

Selective allergy to sheep's and goat's milk proteins

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ABSTRACT

Background: Cow's milk proteins are amongst the most common causes of food allergy in infants, and caseins are probably the main allergens. The existence of a high degree of cross-reactivity between milk caseins from different animals has been reported. We describe a 2-year-old boy who experienced allergic reactions after eating and touching sheep's cheese, but who tolerated cow's milk and cow's milk dairy products. He had never ingested milk or milk derivatives from sheep or goat.

Methods: Skin prick tests were carried out using whey fractions of cow's milk, whole milk and casein from goat, sheep and cow. We also performed skin prick tests with enzymes used in cheese production. Prick-by-prick tests with cheese made from cow, sheep and goat and their corresponding whole milk were also performed. Total serum IgE and specific IgE to cow's milk proteins, whole cow's milk and sheep's milk were determined. Specific IgE against casein and whole milk from the three different species were determined by ELISA. Inhibition of IgE binding to bovine casein was tested for casein and whole milk from all three species. The proteins of three types of casein and whole milk from cow, sheep and goat were separated by SDS-PAGE and were incubated with the patient's serum.

Results: Skin tests were positive to sheep's milk and goat and sheep casein and were negative to all cow's milk proteins and whole cow's and goat's milk. Prick-by-prick tests were positive to goat's and sheep's cheese and were negative to cow's cheese. In ELISA-inhibition, sheep's milk and goat and sheep casein were able to inhibit > 50 % of specific IgE binding to sheep casein. The results of immunoblotting showed that the patient's circulating IgEs recognized only one band in the lanes corresponding to sheep and goat casein.

Conclusions: We report a patient with allergy to sheep's and goat's milk proteins but not to cow's milk proteins. Sheep casein was probably the main allergen causing sensitization in this patient. The results suggest that sheep casein shows a high degree of cross-reactivity with goat casein but not with cow casein. Our patient presented allergic symptoms caused by sheep and goat milk and cheese proteins. However, he was able to tolerate cow's milk and cow's milk dairy products without any ill effects.

Key words: Allergy. Children. Protein. Cow's milk allergy. Food allergy. Casein.

RESUMEN

Introducción: La alergia a proteínas de la leche de vaca es una de las causas más frecuentes de alergia alimentaria en la infancia y las caseínas son probablemente los principales alérgenos implicados. Ya ha sido descrita con anterioridad la existencia de alto grado de reactividad cruzada entre las caseínas de distintos animales.

Describimos un niño de 2 años que presentó reacciones alérgicas tras la ingesta y el contacto con queso de oveja, pero que toleraba el consumo de leche

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de vaca y derivados. Nunca había tomado previamente leche o derivados de oveja o cabra.

Métodos: Se realizaron tests cutáneos mediante prick con leche y caseína de vaca, oveja y cabra así como con las fracciones de la leche de vaca. También se realizaron tests cutáneos con enzimas empleadas en la elaboración de queso. Se llevaron a cabo prick-prick con leche entera y queso de vaca, oveja y cabra.

Se determinó IgE total y específica frente a leche de vaca y sus fracciones y leche de oveja. También se determinó mediante ELISA la IgE específica frente a caseína y leche entera de las tres especies.

Asimismo se calculó mediante ELISA-inhibición el porcentaje de inhibición de la caseína bovina frente a caseína y leche entera de las tres especies.

Se separaron las proteínas de la leche y caseína de vaca, oveja y cabra mediante SDS-PAGE y posteriormente fueron incubadas con el suero del paciente.

Resultados: Los tests cutáneos resultaron positivos frente a leche de oveja y caseína de oveja y cabra, y negativos frente a las proteínas séricas de vaca, así como frente a leche entera de vaca y cabra. Los prick-prick fueron positivos frente a queso de oveja y cabra, siendo negativos frente a queso de vaca.

En el ELISA-inhibición, la leche de oveja y la caseína de oveja y cabra fueron capaces de inhibir más del 50 % de los sitios de unión de la IgE a la caseína de oveja.

