

Allergy to the heat-labile proteins α -lactalbumin and β -lactoglobulin in mare's milk

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Background: Allergy to mare's milk is rare. Recently, however, mare's milk has been recommended for treatment of various ailments by practitioners of "alternative medicine," and it is available in health food stores.

Objective: We report a case of allergic reaction to mare's milk in a 51-year-old woman who was able to tolerate cow's milk.

Methods: The protein composition of mare's milk was determined by methods based on measurement of nitrogen content. The patient underwent prick and intracutaneous tests with commercially available bovine milk proteins and several mare's milk preparations, including mare's milk granulate and boiled mare's milk. RAST and immunoblotting were also performed.

Results: Results of skin testing and RAST with cow's milk were negative but demonstrated an IgE-mediated allergy to mare's milk. Immunoblotting revealed two allergen bands with molecular weights of 16 and 18 kd, most likely representing the whey proteins α -lactalbumin and β -lactoglobulin. The bands disappeared after the mare's milk was boiled, indicating that the proteins are heat-labile.

Conclusion: The results of this study demonstrate the existence of an IgE-mediated mare's milk allergy caused by low molecular weight heat-labile proteins, most likely α -lactalbumin and β -lactoglobulin, which do not cross-react with the corresponding whey proteins in cow's milk. (*J Allergy Clin Immunol* 1996;97:1304-7.)

Key words: Heat-labile allergy proteins, α -lactalbumin, β -lactoglobulin, mare's milk, whey

Allergy to mare's milk is a rare clinical event. We report on a female patient, who showed allergic reactions to mare's milk but was able to tolerate cow's milk. By skin testing and RAST, we could demonstrate an IgE-mediated allergy to mare's milk. Immunoblotting revealed two allergen bands with molecular weights (MWs) of 16 and 18 kd, presumably representing the whey proteins α -lactalbumin and β -lactoglobulin; these bands disappeared after the mare's milk was boiled, indicating that these proteins are heat-labile.

Abbreviations used

MW:	Molecular weight
SDS-PAGE:	Sodium dodecylsulfate-polyacrylamide gel electrophoresis
TBS:	Tris-buffered saline

CASE REPORT

A 51-year-old female patient had had plaque-type psoriasis for several years. A "health food shop" advertisement drew her attention to mare's milk, which is recommended by the so-called "alternative medicine" for various medical indications. For the first time in her life she took, for a short period, mare's milk granulate (Hippolac). After 2 weeks of "treatment," severe anaphylactic symptoms occurred: allergic asthma, angioedema, and severe hypotension requiring immediate emergency treatment. Since then, mare's milk has been strictly avoided. However, cow's milk and cow's milk products are still well tolerated.

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METHODS

Allergy test procedures

Determination of nitrogen content and protein composition of mare's milk and mare's milk granulate. Protein content of native mare's milk and mare's milk granulate (Hippolac) was determined by analyzing nitrogen content by the Kjeldahl method by which total nitrogen, nonprotein nitrogen (after precipitation by trichloroacetic acid), and residual nitrogen of whey protein including nonprotein nitrogen after removal of casein by isoelectric precipitation, respectively, were measured. Protein content was then calculated from the corresponding nitrogen content by applying the empirical factor 6.38, which is valid for cow's milk proteins including carbohydrate.

Skin testing. Prick and intracutaneous tests were performed with commercially available bovine milk proteins (Laboratory Diephuis, Groningen, The Netherlands), as well as prick tests with different mare's milk preparations: mare's milk granulate and fresh and boiled (10 minutes) mare's milk. Control tests were done with fresh mare's milk and mare's milk granulate in 10 nonatopic and nonallergic volunteers.

RAST assays. Native mare's milk and human milk were centrifuged at 5000 rpm, and after resuspension, the pellet was used for production of allergen disks. The cow's milk proteins α -lactalbumin, β -lactoglobulin, and casein were purchased from Sigma Chemie (Deisenhofen, Germany), and disks were prepared with a concentration of 1 mg/ml. The activation of cellulose disks (Schleicher and Schüll, Dassel, Germany) with bromocyanide and the covalent binding of the respective allergens was done according to the method of Ceska et al.¹ The allergen solutions used in this study had a concentration that yielded a protein binding of at least 1 μ g/ml.

