ORIGINAL ARTICLE

Allergy to goat's and sheep's milk in a population of cow's milk–allergic children treated with oral immunotherapy*

Pablo Rodríguez del Río[1], Silvia Sánchez-García[1], Carmelo Escudero[1], Carlos Pastor-Vargas[2], José J. Sánchez Hernández[3], Inmaculada Pérez-Rangel[1] & María Dolores Ibáñez[1]

[1]Department of Allergy, Niño Jesús University Hospital, Madrid, Spain; [2]Department of Immunology, Fundación Jiménez Díaz, Madrid, Spain; [3]Department of Public Health, Faculty of Medicine, Autonomous University of Madrid, Spain

To cite this article: Rodríguez del Río P, Sánchez-García S, Escudero C, Pastor-Vargas C, Sánchez Hernández JJ, Pérez-Rangel I, Ibáñez MD. Allergy to goat's and sheep's milk in a population of cow's milk–allergic children treated with oral immunotherapy. *Pediatr Allergy Immunol* 2012: **23**: 128–132.

Keywords
casein; cow's milk; food allergy; goat's milk; sheep's milk.

Correspondence
Pablo Rodríguez del Río, Allergy Department, Niño Jesús University Hospital, Avenida de Menendez Pelayo 65, 28009 Madrid, Spain.
Tel.: +34915035935
Fax: +34915734012
E-mail: prodriguezd@salud.madrid.org

Accepted for publication 18 January 2012

DOI:10.1111/j.1399-3038.2012.01284.x

Abstract

Background: Cow's milk oral immunotherapy (CMOIT) is a recognized treatment for persistent cow's milk (CM) allergy. However, further data are necessary on tolerance to milk from other mammals.
Objective: To describe the clinical and immunologic features of goat's and sheep's milk (GSM) allergy in patients who tolerated CM after CMOIT.
Methods: Fifty-eight CM-allergic patients who successfully underwent CMOIT in our department were evaluated using skin prick test (SPT), specific immunoglobulin (Ig) E determination, enzyme-linked immunoassay (ELISA), and controlled oral challenge to assess allergy to GSM. Statistical analysis was carried out to identify markers of allergy to GSM.
Results: Fifteen of 58 (25.9%) patients were allergic to either goat's or sheep's milk or to both, as confirmed by a controlled positive oral challenge. Forty-seven percent of all positive oral challenges were classified as anaphylactic reactions. Specific IgE to CM casein, goat's whole milk, and sheep's whole milk was 13.2, 18.0, and 21.4 kU_A/l in the group of GSM-allergic patients and 6.6, 6.5, and 6.5 kU_A/l in the GSM-non-allergic patients ($p < 0.05$). Decision-making cut-off points based on sIgE for diagnosing symptomatic GSM allergy could not be determined. ELISA inhibition assays showed limited cross-reactivity (up to 77.2%) between CM casein and GSM casein in the group of GSM-allergic patients in contrast with almost 100% in GSM-not-allergic patients.
Conclusion: We found a high prevalence (26%) of allergy to GSM in our population of CM–allergic children treated with oral immunotherapy. Therefore, tolerance to GSM should be assessed in order to provide accurate nutritional advice and minimize life-threatening accidental intake. Specific IgE to CM casein, goat's and sheep's whole milk is a good marker of this allergy. Although CM oral immunotherapy is a specific treatment for CM allergy, it may not be effective against allergy to the milk of other mammals.

Abbreviations
CM, cow's milk; GSM, goat's and sheep's milk; GM, goat's milk; SM, sheep's milk; CMOIT, cow's milk oral immunotherapy; SPT, skin prick test; sIgE, specific immunoglobulin E; ELISA, enzyme-linked immunosorbent assay; ROC, receiver operating curve; AUC, area under the curve.

Cow's milk (CM) allergy is the most frequent allergy in early childhood (1), and avoidance has been the only treatment for many years. However, active treatment based on CMOIT is becoming increasingly popular with a high success rate ranging from 65 to 90% (2, 3). Cow's milk most important allergens are caseins and whey proteins, α-lactalbumin (ALA) and β-lactoglobulin (BLG) (4), but casein plays the main role

regarding the cross-reactivity among bovine's milk and other mammal's milk (5). The high cross-reactivity between milk from goats, sheep, and cows described in literature (5) means that goat's and sheep's milk (GSM) is prohibited in CM-allergic patients. Little is known about tolerance to GSM in patients receiving CMOIT; however, there are very few published case reports of allergy to GSM in CM tolerants (6) and also in patients after OIT with CM (7).

