

Clinical Communications

Cross-desensitization to goat and sheep milk following cow's milk oral immunotherapy

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Clinical Implications

- This study demonstrates that almost all Israeli milk-allergic patients, successfully desensitized to cow's milk could tolerate goat's and sheep's milk as well.

TO THE EDITOR:

Milk allergy is among the most common food allergies and frequently persists into adulthood, impairing patients' quality of life.¹ Oral immunotherapy (OIT) is recommended by the European Academy of Allergy and Clinical Immunology as a treatment option for patients with milk, egg, and peanut allergy.² Cross-desensitization to nontreated foods does not typically occur after a single-food OIT. However, cross-desensitization was reported for cross-reactive tree nuts.³ Goat's and sheep's milk (GSM) share 95% protein homology and also have high protein identity, exceeding 84%, with cow's milk (CM), suggesting cross-reactivity.⁴ Indeed, GSM allergy was reported in more than 90% of CM-allergic patients.⁵ However, goat's milk (GM) allergy without accompanying CM allergy, and GSM allergy in CM-desensitized children, was described, attributed primarily to goat-specific casein.^{4,6,7} More importantly, fatal reactions to goat's milk in allergic individuals were reported.⁸ Rodríguez del Río et al⁷ described an allergy rate of 26% to goat's and/or sheep's milk in patients desensitized to 6000 mg CM protein. Validating these findings in a larger set of patients from a different geographic location is important.

The goal of the study was to determine the safety of GSM ingestion after OIT to CM. We examined milk-allergic patients desensitized to 7200 mg CM protein between August 2010 and November 2017. Following desensitization, patients were instructed to consume a regular maintenance dose of at least 4500 mg CM protein. Patients were asked about past GSM consumption and reactions. Skin prick tests (SPTs) were performed to CM and GSM, and patients underwent a graded open oral food challenge (OFC) with fresh GSM to a final dose of 3000 mg GM and 6000 mg sheep's milk (SM) proteins. Serum was obtained from a subgroup of patients and analyzed for the presence of antibodies against GSM proteins. Additional information on study methods is detailed in this article's Online Repository at www.jaci-inpractice.org.

A total of 396 patients were desensitized to milk by November 2017. Of those, 110 patients were evaluated for GSM allergy at a median of 1.4 years (interquartile range, 0.2-2.2 years) after

TABLE I. Demographic and clinical characteristics of the study population

Parameter	Evaluated (n = 110)	Were not evaluated (n = 286)	P value
Age at treatment (y)	7.2 (5.3-10.6)	7.5 (5.2-11)	.90
Sex: male	70 (63.6)	173 (60.5)	.65
HDM sensitivity	56 (52.3)	136 (48.7)	.57
Asthma	41 (37.3)	124 (43.4)	.31
SHTD of CM (mg)	90 (45-480)	90 (45-450)	.71
Treatment duration (mo)	7.6 (3.8-11.7)	7.3 (3.8-11.8)	.92
Epinephrine—induction	20 (18.2)	47 (16.4)	.66
Epinephrine—home	12 (10.9)	16 (5.6)	.08

Categorical variables are presented as number (%) and continuous variables are presented as median (interquartile range).

HDM, House-dust mite; SHTD, single, highest tolerated dose.

reaching maintenance (see Figure E1 in this article's Online Repository at www.jaci-inpractice.org). All were challenged to GM and all but 5 (lack of cooperation or time limitations) were challenged to SM. None of the remaining nonevaluated 286 patients reported a reaction to GSM. Patients who were evaluated and those who were not were comparable in demographic characteristics and OIT course (Table I). Only a single patient described consumption of GSM before the study and had no reactions.

A significant correlation was found between SPT wheal size to GM and SM (see Figure E2, A, in this article's Online Repository at www.jaci-inpractice.org) and between SPT wheal size to CM and SPT wheal size to GSM (Figure E2, B and C). A quarter of the patients had a negative SPT result to CM, suggesting CM tolerance after OIT. The rate of SPT positivity to GSM was 67.3%. A positive SPT result to GM and a positive SPT result to SM were each present in 9 patients (8.9%) with a negative SPT result to CM (see Figure E3 in this article's Online Repository at www.jaci-inpractice.org). One hundred nine of 110 patients passed the GM challenge, and 105 of 105 passed SM OFC without allergic reactions.

