactivity with goat casein but not with cow casein in our patients. In the immunoblot inhibition the 14 kDa sheep casein protein is inhibited with goat and cow caseins, suggesting cross-reactivity in the three types of cheese. This cross-reactivity of the 14 kDa band indicates that it is a common allergen as it is capable of inhibiting itself among different species, but the fact that the patients tolerate cow's milk suggests that they currently present weak sensitisation, with no clinical repercussions. None of the studies published before include a follow-up of their patients but it would be necessary because this high degree of cross-reactivity between milk proteins from different mammals and the possibility of new sensitisations is known. Our hypothesis about the cow's milk tolerance in our patients for instance is that the regular intake of cow's milk induces oral tolerance, so we did not ask them to stop in its ingestion but we think that more studies are necessary for monitoring these patients' evolution.

In conclusion we showed sensitisation to goat's and sheep's cheese in two patients and only to sheep's cheese in another two with good tolerance to cow's milk and its derivatives in all of them.

Ethical disclosures

Confidentiality of data. The authors declare that no patient data appears in this article.

Right to privacy and informed consent. The authors declare that no patient data appears in this article.

Protection of human subjects and animals in research. The authors declare that no experiments were performed on humans or animals for this investigation.

Conflicts of interest

The authors have no conflict of interest to declare.

References

1. Kohno Y, Honma K, Saito K, Shimojo T, Tsunoo H, Kaminogawa S, et al. Preferential recognitions of primary protein structures of casein by IgG and IgE antibodies of patients with milk allergy. Ann Allergy. 1994;73:419–22.
2. Spuergin P, Walter M, Schiltz E. Allergenicity of a-caseins from cow, sheep and goat. Allergy. 1997;52:293–8.
3. Wüthrich B, Johansson SGO. Allergy to cheese produced from sheep's and goat's milk but not to cheese produced from cow's milk. JACI. 1995;96:270–3.
4. Calvani M, Alessandri C. Anaphylaxis to sheep's milk cheese in a child unaffected by cow's milk protein allergy. Eur J Pediatr. 1998;157:17–9.
5. Umpiérrez A, Quirze S, Marañón F, Cuesta J, García-Villamuza Y, Lahoz C, et al. Allergy to goat and sheep cheese with good tolerance to cow cheese. Clin Exp Allergy. 1999;29: 1064–8.
6. Muñoz T, De la Hoz B, Marañón L, González R, Prieto P, Sánchez M. Selective allergy to sheep's and goat milk proteins. Allergol et Immunopathol. 2004;32:39–42.
7. Tavares B, Pereira C, Rodrigues F, Louriro G, Chieira C. Goat's milk allergy. Allergol et Immunolpathol. 2007;35: 113–6.
8. Ah-Leung S, Bernard H, Bidat E, Paty E, Rance F, Scheinnmann P, et al. Allergy to goat and sheep milk without allergy to cow's milk. Allergy. 2006;61:1358–65.
9. Bellioni-Businco, Paganelli R, Lucenti P, Giampietro PG, Perborn H, Businco L. Allergenicity of goat's milk in children with cow's milk allergy. J Allergy Clin Immunol. 1999;103: 1191–4.