Allergenicity of goat's milk in children with cow's milk allergy

Barbara Bellioni-Businco, MD,[a] Roberto Paganelli, MD,[a] Patrizia Lucenti, MD,[b] Paolo G. Giampietro, MD,[b] Hans Perborn,[c] and Luisa Businco, MD[b] *Rome, Italy, and Uppsala, Sweden*

Background: Cow's milk allergy (CMA) is a common disease of infancy and childhood. An appropriate cow's milk (CM) substitute is necessary for feeding babies with CMA. CM substitutes are soy formulas and casein- or whey-based extensively hydrolyzed formulas. In several countries, including Italy, goat's milk (GM) formulas are available, and some physicians recommend them for feeding babies with CMA.

Objective: We sought to investigate, in vitro and in vivo, the allergenicity of GM in 26 children with proven IgE-mediated CMA.

Methods: All the children underwent skin tests with CM and GM; detection of specific serum IgE to CM and GM; and double-blind, placebo-controlled, oral food challenges (DBPCOFCs) with fresh CM, GM, and, as placebo, a soy formula (Isomil, Abbott, Italy). CAP inhibition and immunoblotting inhibition assays were also carried out in 1 of 26 and 4 of 26 children with positive RAST results to both CM and GM, respectively.

Results: All the children had positive skin test responses and CAP results to both CM and GM, all had positive DBPCOFC results to CM, and 24 of 26 had positive DBPCOFCs to GM. In CAP inhibition tests, preincubation of serum with CM or GM strongly inhibited IgE either to CM or to GM. In immunoblotting inhibition assays, preincubation with CM completely extinguished reactivity to GM, whereas GM partially inhibited reactivity to CM.

Conclusions: These data strongly indicate that GM is not an appropriate CM substitute for children with IgE-mediated CMA. A warning on the lack of safety of GM for children with CMA should be on the label of GM formulas to prevent severe allergic reactions in babies with CMA. (J Allergy Clin Immunol 1999;103:1191-4.)

Key words: Cow's milk allergy; cross-reactivity; double-blind, placebo-controlled, oral food challenge; goat's milk; immunoblotting

Cow's milk allergy (CMA) is a common disease of infancy and childhood. The prevalence of CMA is approximately 2.5% during the first 3 years of life.[1] An appropriate cow's milk (CM) substitute is necessary for feeding babies with CMA, whereas in older children other sources of proteins can be given to provide protein requirements. CM substitutes are soy formulas and casein- or whey-based extensively hydrolyzed formulas.[2]

Abbreviations used

CM:	Cow's milk
CMA:	Cow's milk allergy
DBPCOFC:	Double-blind, placebo-controlled, oral food challenge
GM:	Goat's milk

Goat's milk (GM) is prescribed by some physicians as a CM substitute in children with CMA, but in our experience many children with CMA have allergic reactions after ingesting GM. However, in several countries, including Italy, GM formulas are available and recommended for feeding babies with CMA.

The aim of this study was to investigate, in vitro and in vivo, the allergenicity of GM in children with proven IgE-mediated CMA. To our knowledge, no such studies have been done in humans.

METHODS

Patients

Twenty-six children (17 boys and 9 girls), aged 5 months to 7 years (median age, 2 years and 9 months), with CMA (positive double-blind, placebo-controlled, oral food challenge [DBPCOFC] results; positive skin test responses; and positive RAST responses to CM) were enrolled into the study.

Personal histories showed that the main symptoms of the children after ingesting CM were atopic dermatitis in 16 children, urticaria in 5, and diarrhea in 5. All the children underwent skin prick tests with CM and GM, measurement of specific serum IgE to CM and GM, and DBPCOFCs with fresh CM, GM, and, as placebo, a soy formula (Isomil, Abbott, Italy).

Skin prick tests

Skin testing was done by the prick method on the volar surface of the forearm. The skin prick tests results were read after 20 minutes and considered positive when the wheal was greater than 3 mm larger than that produced by the negative control. Children were tested with isotonic saline as a negative control, histamine (10 mg/mL) as a positive control (SARM, Rome, Italy), and undiluted pasteurized fresh CM and GM.

Specific IgE determination

The Pharmacia CAP System (Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden) was used to measure specific serum IgE to CM and GM. Values of allergen-specific IgE below 0.35 kU/L were

From the Division of Allergy and Clinical Immunology, the Departments of [a]Clinical Medicine and [b]Pediatrics, University "La Sapienza," Rome; and [c]Pharmacia & Upjohn Diagnostics AB, Department of Allergy and Asthma, Uppsala.