A single patient had an anaphylactic reaction to GM (3000 mg) and required intramuscular epinephrine. He refused SM OFC. Surprisingly, only this patient reported recurrent uneventful consumption of small servings of goat's cheese after CM-OIT. Moreover, goat's cheese was frequently consumed in this patient's household. On immunoblot analysis (Figure 1), sera from this patient after CM desensitization demonstrated reactivity against GM proteins (lane 1), which was inhibited by pretreatment with GM (lane 2), but only minimally by CM (lane 3). Furthermore, sera pooled from 3 patients who passed the GM OFC after CM-OIT only minimally reacted to GM (lanes 4-5). These findings suggest the presence of an antibody reacting to GM but not to CM in this patient's, but not in other patients', serum.

Nearly all milk-allergic patients in our study, who were successfully desensitized to CM, could tolerate GSM as well. This

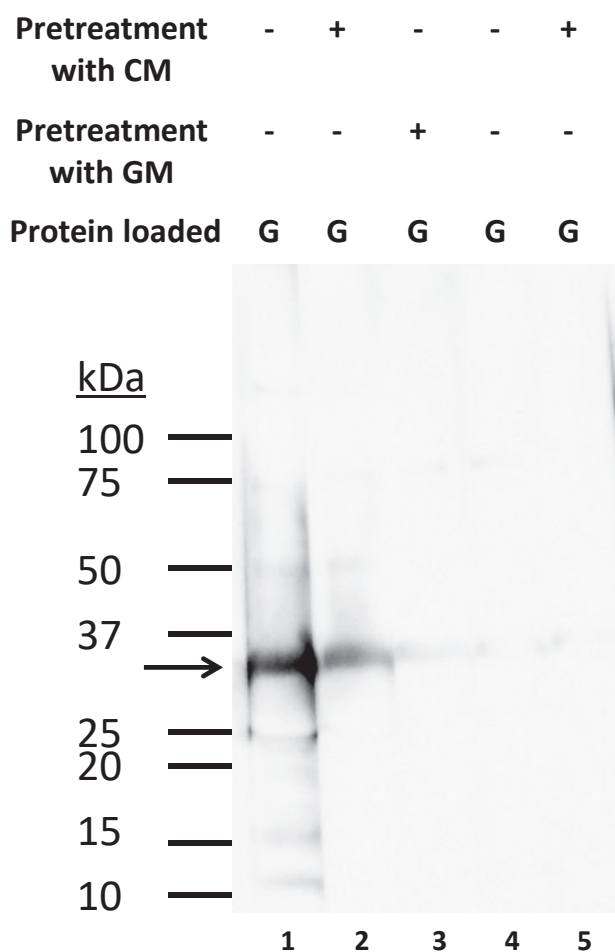


FIGURE 1. Immunoblot analysis against CM and GM in CM-allergic and in CM-desensitized patients. Lanes were loaded with GM (G) as indicated in the figure. Sera were pretreated with CM or GM before immunoblotting. Lanes 1 to 3: sera derived from the patient who reacted to GM after CM-OIT. Lanes 4 and 5 were immunoblotted with pooled sera ($n = 3$) from post-CM-OIT patients who passed the GM-OFC.

finding is in stark contrast to that in the previously described Spanish cohort.⁷ It could be that the quantity of GSM protein used in the Spanish study, although not specified, was higher. However, our challenge protocol included protein amounts previously used to rule out GM allergy,⁵ and none of our patients reported a reaction to GSM on accidental exposure. Alternatively, genetic variability could affect the tendency to develop specific IgE antibodies to unique GSM epitopes. Finally, patients living in societies with high GSM consumption might be more exposed to environmental GSM proteins, which could drive specific GSM allergy, as previously shown for peanut.⁹ In contrast, in countries where GSM consumption is low, sensitization and allergy to GSM might be driven by allergy to CM, resulting in the presence of specific IgE only to proteins that are shared with CM. Indeed, GSM production in Spain approaches

10% of local milk production,¹⁰ whereas GSM consumption in Israel accounts for only 2% of the local milk production.¹¹ The high consumption of goat's cheese in the household of the single patient in our study who reacted to GM, together with his specific GM immune-epitope, might support this theory.

Only a quarter of the eligible patients presented to the GSM OFCs. This is unlikely to be due to discontinued or limited CM consumption given our previous data that most patients (92.5%) who were past 6 months after achieving maintenance continued regular milk consumption¹² and because a comparable rate of patients who were consuming only the daily dose and of patients who were consuming other dairy products present to the GSM OFC (~35% in each group). Rather, it is probably the relatively uncommon use of GSM in Israel and the minimal impact GSM allergy has on patients' lives that led to their low motivation to undergo evaluation for GSM allergy. Another limitation is that patients were not challenged to GSM before milk OIT. However, most were likely GSM allergic based on previous reports.⁵

In conclusion, we demonstrate in the largest group studied so far that almost all milk-allergic patients successfully desensitized to CM could tolerate GSM as well. This enables free CM-based product consumption without concern for cross-contamination with limited amounts of GSM. Potential high-threshold GSM allergy persistence after CM desensitization necessitates GSM challenge in patients who wish to deliberately consume GSM. Further studies are needed to examine whether this finding is unique to Israeli patients.