Los resultados del inmunoblot mostraron que los anticuerpos IgE del paciente reconocían solo una banda en las calles correspondientes a las caseínas de oveja y cabra.

Conclusiones: Presentamos un paciente con alergia a proteínas de la leche de oveja y cabra pero no a las proteínas de la leche de vaca. La caseína de la oveja es probablemente el principal alérgeno causante de la sensibilización de este sujeto.

Los resultados sugieren que la caseína de oveja muestra un elevado grado de reactividad cruzada con la caseína de cabra, pero no con la de vaca.

Nuestro paciente presentaba síntomas alérgicos causados por la exposición frente a las proteínas de la leche y queso de oveja y cabra, pero es capaz de tolerar el consumo de leche y derivados de vaca sin ningún efecto adverso.

Palabras clave: Alergia. Niños. Proteínas. Alergia a la leche de vaca. Alergia alimentaria. Caseína.

INTRODUCTION

Cow's milk proteins are among the most common causes of food allergy in infants, and caseins are pro-

bably the main allergens¹ The existence of high degree of cross-reactivity between milk caseins from different animals have been reported²⁻⁵.

CASE REPORT

A 2-year-old boy developed urticaria and angioedema 15 minutes after eating sheep cheese. Afterwards, experienced contact urticaria touching sheep cheese. He tolerated cow's milk and cow's milk dairy products. He had never ingested milk or milk derivatives from sheep or goat.

MATERIALS AND METHODS

Skin tests

Skin prick test (SPT) were carried out using whey fractions of cow's milk, whole milk and caseins (Sigma Chemical Co.,USA) from goat, sheep and cow. We also performed SPT with *Lactococcus alactis* and *Streptococcus termophilus* (used in cheese manufacture). Prick-by-prick test with cheese made from cow, sheep and goat and their corresponding whole milk were also performed.

Total and specific IgE determination

Total serum IgE and specific IgE (CAP Pharmacia) to cow's milk proteins, whole cow's milk and sheep's milk were determined. Specific IgE against caseins and whole milk from the three different species were determined by ELISA technique using a concentration of 10 µg/ml and 1:10 serum dilution as previously described⁶.

ELISA inhibition

Inhibition of the IgE binding to bovine casein was tested for caseins and whole milk from all three species. The concentration which produced 50 % inhibition was calculated.

SDS-PAGE and Immunoblotting

The proteins of three types of commercial purified caseins and whole milk from three species were separated by SDS-PAGE (sodium dodecylsulphate-polyacrylamide gel electrophoresis), following the Laemmli method⁷.

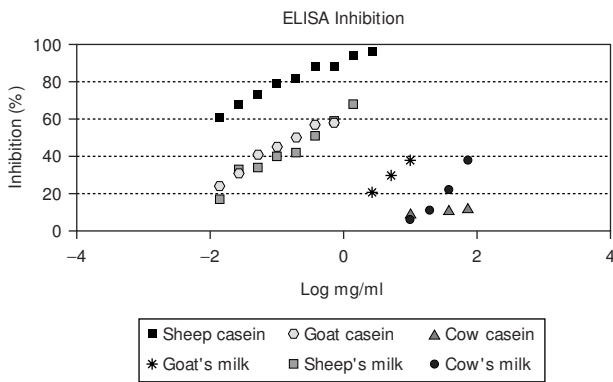


Figure 1.—ELISA Inhibition of sheep casein (solid phase) using caseins and whole milks from sheep, goat and cow as inhibitor allergens.