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Reprint requests: Luisa Businco, MD, Division of Allergy and Clinical Immunology, Department of Pediatrics, University "La Sapienza," Viale Regina Elena 324, 00161 Rome, Italy.

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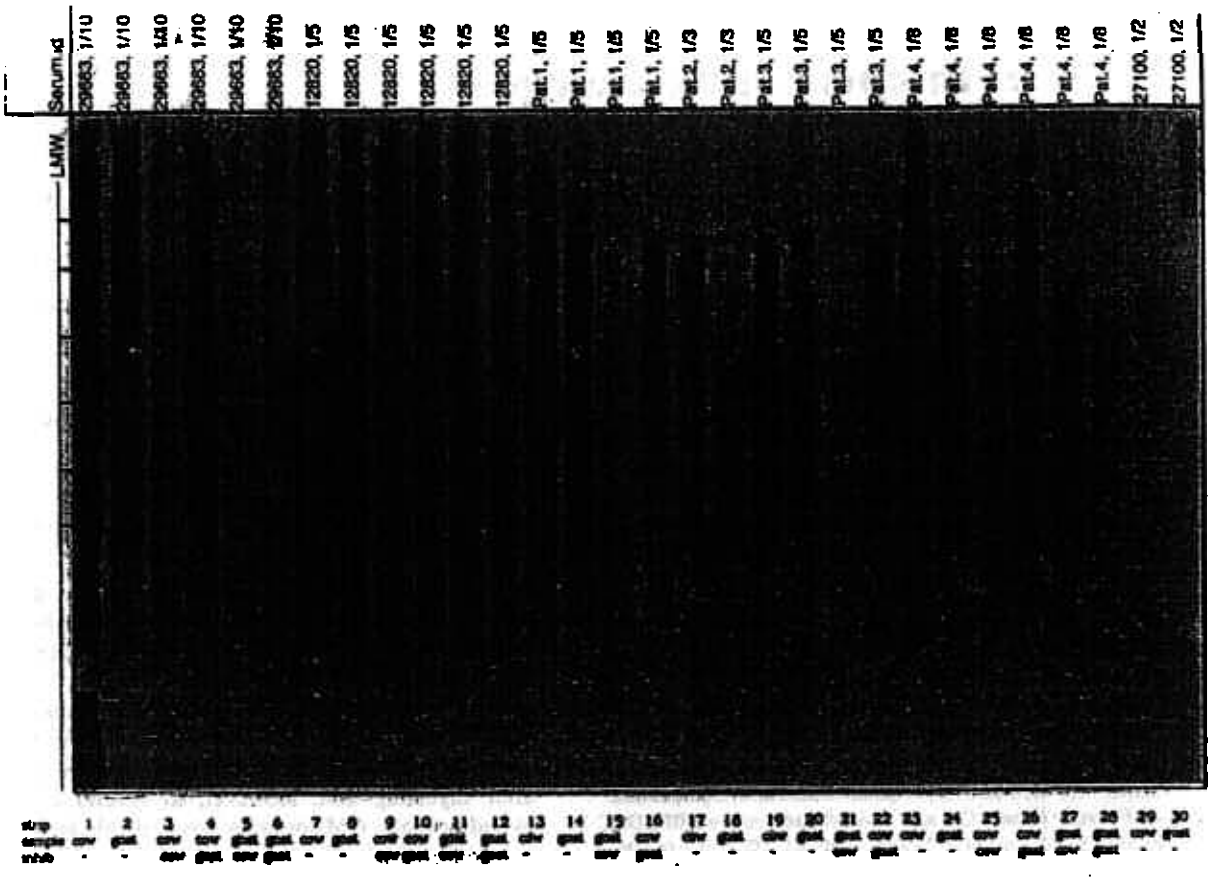

FIG 1. Immunoblotting of CM and GM, including inhibition studies. Patients 1, 2, 3, and 4 are children with CMA who also had positive skin test responses, RAST results, and DBPCOFC responses to GM. Sera identified as no. 29663 and no. 12820 are 2 additional CM RAST-positive sera. Serum identified as no. 27100 is a CM RAST-negative serum.

TABLE I. GM Pharmacia CAP System inhibition in a child with CMA

Type of milk	kU/L	Percent inhibition
Cow	12.02	—
Goat	12.80	—
Goat vs cow	2.38	80.2
Goat vs goat	3.62	71.7
Cow vs goat	2.12	83.4
Cow vs cow	3.29	72.6

considered negative, and values above 0.35 kU/L were considered positive.

