
Anaphylaxis to Goat's and Sheep's Milk in an Adult Who Tolerated Cow's Milk: A Sensitization Profile Study

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Allergy to cow's milk (CM) is a common food allergy in children and can present with the full spectrum of IgE-mediated allergic reactions, including anaphylaxis [1]. Almost all affected individuals become tolerant by their late teenage years [2]. Most CM-allergic individuals are sensitized to casein, the main protein fraction of milk. CM proteins typically share >85% sequence homology with goat's and sheep's milk (GSM) proteins, resulting in a high degree of cross-reactivity in CM-allergic persons. Indeed, goat's milk (GM)-induced allergic reactions occur in more than 90% of CM-allergic individuals [3]. However, rare cases with specific allergy to GSM in CM-tolerant individuals have been reported [4,5]. We report the case of an adult in whom evaluation for sensitization to milk proteins revealed a very uncommon GSM-specific allergy.

A 39-year-old man presented with recurrent episodes of anaphylaxis over 20 years, with 1 episode per year on average. Reactions typically developed within minutes after eating. The episodes were of variable severity and involved severe stomach pain, urticaria, facial angioedema, and—on several occasions—anaphylactic shock. Symptoms resolved in all episodes after treatment with an epinephrine autoinjector, whereas antihistamines alone were insufficient. The patient reported that the episodes occurred after eating feta cheese, traces of GM cheese, or other GSM cheese-containing foods, although he tolerated CM cheese and large quantities of pasteurized CM without symptoms. Notwithstanding this tolerance, the patient recalled reactions after ingestion of concentrated CM-derived whey protein nutritional supplements. He has minor pollen-associated food allergy. Skin prick tests showed sensitization to GSM and GSM cheese, but not to CM or CM cheese (Fig. S1A). Total IgE was 100 IU/mL (<100) and baseline tryptase 2.2 ng/mL (<11.4). Specific IgE testing (ImmunoCAP, Thermo Fisher) revealed sIgE against whole GM (0.53 kU_A/L) and sheep's milk (SM) (0.76 kU_A/L) and against SM whey proteins (0.74 kU_A/L) (Table S1).

Commercial sIgE tests against GM whey proteins and GM and SM caseins were not available. Levels of sIgE against whole CM (0.19 kU_A/L) and CM whey proteins (α -lactalbumin, 0.16 kU_A/L; β -lactoglobulin, 0.11 kU_A/L) were slightly above the limit of detection, while sIgE to CM caseins could not be detected (Table S1). We excluded sIgE-mediated sensitization to LTP, ω -5-gliadin, and galactose- α -1,3-galactose.

We diagnosed recurrent anaphylactic reactions to GSM products associated with tolerance to CM. To further elaborate on the target allergen and cross-reactivity profiles, we performed IgE-Western blot analyses with CM, GM, and SM. We included control sera from patients with distinct molecular sensitization profiles to milk, as follows: isolated sensitization to casein with anaphylaxis to milk from all 3 species (patient #2); sensitization to CM casein, α -lactalbumin, and β -lactoglobulin with mild CM allergy (patient #3); and isolated sensitization to bovine serum albumin with tolerance to milk (patient #4) (Table S1). The serum of patient #1 showed IgE binding to a band at 19 kDa both in GM and SM (Figure, A). This band was at the same molecular weight as the lowest band in patient #2. The known sIgE sensitization profile in patient #2 (isolated sensitization to caseins) suggests that the lowest band represents κ -casein and the remaining bands α - and β -casein. Therefore, we concluded that the band in the case we report was compatible with κ -casein. Specific binding of IgE was not inhibited following serum pre-adsorption with cow casein-loaded beads (maximal signal suppression,

21%) (Figure, B). In contrast, 80% inhibition was observed in patient #2, thus indicating cross-reactivity of IgE to CM and GSM caseins in this case.

