CASE REPORT

Phosphorylation Reduces the Allergenicity of Cow Casein in Children With Selective Allergy to Goat and Sheep Milk

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Abstract

This study aimed to characterize the role of phosphorylation of caseins in selective allergy to goat milk (GM) and sheep milk (SM) in patients with good tolerance to cow milk (CM).
We performed skin prick tests with milk and caseins from CM, GM, and SM and immunoblotting and specific immunoglobulin (Ig) E determinations with milk and casein from cow and GM and SM.
Sensitization to milk and caseins from goat and sheep was demonstrated in all 3 patients by skin tests, determination of specific IgE, or both. Immunoblotting confirmed that GM/SM proteins but not CM proteins were involved in the allergic symptoms. IgE reacted with several protein bands from the caseins and milk extracts of both sheep and goat. Phosphorylation was involved in the different allergenicity of CM caseins.
We report the implication of phosphorylation in the allergenicity of caseins involved in selective allergy to GM and SM.

Key words: Casein. Cow milk allergy. Food allergens. Phosphorylation.

Resumen

El objetivo fue caracterizar el papel de la fosforilación de las caseínas en la alergia selectiva a leche de cabra (GM) y oveja (SM), con tolerancia a leche de vaca (CM).
Se realizaron pruebas cutáneas con leche y caseínas de vaca, cabra y oveja. Se efectuaron determinaciones de IgE a leche y caseína de vaca, leche de cabra y oveja, así como inmunodetección.
Mediante pruebas cutáneas y/o determinación de IgE específica se demostró sensibilización a leche y caseínas de cabra y oveja en los 3 pacientes. La inmunodetección confirmó la implicación de proteínas de GM/SM, y no de CM, en los síntomas alérgicos. La IgE reaccionaba con varias bandas proteicas de caseínas y de la leche de oveja y cabra. La fosforilación de las caseínas de vaca está implicada en su diferente alergenicidad.
Describimos la implicación de la fosforilación de caseínas en alergia selectiva a leche de cabra y oveja.

Palabras clave: Caseína. Alergia a leche de vaca. Alérgenos alimentarios. Fosforilación.

Introduction

Cow milk (CM) allergy occurs predominantly during the first 6 months of life, coinciding with its introduction into the infant's diet. CM contains several allergens, namely, whey proteins (ß-lactoglobulin, α-lactalbumin, and serum albumin) and caseins (α-s1 casein, α-s2 casein, ß-casein and κ-casein). In the initial diagnosis of allergy to CM proteins, sensitization to whey proteins is the most frequent finding, whereas elevated casein-specific immunoglobulin (Ig) E levels are a marker of persistent allergy [1].

The treatment of choice for CM allergy is an exclusion diet and administration of a substitute formula. Allergy to goat milk (GM) and sheep milk (SM) is less common and occurs later than CM allergy, since children are older when they eat cheese or dairy products containing these types of milk.

Allergy to CM, GM, and SM is due to extensive cross-reactivity between their caseins [2], which show high amino acid sequence homology. However, some patients with CM allergy tolerate GM and SM [3], and vice versa [4]. The considerable conservation of the phosphorylated sites emphasizes the importance of phosphorylated residues in the functioning of caseins [5]; this structural feature may play an important role in their allergenicity. With the exception of cow casein, which is hyperphosphorylated, α-s1 caseins from the milk of other mammals are known to be hypophosphorylated [6]. The IgE response to caseins is affected by modifying or eliminating the major phosphorylation site [7].

Case Descriptions

We report 3 CM-tolerant children (2 boys and 1 girl, age 4-5 years) who experienced immediate allergic reactions upon ingestion of cheese made from GM and SM or other dairy products. Two of the patients experienced generalized urticaria and angioedema after the ingestion of minimal amounts of cheese, followed by cough, stridor, and shortness of breath in

Table. Results of Skin Prick Tests and Specific Immunoglobulin E Measurements

	Case 1		Case 2		Case 3	
	SPT, mm	IgE, kU_A/L	SPT, mm	IgE, kU_A/L	SPT, mm	IgE, kU_A/L
Sheep milk	8	1662	6.5	26.6	4.5	164
Sheep casein	11	NA	6	NA	NA	NA
Goat milk	9.5	1464	4.5	24.3	3	129
Goat casein	10.5	NA	6	NA	NA	NA
Cow milk	5.5	<0.35	0	0.61	4.5	7.8
Cow casein	5.5	<0.35	0	<0.35	0	12.2
ß-lactoglobulin	0	<0.35	0	<0.35	0	<0.35
α-lactalbumin	0	<0.35	0	<0.35	0	<0.35

Abbreviations: Ig, immunoglobulin; NA, not available; SPT, skin prick test.