We carried out a prospective study in a population of CM-allergic patients receiving CMOIT to assess the tolerance to GSM, search for predictive markers of GSM allergy, and determine the role of casein.

Patients and methods

Patients

The study population comprised 58 patients who had completed a CMOIT protocol (3) in our department and maintained a minimum daily intake of 200 ml of CM. Mean time between the end of CMOIT and inclusion was 15.7 (SD ± 11.5) months. Thirty-two patients were females (55.2%), and mean age on initiating CMOIT was 6.2 (SD ± 2.96) years. Asthma was present in 24 of 58 (41.4%) patients, and 38 of 58 (65.5%) had other types of food allergy, with egg allergy being the most frequent (27/58, 46.6%). Informed consent was obtained in all cases. All the study procedures were approved by the Ethical Committee of the Hospital.

Skin prick tests

Skin prick tests (SPT) (Diater Laboratories, Madrid, Spain) to ALA, BLG, and casein from CM, as well as prick-prick tests (PPT) with GM and SM cheeses separately, were carried out before an open food challenge with GM and/or SM. PPT were performed with 1-mm lancets in the volar side of the forearm. Wheals 3 mm larger than those of the saline control were considered positive.

Serum-specific IgE

Specific IgE (sIgE) (CAP-Phadia, Uppsala, Sweden) to CM proteins (nBos d 4 α-lactalbumin, nBos d 5 β-lactoglobulin, nBos d 8 Casein), goat's milk (GM) and sheep's milk (SM) was determined before the open food challenge with GSM.

Assessment of goat and sheep cheese allergy

Patients who reported unequivocally good tolerance to GSM were classified as not allergic. Those who were unaware of their tolerance to GSM underwent a controlled open food challenge with fresh GM cheese, cured SM cheese, or both. Cheese was used as the matrix for open food challenge instead of milk because its consumption is more widespread.

Open food challenge was performed by administering four doses of cheese (5, 10, 25, and 60 g) at 20-min intervals until a cumulative dose of 100 g was tolerated (negative, non-allergic patient) or an allergic reaction occurred within the following 2 h (positive, allergic patient). Anaphylaxis was defined following current guidelines (8). If the result of the open food challenge was positive to either or both cheeses, the patient was classified as allergic to GSM (GSM-allergic group).

ELISA inhibition assays

Pooled sera obtained before the open food challenge with GSM from five GSM-allergic and five GSM-not-allergic patients were compared. Wells were coated separately with 1 μg of casein from CM, GM, and SM (Sigma-Aldrich, Madrid, Spain). Sera were inhibited with an excess of casein (500 μg/ml) of all three types separately in order to reach maximum inhibition, regardless of the 50% inhibition dose, and rule out any probability of cross-reactivity between the different caseins (9). Values were expressed in optical densities as the mean of the duplicate determinations minus the blank.

Statistical analysis

Patient characteristics, duration of CMOIT, and time between success in CMOIT and open food challenge with GSM according to allergy status were compared using the Fisher exact test and *t* test or Mann–Whitney test. Logistic regression analysis was used to assess the predictive capacity of sIgE to CM casein, whole GM, and whole SM. The predictive utility of sIgE to CM casein, whole GM, and whole SM was evaluated using receiver operating characteristic (ROC) curve analysis, and the area under the curve (AUC) was measured. Analyses were performed using SPSS version 17 (SPSS Inc, Chicago, IL, USA).

Results

Oral challenges

Eleven of 58 patients regularly consumed GSM-based products and were thus considered for the analysis as GSM-not-allergic. Forty-seven patients underwent open food challenge with GM and SM cheese, and positive results were observed in 15 cases. Overall, 15 of 58 (25.9%) patients were GSM-allergic in our population of CMOIT children. Detailed information on the allergic patients is shown in Table 1.

The mean dose triggering symptoms was 50 g of GM and 65 g of SM cheese. Anaphylaxis occurred in eight of 17 (47%) patients with positive open food challenge results and was treated with intramuscular adrenaline. We found no differences in gender, age, association with other allergies, duration of CMOIT, or time between successful CMOIT and open food challenge with GSM between the groups (GSM-allergic and GSM-not-allergic patients).