Pharmacia CAP System inhibition test

The CAP inhibition test was carried out only in 1 of the 26 children with CMA who had a positive skin prick test response to CM and GM, a positive DBPCOFC response to CM and GM, and positive specific IgE to CM (12.02 kU/L) and GM (12.80 kU/L). Fifty microliters of the patient's serum was preincubated with CM or GM ImmunoCAP and then tested for specific IgE to CM and to GM by using the Pharmacia CAP System.[3]

DBPCOFC

Challenge tests were performed in a day-hospital setting by administering fresh CM, GM, or, as placebo, a soy formula (Isomil) as follows. One drop was put on the inner border of the lower lip, and a further 1 mL was given every 5 minutes. If no symptoms appeared, 20 mL and 100 mL were given after 30 minutes. After the last administration of the tested milk, the children were kept under observation for at least 4 hours and then discharged. The next challenge test was done 1 week later.

SDS-PAGE and immunoblotting

Electrophoresis (SDS-PAGE) and immunoblotting[4] were carried out in the sera of 4 children with CMA who also had positive skin test, RAST, and DBPCOFC responses to GM. Two additional CM RAST-positive sera and 1 negative serum were tested in immunoblotting only.

CM (standard low-fat type) was obtained locally. GM was obtained fresh at a local educational farm from kids about 1 month old. Samples for CM and GM were prepared by centrifugation, gel filtration on PD-10 columns, and freeze-drying.

Samples were separated by molecular weight on a 1.5-mm thick gradient polyacrylamide gel (7.5% to 20%) under reducing conditions. One gel was used for protein staining with Coomassie Brilliant Blue, and the separated proteins on the second gel were transferred electrophoretically to a nitrocellulose membrane, which was then cut into strips and incubated with the appropriate sera.

In those sera that were inhibited before contact with nitrocellulose, 300 μL of either milk sample at 10 mg/mL (dry weight) was incubated with 300 μL of the serum. IgE binding was detected with ^{125}I-anti-IgE followed by autoradiography.

Only the positive control subjects and patient 4 were run in a full

cross-wise inhibition design. A partial cross-inhibition design was performed for patients 1 and 3, and only Western blotting was carried out for patient 2.

RESULTS

Skin tests and DBPCOFCs

All the children had positive skin test responses to both CM and GM, all had positive DBPCOFC responses to CM, and 24 of 26 had positive DBPCOFC responses to GM. At the time of the skin test, the 2 children who tolerated GM had a specific reactivity to CM casein and β-lactoglobulin. There was no difference in the onset, type, or severity of allergic reactions after CM or GM challenge. At the time of challenge with CM, the main symptoms were urticaria in 15 of 26 children, rhinitis and/or wheezing in 7 of 26, and vomiting and rush in 4 of 26. At the time of challenge with GM, the main symptoms were urticaria in 12 of 24 children, respiratory symptoms (rhinitis and/or wheezing) in 5 of 24, angioedema in 3 of 24, and vomiting and rush in 4 of 24. However, the amount of GM triggering the allergic reaction was significantly higher than that of CM (CM, 8 mL [range, 1 to 30 mL]; GM, 38 mL [range, 3 to 100 mL]; $P < .005$). No children reacted to placebo (Isomil).

Specific IgE determination

All the children tested exhibited positive values of specific serum IgE to CM and GM (class range between II and IV).

Pharmacia CAP System inhibition test

In Pharmacia CAP System inhibition assays, preincubation of serum with CM ImmunoCAP strongly inhibited IgE to both GM and CM. At the same time, preincubation of serum with GM ImmunoCAP strongly inhibited IgE either to GM or CM (Table I).

SDS-PAGE and immunoblotting

Protein staining of SDS-PAGE gels shows that the composition differs between CM and GM. GM contains proportionally more α-lactalbumin and somewhat less casein, especially α-casein (35 kd), compared with CM. One band at 13 kd is missing in GM. There are differences also in the interval of 50 to 90 kd. Allergenic components were detected between 10 and 94 kd. The molecular markers show the presence of 4 regions identified by specific IgE binding in both types of milk: albumin (69 kd), caseins (between 33 and 40 kd), β-lactoglobulin (18 kd), and α-lactalbumin (15 kd). Other bands are identified between 22 and 28 kd (Fig 1, *strips 1* and *7* for CM and *strips 2* and *8* for GM).

