

Allergy to goat and sheep cheese with good tolerance to cow cheese

A. UMPIÉRREZ, S. QUIRCE, F. MARAÑÓN*, J. CUESTA, Y. GARCÍA-VILLAMUZA, C. LAHOZ† and J. SASTRE

Servicio de Alergia, Fundación Jiménez Díaz, Universidad Autónoma de Madrid, *CBF Leti and

†Immunology Department, Fundación Jiménez Díaz, Madrid, Spain

Summary

Background We report on a patient who experienced allergic reactions after eating goat cheese and after touching goat and sheep cheese, but not after consuming cow's milk dairy products.

Objective To assess the allergenicity and IgE-binding capacity of the caseins from the three different species.

Methods Skin prick tests were carried out using whole milk and caseins from three different species (goat, sheep and cow), and whey fractions of cow's milk. Total serum IgE and specific IgE to cow's milk proteins were measured by CAP system and specific IgE against caseins and whole milk were determined by ELISA technique. To evaluate allergenic cross-reactivity, inhibition of the IgE ELISA activity to goat's milk and goat casein was tested for the three caseins. SDS-PAGE and immunoblotting was used to determine IgE binding bands in caseins.

Results Skin tests were positive to sheep and goat's milk, sheep and goat casein, as well as to sheep and goat cheese. Total serum IgE was 66 kU/L and IgE determinations by CAP were negative. IgE ELISA against the caseins from goat and sheep was strongly positive, whereas it was negative to cow casein. ELISA inhibition assays revealed a high degree of cross-reactivity between goat casein and sheep casein. Immunoblotting showed three IgE-binding bands in goat casein at 31, 27 and 22 kDa, which may correspond to α -, β - and γ -caseins. A band at about 31 kDa was observed in sheep casein and another band at 34 kDa was recognized in cow casein.

Conclusion This patient developed allergy to goat and sheep cheese with good tolerance to cow's milk. We identified goat casein as the main allergen causing sensitization in this patient as demonstrated by *in vivo* and *in vitro* tests. A high degree of cross-reactivity between goat and sheep casein was observed.

Keywords: casein allergens, casein allergy, cheese allergy, food allergy

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Introduction

Allergy reactions due to cow's milk proteins have been considered one of the most common causes of food allergy among infants [1]. The major allergenic proteins in cow's milk are β -lactoglobulin, α -lactalbumin, caseins and serum

albumin [2]. Recent studies suggest that casein may be the main allergen both in children [3] and adults [4]. Caseins represent 80% of mass in milk, and the existence of cross-reactivity between milk caseins from different animals has been reported [4,5].

We describe the case of a patient who had allergic reactions after eating goat cheese and after touching goat and sheep cheese, but not after consuming cow's milk or cow's milk dairy products. In this study, we have examined

Correspondence: J. Sastre, Servicio de Alergia, Fundación Jiménez Díaz, Av. Reyes Católicos 2, 28040 Madrid, Spain.

the allergenicity and IgE-binding capacity of three caseins from different species by means of immunoblotting, ELISA and ELISA-inhibition assays.

Case report

The patient is a 2-year-old girl with a family background of atopy (her father suffered from hay fever due to grass pollen). She was exclusively breast-fed for the first 2 months of life, and thereafter, she had received a cow's milk formula (Nativa®). Other foodstuffs, which were introduced as recommended, were well tolerated with the exception of legumes. At 1 year of age she experienced an episode of wheezing and cough within 5 min after the ingestion of lentils, and following the ingestion of chick-peas, she developed generalized urticaria. In the last 3 months, she had experienced at least three episodes of cough and wheezy dyspnea, together with swelling of the eyelids and watery eyes, within 10 min after eating goat cheese. However, 1 month ago she had had a small amount of goat cheese without any clinical manifestation. After touching goat cheese, she also noted the appearance of urticaria on her hands which spread to other parts of her body in contact with her hands. Cutaneous symptoms also occurred when the patient touched sheep cheese, although she has never eaten this kind of cheese. These reactions cleared up 1 h thereafter without any medication and she had no further reactions after dietary elimination of these types of cheese. She had no problem when eating cheese made from cow's milk or when drinking cow's milk. Her physical examination at the time of consultation was normal. The patient's weight was 15 kg and the height was 91 cm (both at 97th percentile). Blood differential count showed 510 eosinophils/mm³.