SPT and sIgE

SPT results to CM fractions and GSM were positive in all patients from both groups. The GM cheese and SM cheese PPT result was negative in three of 39 (7.7%) and eight of 41

Table 1 Patient characteristics and results of allergy determinations

N	Sex	Age at OIT (years)	Age at GSM challenge (years)	sIgE* (kU/l)			GM challenge		SM challenge	
				CM Casein	GM	SM	Symptoms	Dose** (g)	Symptoms	Dose** (g)
1	F	8	11	ND	52.1	56.7	AE, RC, AP, V	5	ND	–
2	M	12	14	30.8	55.1	50	A	100	A, RC, U	100
3	F	3	4	6.3	8.08	6.99	ND	–	RC, U	15
4	M	4	5	15.4	15.7	24.8	A	100	ND	–
5	F	4	6	5.64	7.3	7.66	OAS, RC	5	RC, A	100
6	F	10	12	14.8	35.4	32.7	Tolerant	–	RC, A	100
7	F	7	7	25.2	29	45	Tolerant	–	U, RC, V	5
8	M	10	11	3.86	8.04	9.3	ND	–	V	15
9	M	17	19	12	ND	ND	ND	–	D	5
10	M	5	8	21.4	ND	ND	U, V	40	ND	–
11	F	6	8	1.76	0.52	0.38	Tolerant	–	V, RC	100
12	F	6	7	24.7	28.9	40.6	ND	–	V, U	100
13	M	4	6	2.1	1.48	8.58	Tolerant	–	D, AP, RC	100
14	M	7	8	13.5	17.7	18.5	Tolerant	–	U, A	100
15	M	11	12	7.26	7.74	9.55	ND	–	AE, A	40

All positive challenges elicited immediate reactions (<2 h); no delayed reactions were recorded.
AE, angioedema; A, asthma; OAS, oral allergy syndrome; RC, rhinoconjunctivitis; AP, abdominal pain; U, urticaria; V, vomiting; D, dysphagia; –, negative.
*after OIT.
**Cumulative dose expressed in grams of cheese that elicited the reaction.
ND, not done: some patients and families refused the challenge with both cheeses when the first challenge yielded a positive result.

(19.5%) respectively, with no significant differences between the groups.

All serum sIgE determinations were available for only 49 patients. Mean sIgE to CM casein, GM, and SM was higher in allergic patients than in non-allergic patients: 13.2 (SD ± 9.8), 18.0 (SD ± 19.0), and 21.4 kU_A/l (SD ± 19.8) compared with 6.6 (SD ± 7.5), 6.5 (SD ± 10.2), and 6.5 kU_A/l (SD ± 7.3) ($p < 0.05$). No significant differences were detected in sIgE to CM, ALA, or BLG.

The ROC curve was calculated for sIgE to CM casein, whole GM, and whole SM. Only sIgE to CM casein yielded a significant AUC (0.7297), see Fig. 1. The predicted probabilities for a symptomatic sensitization to GSM at a given IgE titer for CM casein, GM, and SM were estimated, but neither 95% nor 90% predictive values could be calculated, as these levels were not reached for any given sIgE titer.

ELISA inhibition assays

ELISA inhibition results are detailed in Table 2. Inhibition with CM casein reached a maximum of 67.1–74.5% in sera from GSM-allergic patients, although it was almost complete in GSM-not-allergic patients. The 100% inhibition with GM and SM casein was noteworthy.