Notably, sIgE to whey proteins in patient #1 were only detected using Western blot at high protein concentrations when using pasteurized GSM (data not shown). In contrast, Western blot analyses using fresh unpasteurized SM and separated casein and whey fractions of SM confirmed sensitization to SM whey proteins, with bands at around 15 kDa and 12 kDa, comparable to those detected in patient #3 with CM allergy and sIgE against CM α -lactalbumin and β -lactoglobulin (Figure, C). We confirmed that the milk processing temperature affects detection of IgE in Western blot also for CM, thus underpinning the relevance of the milk treatment temperatures applied in milk-specific IgE sensitization profile studies (Fig. S2). Combined, our data reveal sIgE in our patient-targeted whey proteins and κ -casein in GSM, with no detectable cross-reactivity to CM casein. The traces of sIgE against CM whey proteins detected by ImmunoCAP may explain the patient's clinical reactions to highly concentrated CM whey protein supplements and indicate dose-dependent *in vivo* cross-reactivity between CM and GSM whey proteins. We performed an *in silico* structural comparison of κ -casein, α -lactalbumins, and β -lactoglobulins from CM, SM, and GM (Supplementary data). We identified several surface-exposed amino acids unique to GSM κ -casein, which could well serve as GSM-specific epitopes (Figure, D).

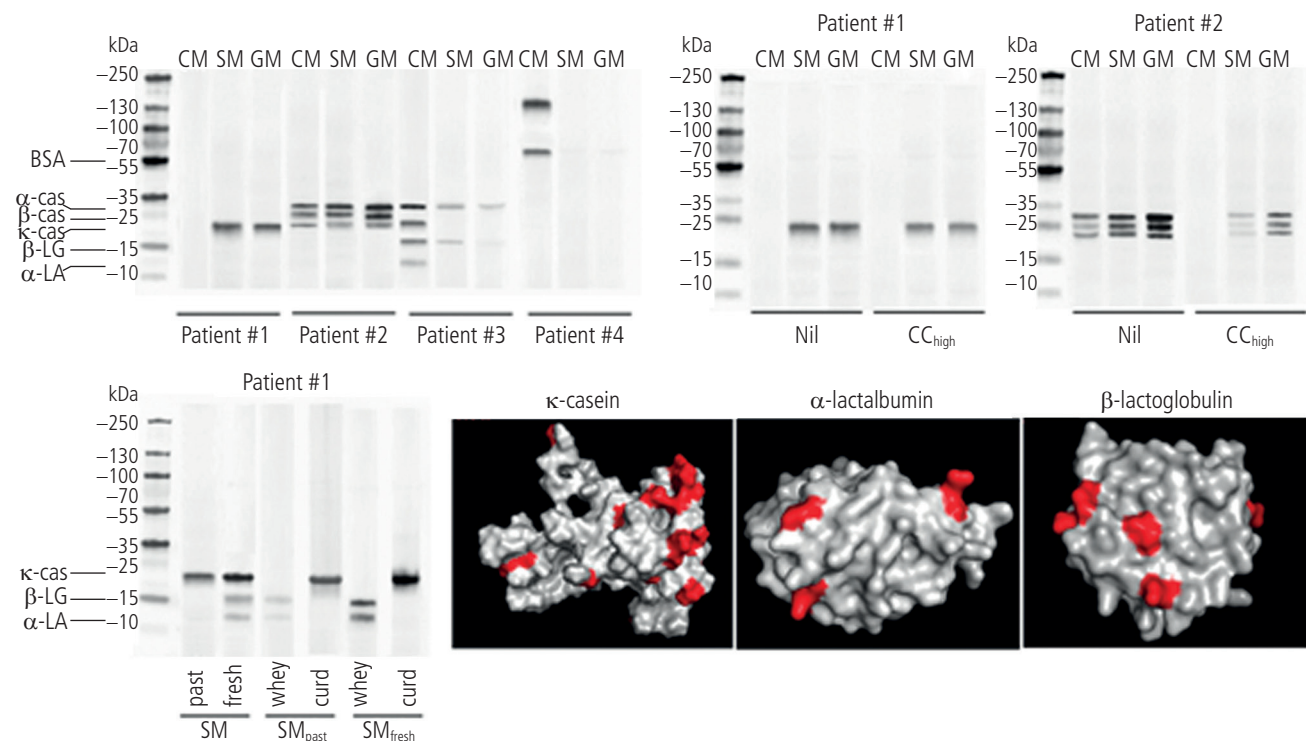


Figure. Milk sensitization profile. A, Western blot of pasteurized CM, SM, and GM using patient sera. The main milk proteins are shown on the vertical axis. B, CM pre-adsorption experiments with CM casein (CC). C, Western blot with pasteurized and unpasteurized SM and whey vs casein-containing curd fraction. D, Visualization of amino acids unique to the GSM proteins (red) as potential non-CM-cross-reactive epitopes. CM indicates cow's milk; SM, sheep's milk; GM, goat's milk.

