Cross-Sensitization between Milk Proteins: Reactivity to a "Kosher" Epitope?

Yitzhak Katz MD[1,2], Michael R. Goldberg MD PhD[1,2], Galia Zadik-Mnuhin MD[2], Moshe Leshno MD PhD[4] and Eli Heyman MD[3]

[1]Institute of Allergy and Immunology, [2]Department of Pediatrics and [3]Department of Neonatology, Assaf Harofeh Medical Center, Zerifin, Israel
[4]Faculty of Management, Sackler Faculty of Medicine, Tel Aviv University, Ramat Aviv, Israel

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Abstract

Background: Immunoglobulin E-mediated allergy to cow's milk protein represents a major problem for infants who are not breast fed. A search for substitute milks revealed a cross-allergenicity to milk derived from goat and sheep but not to milk from a mare. We noted that the cow, goat and sheep species are both artiodactyls and ruminants, defining them as kosher animals, in contrast to the mare.

Objectives: To determine whether patients with IgE-mediated cow's milk allergy are cross-sensitized to milk from other species such as the deer, ibex, buffalo, pig and camel.

Methods: Patients with a clinical history consistent with IgE-mediated cow's milk protein allergy were tested by skin-prick test to validate the diagnosis. They were then evaluated by skin-prick test for cross-sensitization to milk-derived proteins from other species.

Results: All patients allergic to cow's milk tested positive by skin-prick test for cross-reactivity to deer, Ibex and buffalo (n=24, *P* = 0). In contrast, only 5 of the 24 patients (20.83%) tested positive to pig milk and only 2 of 8 (25%) to camel's milk. Cross-sensitization to soy milk was noted in 4 of 23 patients (17.39%), although they all tolerated oral ingestion of soy-containing foods.

Conclusions: A significant cross-sensitization to milk proteins derived from kosher animals exists in patients allergic to cow's milk protein, but far less so compared to the milk proteins from non-kosher animals tested. Patients with proven IgE-mediated allergy to cow's milk can utilize the above findings to predict suitable alternative sources of milk.

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Cow's milk protein allergy is one of the most common food allergies affecting young infants. In the search for an alternate feeding regimen, where lactation is not an option, milk from several other mammals was introduced. This has met with limited success in cases of immunoglobulin E-mediated cow's milk allergy due to the high degree of cross-sensitization between cow's milk and milk from the goat, ewe and buffalo [1-3]. On the other hand, cross-reactivity with milk from other mammals, such as the camel [3], mare [4] and ass [5], was negligent.

One of the major allergeneic proteins in milk are the caseins [6]. A phylogenetic comparison of beta-casein and kappa-casein genes demonstrates that the goat, sheep, cow, deer, and pronghorn casein genes are most closely related when compared to the milk protein of a camel, pig or zebra [7]. We noted that those animals with the more closely related casein genes (i.e., the goat, sheep, cow, deer, pronghorn, buffalo) are "kosher" animals, while the camel, mare and pig represented "non-kosher" animals. "Kosher" animals are listed in the Old Testament (Leviticus 11) as animals that the Jews are allowed to eat, and are generally described as animals that have split hooves (artiodactyls) and chew their cud (ruminants). We examined the hypothesis that once an individual is allergic to cow's milk protein, he or she is sensitized to milk from all kosher animals but not to milk from non-kosher animals. In the present study we examined the sensitization to milk from ibex, deer and pig among 24 patients with cow's milk allergy. The ibex and deer are kosher but the pig is not.

Patients and Methods

The study protocol was approved by the Institutional Review Board at Assaf Harofeh Medical Center according to the Helsinki Declaration. Evaluations were performed in a referral center for allergic disorders at the investigators' institution. The characteristics of the study and control populations are listed in Table 1. Fresh milk from a lactating pig was obtained from Kibbutz Lehavim (Israel). Milk from deer and ibex was obtained from lactating animals at Odem Farms (Israel); these animals were milked specifically for the study. Milk from a camel was obtained from Dr. R. Yagil (Beer Sheva) and milk from buffalo from Moshav Bitzaron (Israel). Fresh milk of the studied species was placed at 4°C and used within 5 days.

Skin-prick tests were performed according to a standard technique with a Wyeth needle (Heinz Herenz, Hamburg, Germany). Allergy extracts to cow's milk protein and soy were purchased from ALK-Abelló, (Port Washington, NY, USA). Milk from different species, commercial allergy extracts, histamine at 1 mg/ml (Center Laboratories, Port Washington, NY) and negative controls (saline) were applied. The mean diameters of the wheal and erythema (flare) were measured after 15 minutes (a planimeter was used to measure the area of the papule). If the wheal or flare diameter exceeded that of the histamine-positive control, the test was regarded as positive.