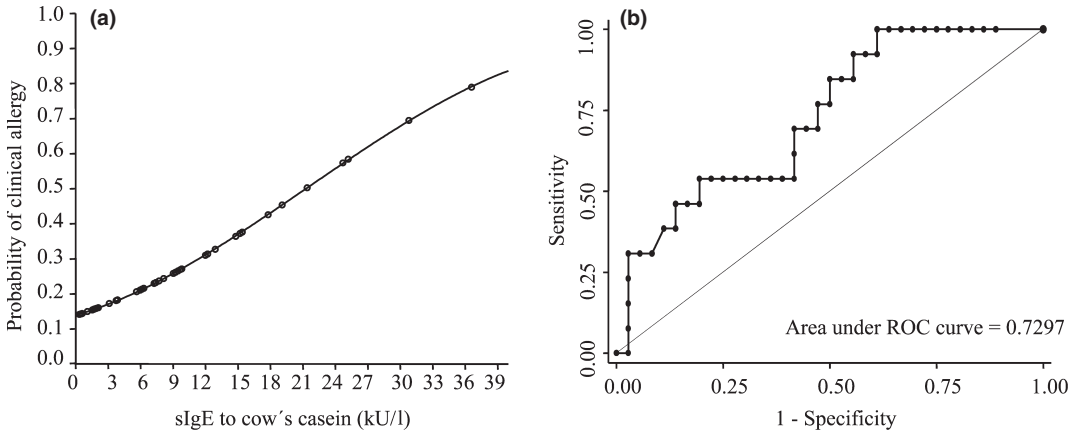

Figure 1 Relationship between specific IgE to cow's milk casein and probability of suffering a clinically relevant sensitization. ROC curve for sIgE to CM casein. Left-hand figure shows how neither 95% nor 90% predictive values are reached in the probability of clinical allergy for the given sIgE cow's casein values. Right-hand figure shows ROC curve for sIgE to CM casein.

Table 2 Results of ELISA inhibition assays

		GSM-not-allergic patients' sera			GSM-allergic patients' sera		
		Solid phase			Solid phase		
		CC (%)	GC (%)	SC (%)	CC (%)	GC (%)	SC (%)
INHIBITOR	CC	99.84	99.63	99.4	100	67.08	77.27
	GC	88.34	100	100	89.94	100	100
	SC	86.57	100	100	88.24	100	100

Solid phase shows the extract used to coat wells in each experiment. Each cell represents the maximum inhibition degree reached at each experiment.
CC, cow's casein; GC, goat's casein; SC, sheep casein.

Discussion

The current recommendations for CM-allergic patients are avoidance of CM and GSM; however, there is a lack of consensus with regard to extending this recommendation to children receiving CMOIT. Our aim was to appraise tolerance to both GM and SM in order to allow a non-restrictive diet or limit milk intake to CM. In our population of 58 patients who tolerated CM after CMOIT, allergy to GSM was confirmed by a positive open food challenge in 15 (25.9%) cases.

Most symptoms in open food challenges were moderate to severe, and 47% of patients suffered anaphylaxis, thus emphasizing the relevance of assessing tolerance to GSM in this population. Doses eliciting symptoms were higher than previously reported (10) in GSM-allergic patients, probably as a result of the partial cross-reactivity between casein from CM and GSM observed after ELISA inhibition in our population.

Established 95% predictive decision points based on CM sIgE have been proposed as a useful test for diagnosing symptomatic milk allergy (11). Our attempts to establish these points failed, probably because of the small number of patients, but more likely because their special condition of being CMOIT-treated patients, with higher levels of sIgE to CM and GSM proteins, biased our results. However, higher sIgE levels to CM and GSM casein were associated with GSM allergy and can be used as markers of this allergy. Moreover, sIgE to CM casein proved to be a useful diagnostic tool in GSM allergy, as demonstrated with the ROC curve (AUC 0.7297). As previously described (12), we support the use of controlled oral challenges to avoid the misclassification of patients and the subsequent detriment to quality of life and nutrition.

To our knowledge, there is only one report of two patients who, having successfully completed CMOIT, suffered oral pruritus and generalized urticaria after eating GSM cheeses. Casein was shown to be the causative allergen (7).

Our ELISA inhibition assays demonstrated that sIgE antibodies to GSM casein were inhibited by CM casein to a lesser extent in GSM-allergic patients than in GSM-not-allergic individuals. This finding indicates the existence of IgE that is highly specific to casein from GSM and that shows lower cross-reactivity with CM casein. An inhibition of 67–77% is still higher than that observed elsewhere in CM-tolerant GSM-allergic patients (13); however, considering the unique profile of our patients and the fact that we performed our experiments with saturation of inhibitor, we think our results are significant.

It could be hypothesized that GSM-allergic patients were primarily sensitized to GSM, as they can recognize epitopes in GSM casein but not in CM casein (14), although we cannot say whether this sensitization was before or after CMOIT or specify the sensitization route. In the group of GSM-allergic patients, CMOIT was allergen-specific for CM but not for GSM.