Materials and methods

Skin tests

Skin tests were carried out with the skin prick method as described [6] using a battery of common inhalant and food allergens, as well as whole milk and caseins (Sigma Chemical Co., St Louis, MO, USA) from three different species (goat, sheep and cow), and whey fractions of cow's milk (α -lactalbumin, β -lactoglobulin and albumin) (ALK-Abelló, Madrid, Spain). Skin tests with lysozyme and pepsine (Sigma Chemical Co) that are used for the production of cheese, were also carried out. All milk-derived proteins were tested at a concentration of 1 mg/mL. We also performed skin prick-by-prick test with cheese made from cow, sheep and goat and their corresponding whole milk.

Histamine hydrochloride at 10 mg/mL and saline solution were used as positive and negative controls, respectively.

Results of skin prick tests were expressed as the two largest weal orthogonal diameters in mm. A weal with a mean diameter of 3 mm, or greater, together with erythema occurring 15 min after testing was defined as a positive reaction, being negative the skin prick test with the saline at the same time.

Total and specific IgE determination

Total serum IgE and specific IgE to cow's milk proteins were measured with the Pharmacia CAP system IgE FEIA (Uppsala, Sweden), according to the manufacturer's instructions. The results were expressed in kilounits per litre (kU/L) and CAP scores higher than 0.35 kU/L were regarded as positive. Specific IgE against caseins and whole milk from the three different species were determined in duplicate by ELISA technique using a concentration of 10 μ g/mL and serum dilution 1 : 2, as previously described [7]. A result was considered positive when a serum bound four times more specific IgE than the titre of one atopic negative control.

ELISA inhibition

For determination of allergenic cross-reactivity, inhibition of the IgE binding to goat's milk and goat casein was tested for caseins from all three species. The concentration which produced 50% inhibition was calculated, as compared with a saline control.

SDS-PAGE and Immunoblotting

Sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out following the Laemmli method [8]. Polyacrylamide concentrations of 12 and 4% were used for separating and stacking gels, respectively. Three types of commercial purified caseins (Sigma Chemical Co.) were separated by SDS-PAGE: cow casein, goat casein and sheep casein. A 30 μ g protein amount was applied per lane. Electrophoresis was carried out applying 100 V for 1 h, and the protein bands were visualized with Coomassie brilliant blue.

Electrotransfer of proteins to the nitrocellulose membrane (Bio-Rad, Richmond, CA, USA) was carried out according to Towbin *et al.* [9]. The membrane was then incubated with the patient serum diluted 1 : 6 in 10 mmol/L TRIS-buffered saline containing 1% Polyoxyethylenesorbitan monolaurate (TWEEN 20) and 3% bovine serum albumin. After washing, the membrane was incubated with antihuman IgE conjugated with peroxidase (The Binding Site, Birmingham, UK). The protein bands were developed by chemoluminescence with ECL (Amersham International plc, Amersham, UK).

Results

Skin tests

Skin prick tests were positive with sheep's milk (8×6 mm), sheep casein (11×5 mm) and goat casein (8×5 mm), with lentil and chickpea (5×3 mm and 6×4 mm, respectively). All the other extracts tested were negative, including the common inhalant allergens.

Skin prick-by-prick tests were positive with sheep whole milk (15×6 mm), sheep cheese (6×5 mm), goat whole milk (4×4 mm) and goat cheese (8×4 mm). Cow whole milk and its corresponding casein elicited no reaction. The same tests carried out in three healthy volunteers were all negative.

Total and specific IgE determination

Total serum IgE was 66 kU/L, and specific IgE to cow's milk proteins by CAP (α -lactalbumin, β -lactoglobulin and casein) were negative (<0.35 kU/L). Specific IgE determinations were positive to lentil (65.1 kU/L), chickpea (3.54 kU/L) and white bean (2.38 kU/L). IgE ELISA results to the caseins and whole milk, expressed in optical densities as the mean of duplicate determinations, were positive to sheep casein (1.08), sheep's milk (0.44), goat casein (0.89) and goat's milk (0.42), and were negative to cow casein (0.01) and cow's milk (0.01). Negative control was ≤ 0.01 optical densities for all the extracts aforementioned.