In total, 32 patients were evaluated by skin-prick testing using the milks derived from the various species listed. Twenty-four of these patients had a clinical history remarkable for cow's

Ig = immunogloublin

milk protein allergy and tested positive on a skin-prick test for cow milk protein. Eight volunteers were used as negative controls.

Statistical analysis

The Fisher exact test was used to determine whether pig's milk allergy was dependent on allergy to cow's milk.

Results

A total of 24 patients with known cow's milk allergy and a positive skin-prick test to cow's milk protein were tested for cross-sensitization to milk proteins derived from deer, ibex, buffalo, pig and camel. The clinical characteristics of the 24 subjects are listed in Table 1. Thirteen of the patients studied were male and 11 were female. Nineteen patients (79%) developed a rash after ingesting cow's milk, either as the sole symptom (n=2) or as part of a constellation of symptoms as listed in Table 1. With the exception of one patient (# 6), the time from ingestion to the onset of clinical manifestations was less than 20 minutes.

Table 1. Clinical characteristics and symptoms associated with cow's milk protein allergy

Patient no.	Gender	Age at onset (mos)	Age at exam (mos)	Onset of reaction (min)	Clinical manifestations
1	M	1	30	15	Rash, vomiting
2	F	< 1	13	< 5	Rash, anaphylaxis, vomiting
3	M	5	180	< 5	Rash, anaphylaxis
4	M	1.75	22	< 5	Rash, vomiting, shortness of breath
5	M	6	31	10	Rash, vomiting
6	F	4	26	120	Vomiting
7	F	6	22	10	Rash, vomiting
8	F	1	2.5	20	Rash, edema
9	M	9	20	5	Anaphylaxis, vomiting, shortness of breath, edema
10	M	4	192	5	Rash, edema
11	M	48	96	20	Rash
12	F	2	174	5	Rash, anaphylaxis
13	F	6	48	5	Rash, vomiting
14	M	2	47	20	Rash, vomiting, edema
15	F	3	96	5	Rash, anaphylaxis, vomiting, shortness of breath, edema
16	M	0.1	54	20	Rash, vomiting
17	M	1	31	5	Rash
18	F	1	31	20	Rash, vomiting, anaphylaxis, shortness of breath
19	M	6	108	5	Rash, vomiting, anaphylaxis, edema
20	M	6	20	20	Rash, edema localized to the ear
21	M	1.5	9	5	Rash, anaphylaxis, diarrhea
22	F	1.5	36	5	Vomiting, shortness of breath
23	F	6	39	5	Vomiting
24	F	2	42	5	Vomiting

Table 2. Skin-prick test results in cow's milk protein allergic patients

Patient no.	Histamine	Cow milk	Deer milk	Ibex milk	Buffalo milk	Pig milk	Camel milk	Soy
1	2/3	6/12	7/12	3/5	8/12	0	ND	ND
2	6/10	7/30	17/30	13/30	16/40	3/3	ND	4/8
3	4/4	12/22	13/27	12/35	12/35	2/2	ND	4/4
4	6/8	13/19	14/21	16/30	12/20	2/2	ND	2/2
5	2/6	2/8	4/10	4/10	2/8	2/3	ND	1/5
6	5/7	10/20	15/37	15/30	15/30	0	ND	0
7	4/12	6/15	8/20	10/22	12/25	10/25	ND	0
8	7/12	8/16	11/19	9/22	11/28	0	ND	0
9	6/8	12/19	16/30	18/28	18/26	4/4	ND	6/8
10	5/7	9/19	16/22	15/25	13/25	0	ND	0
11	3/14	4/15	7/19	9/20	14/26	0/4	ND	0/4
12	10/12	12/22	25/40	24/40	22/32	12/12	ND	12/20
13	3/7	12/18	15/25	13/20	14/21	2/4	ND	0
14	6/9	9/16	21/33	20/36	19/27	6/9	ND	5/6
15	6/8	11/20	10/20	9/20	11/24	4/4	ND	4/6
16	2/4	12/13	18/27	15/20	14/26	13/18	ND	4/6
17	8/15	21/30	16/30	26/40	20/30	11/15	0	18/25
18	5/7	14/24	20/30	17/30	16/22	5/5	8/14	5/7
19	6/8	14/18	12/20	14/25	16/26	0	0	4/5
20	4/6	8/13	13/20	12/17	11/18	0	2/2	6/6
21	10/12	10/16	12/18	20/24	12/20	0	0	0
22	7/9	11/23	23/30	17/20	17/27	2/2	4/4	4/4
23	8/10	10/14	16/30	12/22	22/26	21/25	14/20	6/6
24	6/8	12/18	18/22	18/30	16/20	3/3	4/4	4/4
Total		24/24	24/24	24/24	24/24	5/24	2/8	4/23