Although high in vitro cross-reactivity between casein from GM and SM has been reported (10) and confirmed in our experiments, five patients were allergic to SM but not to GM. A possible explanation for this observation could be the different allergenicity of GSM depending on genetic polymorphisms of the α_{s1}-casein (15).

To our knowledge, this is the first report on the prevalence of GSM allergy in a CMOIT-treated population. We found that 26% of our patients were allergic to GSM and that 47% of positive oral food challenges resulted in anaphylactic reactions; consequently, tolerance to GSM must be assessed in all allergic patients successfully treated with CMOIT. Specific IgE levels to CM casein and GSM correlated with allergy to GSM, although food challenge is the only reliable diagnostic tool. Highly specific epitopes in GSM casein seem to be involved in this allergy, thus reflecting the allergen specificity of CMOIT for CM but not for GSM.

Acknowledgments

Authors are most grateful for the extensive work of our nurses and nurse assistants in our daily practice and especially for their contribution to the clinical work of this manuscript: Catalina Quioneros, Esperanza Doval, Ascensiœn Lopez, Carmen Ballesteros, Inmaculada Sanz and Maria Jesús Jiménez.

References

1. Høst A, Halken S, Jacobsen HP, Christensen AE, Herskind AM, Plesner K. Clinical course of cow's milk protein allergy/intolerance and atopic diseases in childhood. *Pediatr Allergy Immunol* 2002: **13** (Suppl. 15): 23–8.
2. Patriarca G, Nucera E, Roncallo C, et al. Oral desensitizing treatment in food allergy: clinical and immunological results. *Aliment Pharmacol Ther* 2003: **17**: 459–65.
3. Martorell A, De la Hoz B, Ibáñez MD, et al. Oral desensitization as a useful treatment in 2-year-old children with cow's milk allergy. *Clin Exp Allergy* 2011: **41**: 1297–304.
4. Natale M, Bisson C, Monti G, et al. Cow's milk allergens identification by two-dimensional immunoblotting and mass

spectrometry. *Mol Nutr Food Res* 2004: **48**: 363–9.
5. Järvinen KM, Chatchatee P. Mammalian milk allergy: clinical suspicion, cross-reactivities and diagnosis. *Curr Opin Allergy Clin Immunol* 2009: **9**: 251–8.
6. Eigenmann PA. The spectrum of cow′s Milk allergy. *Pediatr Allergy Immunol* 2007: **18**: 265–71.
7. Alonso-Lebrero E, Fuentes V, Zapatero L, Pérez-Bustamante S, Pineda F, Martinez-Molero MI. Goat's milk allergies in children following specific oral tolerance induction to cow's milk. *Allergol et Immunopathol* 2008: **36**: 180–1.
8. Lieberman P, Nicklas RA, Oppenheimer J, et al. The diagnosis and management of anaphylaxis practice parameter: 2010 update. *J Allergy Clin Immunol* 2010: **126**: 477–80.
9. Cases B, Pastor-Vargas C, Dones FG, et al. Watermelon profilin: characterization of a major allergen as a model for plant-derived food profilins. *Int Arch Allergy Immunol* 2010: **153**: 215–22.
10. Ah-Leung S, Bernard H, Bidat E, et al. Allergy to goat and sheep milk without allergy to cow's milk. *Allergy* 2006: **61**: 1358–65.
11. Sampson HA. Utility of food-specific IgE concentrations in predicting symptomatic food allergy. *J Allergy Clin Immunol* 2001: **107**: 891–6.
12. Mehl A, Verstege A, Staden U, et al. Utility of the ratio of food-specific IgE/total IgE in predicting symptomatic food allergy in children. *Allergy* 2005: **60**: 1034–9.
13. Muñoz Martín T, De la Hoz Caballer B, Marañón Lizana F, González Mendiola R, Prieto Montaño P, Sánchez Cano M. Selective allergy to sheep's and goat's milk proteins. *Allergol et Immunopathol* 2004: **32**: 39–42.
14. Umpiérrez A, Quirce S, Marañon F, et al. Allergy to goat and sheep cheese with good tolerance to cow cheese. *Clin Exp Allergy* 1999: **29**: 1064–8.
15. Ballabio C, Chessa S, Rignanese D, et al. Goat milk allergenicity as a function of αS1-casein genetic polymorphism. *J Dairy Sci* 2011: **94**: 998–1004.