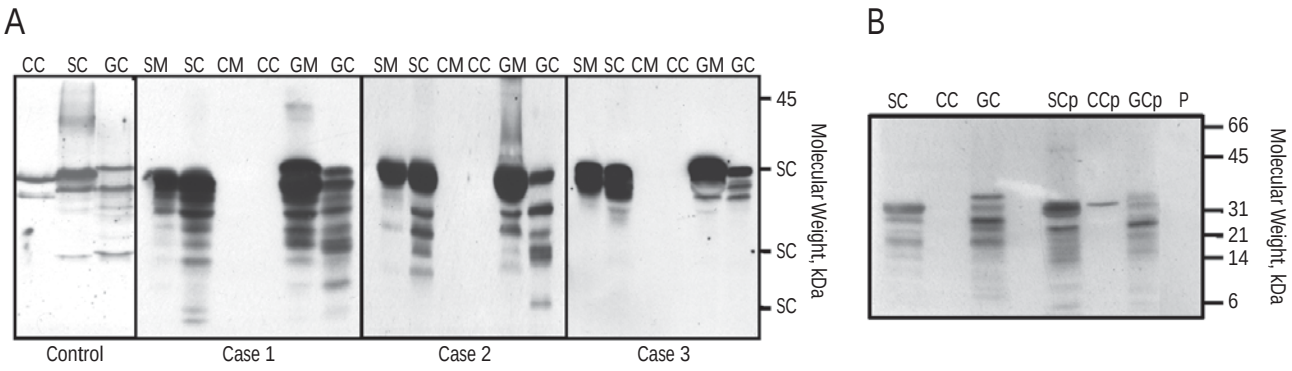

Figure. A, Immunoblotting analysis of individual patient sera. Serum from a patient with classic cow milk allergy was used as a control. Sera diluted 1/10 in NET buffer were incubated with sheep milk (SM), sheep commercial casein (SC), cow milk (CM), cow commercial casein (CC), goat milk (GM), and goat commercial casein (GC).
Panel B: Immunoblotting of the commercial caseins treated with phosphatase (SCp, CCp, GCp) and not treated (SC, CC, GC) with phosphatase (P) using a pool of patient sera. Fifty micrograms were loaded in each lane.

one case and by systemic anaphylaxis requiring emergency treatment in the other. The third patient experienced contact urticaria, nausea, and general discomfort when close to cheese made from GM or SM and refused to eat these dairy products.

Immunologic studies were performed to characterize the proteins involved in GM/SM allergy. All types of caseins were present in both fresh milk and commercial casein extracts, as confirmed by mass spectrometry [8]. Skin prick tests (SPT) were performed with CM (ALK-Abelló, Madrid, Spain) and its proteins (casein, ß-lactoglobulin, and α-lactalbumin: Sigma Chemical, St. Louis, Missouri, USA), GM, SM, GM casein (GC), and SM casein (SC) (Bial Aristegui, Bilbao, Spain) at a concentration of 10 mg/mL. The SPT results were positive for SM and GM in all 3 cases, and in cases 1 and 2 they were also positive for SC and GC. Specific IgE measurements (ImmunoCAP, Phadia, Uppsala, Sweden) to SM and GM were positive in all 3 patients, whereas specific IgE to CM was negative in case 1, slightly positive in case 2, and positive in case 3. Specific IgE to CC was negative in cases 1 and 2 and positive in case 3 (Table).

CM, GM, and SM extracts and commercially available caseins were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis and immunoblotting with patient sera.

The immunoblotting assays revealed strong IgE reactivity to SM and GM proteins in all patients, but not to CM proteins, (Figure, A). In particular, IgE reacted with several protein bands in the commercial casein and milk extracts from both goat and sheep in the 15 to 60-kDa range, corresponding to α-s1 casein, α-s2 casein, and κ-casein, as determined by mass spectrometry or mass spectrometry in tandem.

Caseins were also treated with a Tyr/Ser/Thr protein phosphatase (New England Biolabs, Ipswich, Massachusetts, USA). After removal of the phosphorus groups attached to caseins, cow casein was able to bind to IgE (Figure, B).

Discussion

We report 3 patients who showed strong IgE reactivity to GM and SM proteins, but not to CM proteins, despite the fact that these proteins were also identified as caseins by mass spectrometry in tandem and have high sequence homology with GC and SC. Notwithstanding, the similarity of CC and GC/SC primary structures is lower than that of GC and SC, which are nearly identical [9]. The lack of IgE reactivity to CM proteins in these patients could mean that the epitopes responsible for IgE binding in GC and SC differ in their degree of phosphorylation, as compared to those involved in CM allergy. These differences may affect IgE epitope sequences, thus modifying their allergenic capability. Although patient 3 was sensitized to CM, she tolerated it with no adverse reactions, a finding that is commonly observed in daily clinical practice. In this patient, it seemed that the results observed in the immunoblotting assays were more closely related to the clinical findings (tolerance to CM) than the results of IgE measurement. Immunoblotting was carried out using natural untreated casein. Therefore, cow casein would be hyperphosphorylated, as previously described [5]. We do not know whether the chemical treatment to bind casein to IgE causes dephosphorylation, although this would explain the differences observed between the CAP and immunoblotting results. It would be interesting to analyze this process in future studies.

In summary, differences in the degree of phosphorylation of caseins from cow, sheep, and goat may explain the different allergenicity patterns in patients with selective hypersensitivity to SM and GM. These differences in the allergenic capability of caseins might be caused by different posttranslational modifications and not by differences in IgE epitope sequences.

Acknowledgments

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