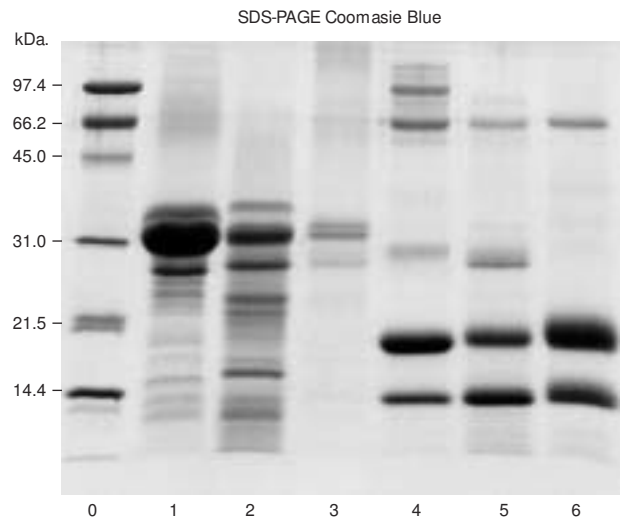


Figure 2.—SDS-PAGE of sheep casein (lane 1), goat casein (lane 2), cow casein (lane 3), sheep's milk (lane 4), goat's milk (lane 5) and cow's milk (lane 6). Lane 0: molecular weight markers.

After SDS-PAGE, transfer of proteins from the gel onto the nitrocellulose membrane was carried out according to Towbin et al⁸. The membrane was then incubated with the patient serum. After washing, the membrane was incubated with antihuman IgE conjugated with peroxidase.

RESULTS

Skin tests

SPT were positive to sheep's milk and goat and sheep caseins and negative to all cow's milk proteins and whole cow's and goat's milk.

Prick-by-prick tests were positive to goat's and sheep's cheese and negative to cow's cheese.

Total and specific IgE determination

Total serum IgE was 40 KU/l. Specific IgE antibodies by CAP were negative (< 0.35 KU/l) to all cow's milk proteins and positive to sheep's milk (2.85 KU/l). IgE ELISA results were positive (> 0.250 expressed in optical densities) to sheep casein (0.548 OD), goat's milk (0.262 OD) and goat casein (0.317 OD).

ELISA inhibition

When sheep casein was used in the solid phase, sheep's milk and goat and sheep casein were able to inhibit > 50 % the specific IgE binding to sheep casein. The lowest inhibition was observed with cow casein (12.4 %) (fig. 1).

BLOT IgE

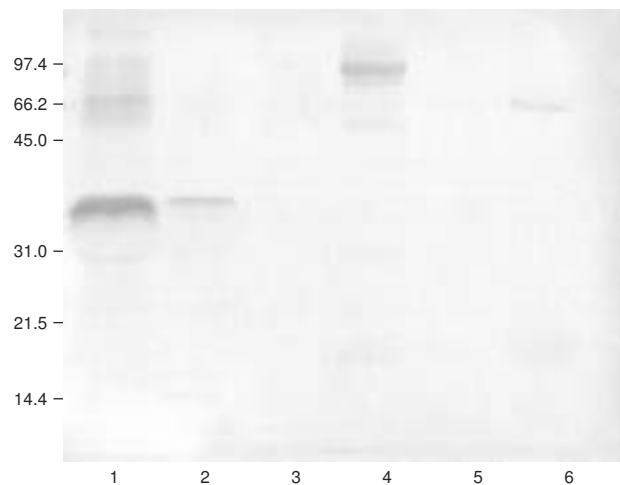


Figure 3.—IgE immunoblots of sheep casein (lane 1), goat casein (lane 2), cow casein (lane 3), sheep's milk (lane 4), goat's milk (lane 5) and cow's milk (lane 6).

SDS-PAGE and Immunoblotting

The results of SDS-PAGE are shown in figure 2. Bands corresponding with 34 and 24 kDa were observed in casein lanes. Three bands that may correspond to α -lactalbumin, β -lactoglobulin and serum albumin were found in lanes corresponding to whole milks.

Figure 3 shows the results of Immunoblotting analysis. Circulating IgEs of patient recognize only one band in caseins of sheep and goat lanes with molecular weight about 33-34 kDa.

FOLLOW UP

The patient was able to eat cow milk dairy products and after one year of following up the tolerance persists.