Allergen-specific IgE antibody levels were measured with conventional Phadebas RAST (Pharmacia Diagnostics AB, Uppsala, Sweden). Disks with new allergens were compared with negative control sera obtained from nonatopic and nonallergic persons. Only disks that rated RAST classes 0 to 4 were used (according to Pharmacia RAST system) when the negative control sera resulted in counts per minute clearly below RAST class 1. The assay was performed according to the manufacturer's instructions.²

Sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The mare's milk was centrifuged for 20 minutes at 4000 rpm (2000 g). A part of the skim milk was additionally boiled at 92° to 97° C for 10 and 25 minutes. Unboiled and boiled mare's milk were diluted 1:150 with nonreducing probe buffer consisting of 1 gm SDS, 0.01 gm NaN₃, 10 mg bromophenol blue, 2.5 ml stock buffer (pH, 6.8; 6.06 gm Tris, 0.4 gm SDS, 0.01 gm NaN₃, distilled water to 80 ml), distilled water to 100 ml. Samples were then transferred to the gel and separated according to the manufacturer's recommendations (material: Exelgel SDS, Gradient 8-18; Pharmacia, Freiburg,

Germany; SDS buffer strips, Pharmacia; Multiphor II, LKB/Pharmacia; conditions: 600 V, 15 W, 30 mA, 60 minutes).

Immunoblot. The milk proteins on unstained SDS-PAGE gels were transferred to nitrocellulose membranes. The blot unit was used according to Pharmacia recommendations for Novablot and with a noncontinuous buffer system: anode buffer 1 consisting of 18.15 gm Tris (= 0.3 mol), 100 ml methanol, distilled water to 500 ml; anode buffer 2 consisting of 1.52 gm Tris (= 25 mmol), 100 ml methanol, distilled water to 500 ml; cathode buffer consisting of 1.52 gm Tris (= 25 mmol), 100 ml methanol, 2.63 gm 6-amino-hexanoic acid, distilled water to 500 ml. Transfer conditions were 10 V, 5 W, 0.8 mA, 50 minutes.

After transfer, the nitrocellulose membrane was covered briefly with 0.2 N NaOH and then washed with Tris-buffered saline (TBS)-Tween. To verify transfer, one part of the membrane was stained with india ink. The other part was blocked with TBS-Tween in 3% gelatin and then fixed by using a Cross-Blot device (Schleicher and Schüll, Dassel, Germany). Diluted patient sera 1:4 with TBS-Tween were added to the chambers and incubated overnight at 4° C. Then the membrane was incubated for 4 hours at room temperature with alkaline phosphatase-conjugated rabbit anti-human IgE (Ventrex; Hycor Biomedical Inc., Irvine, Calif.). IgE binding was detected by ELISA.

Development was done with nitroretetrazolium blue/5-bromo-chloro-indolyl-phosphate in ethanolamine buffer, pH 9.5, and dimethylformamide. Development was stopped with ethylenediaminetetraacetic acid in phosphate-buffered saline, and the membrane was dried at room temperature.

RESULTS

Protein content of mare's milk

Total protein content was calculated as 1.64% $\pm$ 0.01% in native mare's milk and 17.4% $\pm$ 0.2% in mare's milk granulate. The main protein fraction in both cases was whey protein (mare's milk, 71%; mare's milk granulate, 64%; both referenced to total protein).

Skin testing

Results of prick and intracutaneous tests with bovine milk proteins were negative (Table I). However, prick testing with mare's milk granulate and fresh mare's milk produced strong positive reactions. An additional test with boiled mare's milk (10 minutes) caused an anaphylactic reaction. Results of control tests with fresh mare's milk and mare's milk granulate in 10 volunteers were all negative.

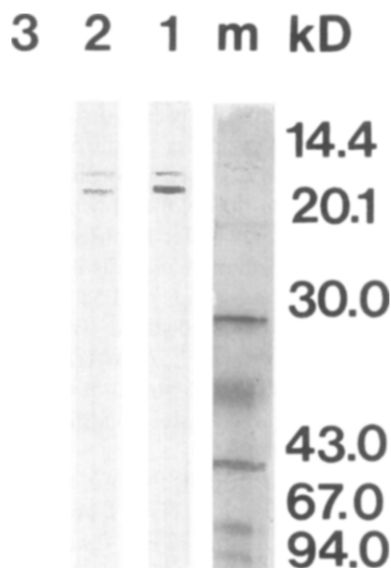


FIG. 1. Immunoblot with mare's milk. *Lane 1*, Native mare's milk; *lane 2*, boiled mare's milk (10 minutes); *lane 3*, boiled mare's milk (25 minutes). *m*, Marker protein (MW in kilodaltons).