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ONLINE REPOSITORY**METHODS****Patients**

Patients who were desensitized to CM (7200 mg protein) and who were consuming either a regular (at least 3 times per week) maintenance dose of at least 150 mL milk (4500 mg milk protein) or dairy products containing at least 4500 mg protein, as previously described,^{E1} were eligible. Patients who were desensitized to 7200 mg CM protein after March 2012 were informed about the risk of reaction to GSM, and ad lib consumption was limited to CM products only. Patients who were desensitized to this amount before March 2012 were not specifically instructed to avoid GSM products initially and were informed on March 2012 regarding the new information and the need to avoid GSM. These patients were evaluated for GSM allergy several months later. History was obtained from each patient regarding CM consumption and allergic reactions to milk after completion of OIT. Patients who experienced reactions to CM in the week preceding the evaluation, and patients with an intercurrent infection (fever, vomiting, or diarrhea) in the 2 days preceding the evaluation, were excluded. The Helsinki institutional review board committee approval for the study was obtained. Informed parental or patient (for those aged >18 years) written consent was obtained from all participants.

Oral immunotherapy

Inclusion criteria for OIT were age 48 months or more, evidence for IgE-mediated allergy to CM by positive SPT result and/or specific serum IgE together with positive OFC or clinical history of a reaction after accidental ingestion in the past year. High-risk patients, including those with a history of anaphylaxis, were not excluded. Patients with stable asthma being treated with daily controller inhaled steroid therapy, or as needed, were included. Exclusion criteria were uncontrolled asthma, a medical contraindication to receive epinephrine, or the inability to adhere to protocol.

Skin prick tests

SPTs were performed using a commercial CM extract (1:10 w/v Alk-Abelló, Port Washington, NY) and with fresh cow, goat, and sheep milk, on the volar surface of the forearm with single-peak lancets (Heinz Herenz, Hamburg, Germany) and wheal diameter was measured after 15 minutes. Histamine 1 mg/mL (Alk-Abelló) and saline were used as positive and negative controls, respectively. A positive SPT result was defined as a

wheal diameter (that was) at least 3 mm more than that of the negative control.

Oral food challenges

OFCs were performed in 2 steps. In the first step, patients were given 10 mL of milk corresponding to 300 mg goat's milk and 600 mg SM and observed for 30 minutes. If no reaction occurred, patients were given 100 mL of GSM milk as described earlier, and observed for 90 minutes. To reduce the risk of bias, a challenge was considered positive only in the case of objective signs of allergic reaction (urticarial rash, rhinitis/sneezing or conjunctival congestion, cough/wheezing, persistent abdominal pain/vomiting, or a decline in blood pressure). Subjective or local oral and perioral signs and symptoms only were not used as criteria for a positive challenge.

Immunoblotting

Pasteurized CM and GM were separately mixed with sample loading buffer (1% SDS final) in the presence of 5% β -mercaptoethanol (Sigma, St Louis, Mo) and heated for 2 minutes at 95°C. Proteins were run on a 4% to 20% SDS-PAGE gel (BioRad, Hercules, Calif) at 200 V for 45 minutes and transferred to nitrocellulose membranes. Individual lane strips were cut after Ponceau S staining and blocked with 5% BSA in PBS plus 0.1% Tween (Sigma) overnight. Serum from the single GM-allergic patient, as well as pooled sera from patients desensitized to GM after CM-OIT, were incubated at room temperature for 60 minutes with or without pretreatment with CM and GM (300 μ g/mL). After incubation, the sera were diluted 1:7 in buffer (PBS +1% BSA + 0.1% Tween20) and applied to the membrane strips overnight at 4°C with gentle shaking. After washing in PBS plus 0.1% Tween, the strips were probed with horseradish peroxidase-conjugated mouse antihuman IgE-Fc (Southern Biotech, Birmingham, Ala) as a secondary antibody for 1 hour. The strips were washed, and then treated with chemiluminescent substrate (Thermo Scientific, Rockford, Ill). Images were acquired by using the MicroChemi Imager (DNR Bio-Imaging Systems, Jerusalem, Israel).

