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 alergenos 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...
de vaca y derivados. Nunca había tomado previa-
mente 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 fren-
te 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
deva, oveja y cabra mediante SDS-PAGE y posterior-
mente fueron incubadas con el suero del paciente.

**Resultados:** Los tests cutáneos resultaron positi-
vos 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 caseí-
na 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 immunoblot mostraron que los
anticuerpos IgE del paciente reconocían solo una
banda en las calles correspondientes a las caseínas
de oveja y cabra.

**Conclusions:** Presentamos un paciente con aler-
gia a proteínas de la leche de oveja y cabra pero no a
las proteínas de la leche de vaca. La caseína de
oveja es probablemente el principal alergeno causan-
te 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
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

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**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 de-
gree 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 alac-
tis and Streptococcus thermophilus (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 concentra-
tion of 10 μg/ml and 1:10 serum dilution as pre-
viously described6.

**ELISA inhibition**

Inhibition of the IgE binding to bovine casein was
tested for caseins and whole milk from all three spe-
cies. The concentration which produced 50 % inhibi-
tion was calculated.

**SDS-PAGE and Immunoblotting**

The proteins of three types of commercial purified
caseins and whole milk from three species were se-
parated by SDS-PAGE (sodium dodecylsulphate-po-
yacrylamide gel electrophoresis), following the
Laemmli method7.
After SDS-PAGE, transfer of proteins from the gel onto the nitrocellulose membrane was carried out according to Towbin et al. 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).

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 rare\(^5\). Reports published to date are few. The first article published in 1995\(^3\) showed the existence of cross-reactivity between sheep's and goat's casein, but no with cow casein demonstrated by RAST inhibition. The last one appeared in 1999\(^5\) 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 \(\alpha\)-lactalbumin, \(\beta\)-lactoglobulin, bovine serum albumin and the caseins\(^9\). Recent studies suggest that casein may be the main allergen implicated in the IgE-mediated milk allergy, both in children\(^1\) and adults\(^10\). Casein is composed of different proteins with several sequences: \(\alpha\)-(subdivided into two fractions: \(\alpha_s1\) and \(\alpha_s2\)), \(\beta\), \(\kappa\) and \(\gamma\)-caseins. From these, \(\alpha\)-casein is quantitatively more important and it is one of the major allergens of cow's milk\(^11\). 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-reactivity\(^2\).

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 non 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 sensitisations. We recommend periodic allergological controls with the purpose of detecting new sensitisations 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

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