All tested sera appeared to react more strongly with CM than with GM. There were differences in allergen composition (eg, the casein interval stained more densely for GM), but samples were still largely similar.

Sera that were inhibited by milk showed a reduction in staining or in some cases an almost total extinction (Fig 1).

Serum from patient 4 extensively reacted with CM (Fig 1, *strip 23*), whereas only caseins and 2 bands between 20 and 30 kd are identified in GM by specific IgE binding (Fig 1, *strip 24*). Preincubation with CM inhibited reactivity to itself and to GM (Fig 1, *strips 25* and *27*), whereas GM inhibited reactivity to itself (Fig 1, *strip 28*) and partially inhibited reactivity to CM (Fig 1, *strip 26*), with the reductions being related only to the bands evidenced in GM. Similar patterns of cross-inhibition were observed also in sera from patients 1 and 3 (Fig 1, *strips 13* to *16* and *19* to *22*).

In conclusion, reactivity patterns to CM appear homogeneous for all sera tested, but there are clear differences in reactivity patterns to GM. In general, preincubation with CM completely inhibits reactivity to GM, but the opposite occurs only partially.

DISCUSSION

This study focuses on the problems with finding an appropriate CM substitute in children with IgE-mediated CMA. Our selected cases were confirmed by DBPCOFC results, and the majority reacted also to the proposed substitution with GM but not to soy milk. This is in agreement with previously published studies that soy milk is a suitable CM substitute for feeding babies with CMA,[5,6] and that extensive cross-reactivity between CM and GM allergens does occur.[7-12] There is no doubt that all the children were initially sensitized to CM proteins, and GM allergy was caused by the presence of CM-specific IgE antibodies that cross-react with GM. In fact, no child had previously been fed any GM-containing food. In addition, sensitization in utero or during breast-feeding may be ruled out because the mothers stated that they had not eaten GM or cheese produced from GM during pregnancy and breast-feeding.

The evidence for cross-reactions has been pursued in both animal studies[1,13-16] and clinical and immunochemical studies in children affected by CMA.[7,9,14-16] The major CM allergens are the whey proteins, casein, β-lactoglobulin, α-lactalbumin, and BSA. Caseins constitute 80% of the total bovine milk proteins. It is known that α-s1 and α-s2 caseins from cows, goats, and sheep share 87% to 98% identical amino acids.[17] These data are not surprising because of the biochemical similarity connected to the same phylogenetic origin of these animal species.

Recently, the allergenic potential of α-caseins from cows, sheep, and goats was compared by ELISA and inhibition ELISA assays in children with CMA.[17] In this study the inhibition of the IgE binding to bovine α-casein with α-casein from cows, goats, and sheep confirmed that the α-caseins from these species are highly cross-reactive. Nevertheless, single observations of allergy to goat and sheep casein in the absence of CMA have been reported.[18,19]

Our results, determined by using immunoblotting techniques, show that although CM appears to be a more potent allergen than GM, the latter is still highly allergenic. The significant amount of such allergenic proteins

triggers severe symptoms in children with CMA. Only 2 of 26 of the children with CMA appeared to tolerate GM in DBPCOFCs.

We observed that the amount of GM triggering a reaction after DBPCOFC was significantly higher than that of CM, thus confirming the lower concentration of cross-reacting allergens in GM. However, we stress that for children with CMA, even minute amounts of CM proteins or cross-reacting proteins can induce symptoms, as shown in children fed with CM hydrolysate products.[2,20]

In our study serologic data were evaluated by cross-inhibition of immunoblotting with either CM- or GM-derived proteins. In general, reactivity patterns for CM seemed to be homogeneous for all cases tested and more reactive than those for GM. However, some differences between CM and GM were clearly detectable, as well as differences in cross-inhibition tests. In particular, caseins were more strongly reactive for IgE antibodies in both CM and GM samples, with varied reactivity to other allergenic proteins for either milk sample. CM strongly absorbed any reactivity to CM proteins in immunoblots, as well as those to GM; however, the reverse was not true. GM absorption and inhibition of CMA sera did not result in full disappearance of immunoblot reactivity. Our data seem to confirm those of a recent study performed in weaning rats,[21] which demonstrated that clinical reactivity to GM occurs in 100% of those sensitized to CM.

Because in many countries GM formulas are now available, we recommend that GM should never be given to children with CMA and that the lack of safety of GM for children with CMA be reported on the label to prevent reactions that may be life-threatening in highly sensitized children.

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