ND = no data

For patient # 6, 120 minutes elapsed until clinical symptoms appeared. Eight of 24 patients (33%) developed anaphylaxis. Six developed generalized edema and in one patient the edema was restricted to the ear. Sixteen of 24 patients (67%) vomited as part of their clinical constellation of presenting symptoms. Only 5 of 24 patients (22%) developed shortness of breath as part of their presenting symptoms. The age of onset was within the first 9 months of life for all patients except for one (patient #11) who developed the allergy at age 4 years. All of these patients were still allergic to cow's milk, as judged by incidental exposure.

The results of the skin-prick test are listed in Table 2. Reactivity to milk derived from the deer, ibex, buffalo, pig, and camel was tested. In addition, the response to commercial allergy extracts to cow's milk and soy was evaluated. The response to histamine served as an internal positive control. All 24 patients who tested positive to cow's milk protein were likewise positive to buffalo, deer and Ibex milk ($P = 0$). On the other hand, only 5 of 24 patients (20.8%) were positive to pig milk. One of the eight control patients had a positive skin-prick test to pig milk but all control subjects were negative to cow, ibex and deer milk [Table 3]. Thus, there was no connection between a skin prick-positive test to pig's milk and an allergy to cow's milk protein, using the Fisher exact test ($P = 0.52$). Four of 23 patients

Table 3. Skin-prick test results in the control group

		Age at examination (mos)	Histamine	Cow milk	Deer milk	Ibex Milk	Buffalo milk	Pig milk	Camel milk	Soy
1	M	17	10/20	0	0	0	ND	4/4	ND	ND
2	F	4	7/10	0	0	0	ND	0	ND	ND
3	M	30	4/6	0	0	0	ND	0	ND	ND
4	F	192	3/5	0	3/3	0	ND	4/12	ND	ND
5	M	36	3/4	0	3/3	0	ND	0	ND	ND
6	F	5	5/8	0	2/3	0	ND	0	ND	ND
7	F	30	4/4	0	0	0	ND	0	ND	ND
8	F	360	3/3	2/2	2/2	0	ND	2/1	ND	ND
Total				**0**	**0**	**0**	**ND**	**1/8**		

ND = no data

(17.4%) cross-sensitized to soy, of whom 3 were also positive to pig-derived proteins (patients 12, 16 and 17). It is interesting to note, however, that all the patients with positive skin-prick test to soy, in the past had tolerated oral soy without any clinical reaction. In general, the size of the wheal and flare in those who tested positive to deer and ibex milk was large compared to the reaction to cow's milk extract [Table 2]. Upon the skin-prick text in five patients allergic to fresh cow milk, however, the size of the reaction was similar to that of the commercial extract (data not shown).

Discussion

This is the first study to investigate the cross-sensitization of milk protein from deer, Ibex and pig in patients with cow's milk protein allergy. The study population consisted of patients with a convincing history of clinical milk allergy based on a positive skin-prick test and a significant clinical reaction. In the majority of cases, the clinical reaction to the ingestion of milk included a rash appearing within 20 minutes from the time of exposure, while in 3 of 24 patients (12.5%) gastrointestinal symptoms predominated. A positive skin-prick test in all our patients, however, ascertains that our patients have true IgE-mediated cow milk protein allergy, and not cow's milk intolerance. The latter, which can be classified into the group of food protein-induced enterocolitis syndrome, is a clinical reaction that occurs 2–48 hours following the exposure and is confined to the gastrointestinal system with severe vomiting and/or diarrhea. This reaction is thought to be immunologically mediated but not via IgE, and the skin-prick test is negative.

An oral food challenge was not performed for several reasons. First, the main purpose of this study was to evaluate for cross-sensitization among milks from different species. Second, an oral challenge with milk from ibex and deer is impractical due to the difficulty in obtaining significant amounts of milk from them for the test. Finally, an oral challenge to pig's milk is not realistic in a largely "kosher" population. Nevertheless, the significant clinical history of allergy to cow's milk along with the positive skin-prick test and the size of the reaction [8] confirm clinical allergy to cow's milk protein. Similarly, the size of the reaction to deer and ibex milk in most of the patients suggests a likely clinical reaction to milk derived from these animals [8,9]. Regarding buffalo milk, cross-reactivity in cow's milk protein-allergic patients to buffalo, as measured by immunoblotting, was previously described [3]. The fact that all four patients who tested positively to soy extract tolerated oral soy is consistent with other reports in the literature [10].