RAST assays

Results of RAST assays with cow's milk proteins were negative (Table I). RAST identified specific IgE (class 2) in native and boiled (10 minutes) mare's milk. After 30 minutes of boiling, the allergenicity of mare's milk decreased.

Immunoblot

The immunoblot with native mare's milk revealed a strong band with an MW of approximately 18 kD and a weaker band with an MW of approximately 16 kD (Fig. 1). After mare's milk was heated for 10 minutes, the two bands were reduced, and after milk was heated for 25 minutes, the bands disappeared.

DISCUSSION

Proteins represent the main milk allergens of all species. One fraction is synthesized in mammary

TABLE I. Results of skin tests and RAST in a patient with mare's milk allergy

	Prick test	IC test	RAST class
Cow's milk proteins	10%	0.1%	
α -Lactalbumin	—	—	0
β -Lactoglobulin	—	—	0
Casein	—	—	0
Mare's milk preparations			
Pure mare's milk granulate	++++	ND	ND
Native mare's milk	++++	ND	2
Boiled mare's milk (10 min)	++++*	ND	2
Boiled mare's milk (30 min)	ND	ND	0-1

IC, Intracutaneous; ND, not done.

*Together with anaphylactic reaction.

glands and consists of casein, α -lactalbumin, and β -lactoglobulin (whey proteins). Casein is heat-resistant, and α -lactalbumin and β -lactoglobulin are heat-labile.

In our patient we could exclude, by in vivo and in vitro investigations, an allergy to cow's milk proteins. However, by using skin tests and RAST, an IgE-mediated allergy to native mare's milk could be verified. RAST and skin test results showed that the allergenicity of native mare's milk could not be reduced after a boiling period of 10 minutes. Only after a boiling period of 30 minutes did the allergenicity disappear, as determined by RAST. These findings are partly confirmed by immunoblot with mare's milk. After mare's milk was heated for 10 minutes, the allergen bands were only weakened; after milk was heated for 25 minutes, the bands were absent. With our procedure, first boiling the mare's milk and then loading with nonreducing probe buffer containing SDS, we prevented denaturation of proteins and loss of conformation. Reduction of IgE-binding activity shown by immunoblotting is therefore only due to boiling mare's milk for 10 and 25 minutes with destruction of the heat-labile whey proteins. Actually, there is a discrepancy in the allergenicity of mare's milk boiled for 10 minutes as determined by skin test and immunoblot. Skin test revealed a strong reaction and caused an anaphylactic reaction in the patient. Allergenicity was boosted by repeated skin testing. Reduction of IgE-binding activity of mare's milk boiled for 10 minutes, as demonstrated by immunoblotting, is partly due to destruction of the heat-labile whey proteins.

The immunoblot with mare's milk revealed two

allergen bands: a strong band with an MW of 18 kd and a weak band with an MW of 16 kd. The allergen band of 18 kd most likely represents the whey protein fraction β -lactoglobulin.³ The weak allergen band of 16 kd observed by us in mare's milk obviously corresponds to the glycosylated variant of α -lactalbumin with an MW of 16.8 kd.⁴

In summary, our findings demonstrate the existence of an IgE-mediated mare's milk allergy caused by low MW heat-labile proteins, most likely the whey proteins α -lactalbumin and β -lactoglobulin, which show no cross-reaction with the corresponding whey proteins from cow's milk. This comprehensive case report describes a single subject with mare's milk allergy. Meanwhile, two additional patients had specific IgE to mare's milk that could be detected by RAST (class 2 and 4) with clinical relevance (personal communication C. M. Kalveram). These patients had treated atopic eczema with mare's milk. After drinking mare's milk, the patients experienced urticaria and angioedema and anaphylactic reaction with severe hypotension requiring immediate emergency treatment, respectively. Because mare's milk is offered

as an "alternative medicine" for treatment of psoriasis and atopic eczema, we expect increasing numbers of allergic reactions to mare's milk in the future. Because of strong cross-reactivity of cow's milk with goat's and sheep's milk, allergic reactions to the milk proteins of these species may be possible.⁵

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