Statistical analysis

Patients who arrived for evaluation were compared with those who did not. Fisher exact test and the Mann-Whitney test were used to compare groups. A *P* value of less than .05 was considered significant. Spearman correlation coefficient was used to analyze correlation between SPT to cow's, goat's, and sheep's milk.

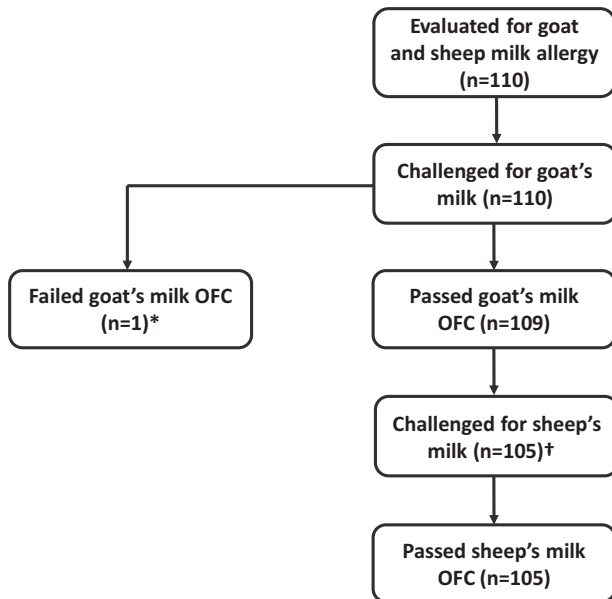


FIGURE E1. Patients' enrollment and outcome. A flow diagram is presented demonstrating patient enrollment and GSM-OFC outcomes. *This patient refused to undergo SM challenge. †Four patients did not undergo SM challenge because of lack of cooperation or time limitations.

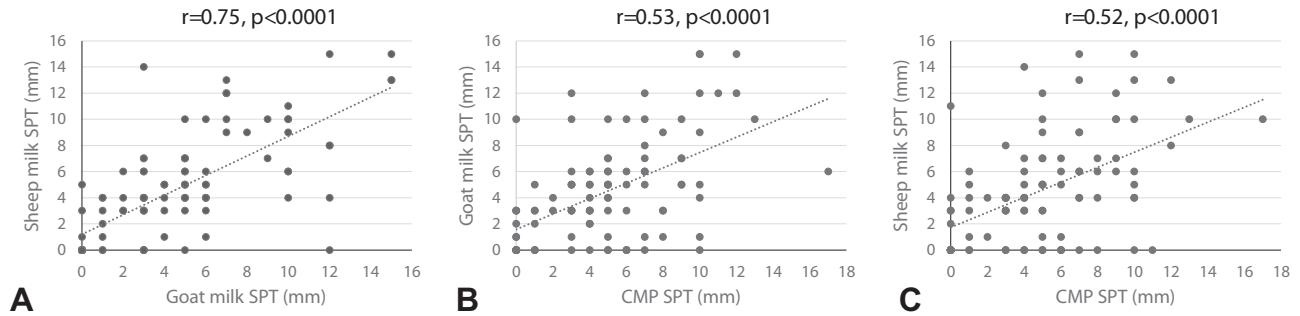


FIGURE E2. Correlations between SPT to CM, GM, and SM. A scatter plot demonstrating the correlation between wheal size obtained by SPTs performed with the various tested milks: GM, SM, and CM. Data available for 100 patients for GM and for 100 patients for SM. *CMP*, Cow milk protein.

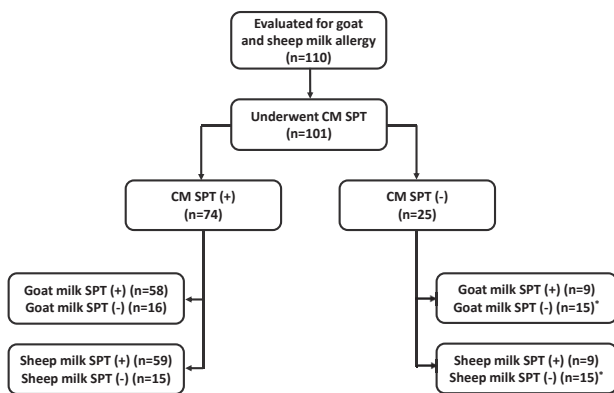


FIGURE E3. Patients' SPT positivity for the different milks. A flow diagram is presented demonstrating patient SPT positivity for CM, GM, and SM. *One patient had SPT for CM but not for GSM.

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