The immunogenic epitopes in milk causing IgE-mediated cow's milk allergy may be diverse although, as reported by Docena et al. [6], anti-casein-specific IgE antibodies were present in all (80/80) sera examined from patients with a compatible history for cow's milk protein allergy and positive IgE against cow's milk protein [6]. In contrast, only 10 of 80 showed reactivity to beta lactoglobulin. Jarvinen et al. [11] reported that the presence of IgE antibodies against at least one of three epitopes present on either α(s1)-casein, α(s2)-casein, or κ-casein identified all patients with persistent cow's milk allergy [11]. Furthermore, lymphocyte proliferative responses to the caseins were only observed in clinically reactive IgE-mediated cow's milk protein allergy, but not in non-IgE-mediated cow's milk allergy [12]. Additional evidence implicating the caseins in cow milk protein allergy is that a decreased proliferative response to κ-casein noted in tolerized IgE-mediated cow's milk allergy patients was abrogated by removing T regulatory cells [12]. It was of interest, therefore, when we noted the reported phylogenetic relationship of β-casein (exon 7) and κ-casein (exon 4) among various mammals [7]. The goat, sheep, cow, deer, giraffe and pronghorn are most closely related compared to the camel, pig, tapir and zebra. The former are all kosher animals while the latter are not. We were unable to obtain milk from a giraffe, although it would be of interest to test giraffe milk to complete our cross-sensitivity studies. Perhaps not surprisingly, the casein genes from non-kosher animals are more closely related to the human-derived casein sequence, and, in general, patients allergic to cow's milk tolerate breast milk. Previous studies analyzing the cross-reactivity to milk among patients allergic to cow's milk also fall along the same divisional lines. Patients allergic to cow milk protein are highly cross-reactive to milk from sheep and goats but not to milk from an ass, mare or camel [3-5]. Individual exceptions are noted in the literature. For example, Wuthrich and Johansson [13] report a case of allergy to sheep and goat cheese but not to cheese from cow's milk. In our study, patients with cow's milk allergy were also shown to be cross-reactive to buffalo, deer (*Cervus aloposs*) and ibex milk, but in the majority of cases not to pig or camel milk.

This study sheds some light on the potential alternatives to milk in IgE-mediated allergy to cow's milk protein. Tolerated alternative milk sources can be sought from non-kosher animals, although the nutritional value of these milks needs to be carefully evaluated. Furthermore, economic considerations may limit the development of these alternative milk sources. Pigs generally do not become pregnant while lactating, in contrast to cows [14,15]. The use of soy may be a potential alternative, although the true percentage of cross-reactivity to soy in patients with cow's milk allergy requires a larger prospective trial. We

noted that 17.4% of our patients had a positive reaction to the skin-prick test to soy, but despite this cross-sensitization all of them consumed soy without complications. In one prospective multicenter study, the prevalence for soy allergy in IgE-mediated patients allergic to cow's milk was 14% [16].

In summary, patient's with an allergy to cow's milk protein are cross-sensitized to milk derived from other kosher animal species, but less so to the non-kosher animals tested. Thus, in addition to their external physical characteristics described in the Old Testament, kosher animals also share internal structural similarities in their milk components. These structural similarities have wide-ranging clinical implications regarding the type of milk that may be tolerated. It seems like an odd coincidence that animals with such diverse features, which share characteristics that qualify them to be kosher, should also share a common milk protein epitope. While the teleological basis of this finding is intriguing, our research focuses on its practical ramifications. From a scientific standpoint, there is likely no substitute better than human breast milk for an infant, given its nutritional and immunological values. The categorization of animals into "kosher" and "non-kosher" and the fact that cow's milk-allergic patients tend to react to milk derived from any "kosher" animal may help alleviate the confusion among patients, dietitians and even pediatricians seeking a substitute for cow's milk [17].

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Correspondence: Dr. Y. Katz, Director, Institute of Allergy and Immunology, Assaf Harofeh Medical Center, Zerifin, 70300, Israel.
Phone: (972-8) 977-9820
Fax: (972-8) 924-7124
email: ykatz@post.tau.ac.il