ELISA inhibition

When goat's milk was used in the solid phase, goat casein and sheep casein were able to inhibit by 90% the specific IgE binding to goat's milk, showing a great affinity. Although cow casein was also able to inhibit IgE binding to goat's milk, it was in a dose-dependent manner. Thus,

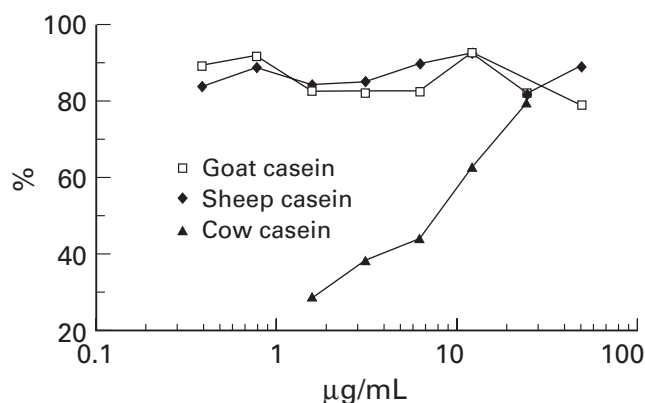


Fig. 1. ELISA inhibition of goat's milk (solid phase) using caseins from goat, sheep and cow as inhibitor allergens.

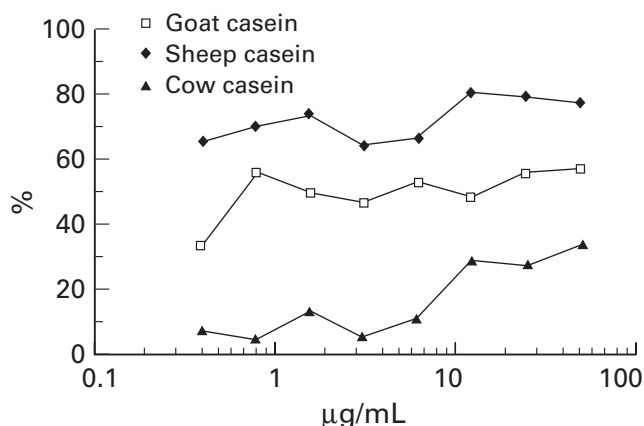


Fig. 2. ELISA inhibition of goat casein (solid phase) using caseins from goat, sheep and cow as inhibitor allergens.

cow casein at a concentration of $6.04 \mu\text{g/mL}$ produced a 50% inhibition of ELISA activity to goat's milk (Fig. 1).

When goat casein was used in the solid phase, significant degrees of inhibition were achieved with sheep casein (maximum inhibition 80%) and goat casein (60%), whereas the lowest inhibition was observed with cow casein (30%) (Fig. 2).

SDS-PAGE and immunoblotting

Figure 3 shows the results of SDS-PAGE analysis of the casein proteins. In lane 3 (goat casein), several bands were observed in the region between 15 and 45 kDa. Four bands found within that range may correspond to α -casein (about 31 kDa), β -casein (≈ 27 kDa), γ -casein (22 kDa) and

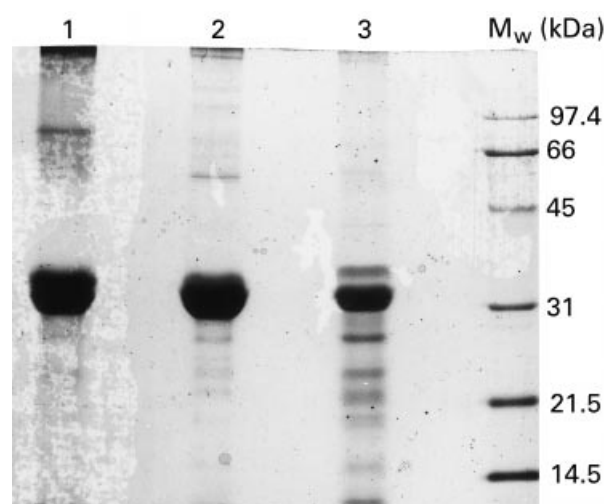


Fig. 3. SDS-PAGE of cow casein (lane 1), sheep casein (lane 2) and goat casein (lane 3). M_w : molecular weight markers.