DISCUSSION

Selective allergy to sheep's milk products in the absence of cow's milk allergy is quite rare³⁻⁵. Reports published to date are few. The first article published in 1995³ showed the existence of cross-reactivity between sheep's and goat's casein, but not with cow casein demonstrated by RAST inhibition. The last one appeared in 1999⁵ included in its study SDS-PAGE of milk caseins from cow, sheep and goat and immunoblotting which identifies a number of IgE-binding bands in goat and sheep casein but not in cow casein.

We report on a patient with allergy selective caused by sheep's and goat's milk proteins, but not to cow's milk proteins. We demonstrated by *in vivo* and *in vitro* tests that sheep casein is probably the main allergen causing sensitization in this patient.

The mayor cow's milk antigens are the whey proteins α -lactalbumin, β -lactoglobulin, bovine serum albumin and the caseins⁹. Recent studies suggest that casein may be the main allergen implicated in the IgE-mediated milk allergy, both in children¹ and adults¹⁰. Casein is composed of different proteins with several sequences: α -(subdivided into two fractions: α s1- and α s2-), β -, κ - and γ -caseins. From these, α -casein is quantitatively more important and it is one of the major allergens of cow's milk¹¹. The analysis of the amino acid sequences of the four fractions of caseins from ruminant species evidence high sequence homologies varying from 80% to more than 90%; this large identity facilitates immunological cross-reactivity².

However, the results of the ELISA inhibition suggest that sheep casein show a high degree of cross-reactivity with goat casein but not with cow casein in this case. Nevertheless, this child never showed any clinical reaction to cow's milk, and skin prick-by-prick test with whole cow's milk and its casein elicited no reaction. Immunoblotting showed IgE-binding bands of similar molecular weight in lanes corresponding with sheep and goat caseins, whereas any band was observed in cow casein. All of our results point out a selective immunological recognition to specific epitopes in caseins from sheep and

goat but not to casein from cow explaining the clinical tolerance to cow milk products in this patient. Few structural variants in the amino acids sequence may affect the allergenicity of caseins.

None of the studies published before include a follow of patients. However, it would be necessary a continuous evaluation of the patient because there is a high degree of cross-reactivity between milk proteins from different mammals (not only caseins but also other milk proteins) and the possibility of new sensitizations. We recommend periodic allergological controls with the purpose of detecting new sensitizations to cow's and other mammal's milk and dairy products as soon as possible. We allowed the ingestion of milk and derivatives from cow but not from another mammals. It is very important make sure that the tolerance persists.

In conclusion, our patient was diagnosed from selective allergy caused by casein from sheep's and goat's milk.

REFERENCES

1. Kohno Y, Honmak et al. Preferential recognition of primary protein structures of casein by IgG and IgE antibodies of patients with milk allergy. *Ann Allergy* 1994;73:419-22.
2. Spuergerin P, Walter M, Schiltz E. Allergenicity of α -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, Quirce S, Marañón F et al. Allergy to goat and sheep cheese with good tolerance to cow cheese. *Clinical and Experimental Allergy* 1999;29:1064-8.
6. Fernández-Caldas E, Quirce S, Marañón F, Díez Gómez ML et al. Allergenic cross-reactivity between third stage larvae of *Hysterothylacium aduncum* and *Anisakis simplex*. *JACI* 1998;101:554-5.
7. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970;227:680-5.
8. Towin H, Staehelin Y, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitro-cellulose sheets: procedure and some applications. *Proc Natl Acad Sci USA* 1979;76:4350-4.
9. Räsänen L, Lehto M, Reunala T. Diagnostic value of skin and laboratory tests in cow's milk allergy/intolerance. *Clin Exp Allergy* 1992;22:385-90.
10. Stöger P, Wüthrich B. Type I allergy to cow milk proteins in adults. A retrospective study of 34 adult milk- and cheese-allergic patients. In *Arch Allergy Immunol* 1993;102:399-407.
11. Yunginger JW. Food antigens. In: Metcalf DD, Sampson HA, Simon RA, eds. *Food allergy: Adverse reactions to foods and food additives*. Oxford: Blackwell Scientific 1991; pp. 36-51.