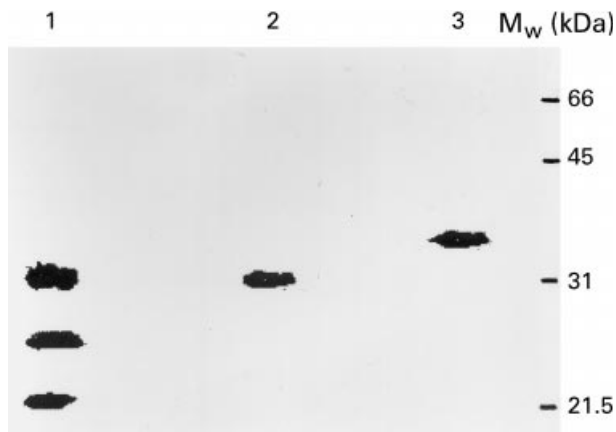


Fig. 4. IgE immunoblots of goat casein (lane 1), sheep casein (lane 2) and cow casein (lane 3). M_w : molecular weight markers.

κ -casein (19 kDa). Bands corresponding with 31 and 27 kDa were also observed in lanes 1 and 2 (cow casein and sheep casein, respectively). Weak bands of molecular weight greater than 66 kDa were observed only in lanes 1 and 2.

Immunoblotting analysis of the three kinds of caseins is shown in Fig. 4. Immunoblots with patient serum showed IgE-binding bands of molecular weight between 22 and 45 kDa. In lane 1 (goat casein) three bands with apparent molecular weight about 31, 27 and 22 kDa were observed, which may correspond with α -, β - and γ -caseins. A band of molecular weight similar to goat α -casein (31 kDa) was observed in lane 2 (sheep casein) and another band corresponding to 34 kDa was recognized in lane 3 (cow casein).

Discussion

Casein is composed of different proteins with defined and completely different sequences: α -, β -, κ - and γ -caseins. α -casein is subdivided into two fractions: α_{s1} - and α_{s2} -casein. The former is quantitatively more important and it is one of the major allergens of cow's milk [10]. Cross-reactivity among caseins from cow, goat and sheep is due to the similarity of the amino acid sequence [4,5,11]. However, allergy to goat and sheep's milk in the absence of cow's milk allergy has been reported [12,13]. A case of allergy reaction to mare milk has also been described in a woman who was able to tolerate cow's milk [14]. Our patient suffered from allergic symptoms caused by goat and sheep cheese. However, she was also able to tolerate cow's milk and cheese without any ill effect. Skin prick test and specific IgE results were consistent with a typical IgE-mediated reaction to casein from goat and sheep, whereas results of the same immunological tests with cow's milk and its fractions were negative. Although the double-blind challenge test is considered the 'gold standard' for the

diagnosis of food allergy, this challenge may be omitted when a patient with evidence of food-specific IgE has a convincing history of a severe allergic reaction to food [15].

The results of the IgE ELISA showed differences in the IgE-binding capacity of milk and caseins for the three species. Serum IgE antibodies bound less to cow's milk and its casein than to goat's and sheep's milk and their caseins. The results of ELISA inhibition suggest that goat casein shows a high degree of cross-reactivity with sheep casein and, to a much lesser extent, with cow casein. Moreover, goat casein seems to be responsible for most of the allergic activity of goat's milk. Immunoblotting showed IgE-binding bands of similar apparent molecular weight in caseins from goat and sheep (α -casein), whereas a band of different molecular weight was observed in cow casein. Taken together, these results point out that caseins from different species may be the source of common and species-specific IgE epitopes. In conclusion, we identified casein from goat's milk as the main allergen causing sensitization in this patient. Since individual milk proteins (i.e. casein) are present as hidden allergens or additives in several foods, drinks, and cosmetics, the need to use purified preparations of these proteins for the correct diagnosis of allergic reactions and avoidance of allergen should be emphasized.

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