The whey proteins showed a higher degree of interspecies structural similarity.

To date, only a few cases of adults with GSM allergy who tolerated CM have been reported (Table S2). Molecular investigations identified sIgE against whey proteins and caseins [4,6]. GSM κ -casein was likely the main allergen in the case we report, since the patient had clinical, skin-prick-test, and IgE reactions to GSM cheese, which mainly contains caseins. The lower interspecies cross-reactivity for κ -casein than for other casein proteins [7] may explain the tolerance to CM in the case we report.

Knowledge of isolated GSM allergy is clinically relevant. GSM dairy products are important components of the human diet [8]. In high-income countries, GSM products are becoming popular foods. Moreover, casein-containing GM or SM powders are being used as nutritional supplements. In the present case, severe allergic reactions occurred with traces of GSM products, while CM was tolerated at high doses. Larger cohorts are needed to assess whether sensitization to GSM-specific κ -casein and whey protein is associated with severe anaphylaxis to GSM in general.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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SUPPLEMENTARY MATERIAL

IgE Western blot

Commercially available pasteurized (75°C for 15 seconds), and ultra-high temperature (140°C for 1-2 seconds) processed CM, pasteurized SM (72-73°C for 15 seconds), and GM (122°C for 5 second) were used. Additionally, we used fresh, unpasteurized SM from a Lacaune sheep (Furter-Farm, Bubendorf, Switzerland). We incubated 40 ml of milk with 100 µl goat rennet (Labextrakt La Chevette, Winkler AG, Konolfingen, Switzerland) at 45°C for 60 min. to separate the whey from the casein (curd) fraction. Milk protein concentrations were determined by Bicinchoninic Acid Assay (BCA) using the Pierce™ BCA protein assay kit (ThermoFisher) according to the manufacturer's instructions. We used 1 ng of CM, SM, or GM total protein diluted in 4x Laemmli buffer, incubated at 95°C with 1000 rpm for 10 min, and run on a 4-20% Mini-PROTEAN® TGX Stain-Free™ 1-D polyacrylamide gel (Bio-Rad Laboratories). Following electrophoresis, gels were transferred on a 0.2 µm nitrocellulose Trans-Blot Turbo membrane (Bio-Rad Laboratories, Inc.). To prevent unspecific binding, membranes were incubated with TBS/T with 5% SureBlock™ (Lubio Sciences) for 1h at RT. Then, blots were incubated with 1/100 of human serum from patients and controls in TBS/T rotating overnight at 4°C. Bound IgE was detected using a biotin mouse anti-human IgE (Biolegend) for one hour, followed by a streptavidin-horseradish peroxidase complex (Biolegend) for one hour. The bands were visualized by Pierce™ ECL Western Blotting Substrate (ThermoFisher) and analyzed using Image Lab (v.6.0.1, Bio-Rad Laboratories, Inc.).

Cow casein pre-adsorption experiments

We did a pre-adsorption experiment to test for cross-reactivity between CM casein-specific IgE and IgE against SM or GM. We coated 1 ng or 10 ng CM casein (Fluka) on Magnetic MagPlex-microspheres (Luminex). Empty beads served as the negative control. To pull down casein antibodies, serum samples were incubated with the casein-coated beads or empty beads for 2 h under constant rotation. Then, samples were placed in a magnet for 1 min to remove the cow casein-specific antibodies. The cow casein antibody-depleted sera were then analyzed by Western blotting.

Protein sequence and structure analysis

Protein sequences of κ-casein (cow: NP_776719; sheep: NP_001009378, goat: NP_001272516), α-lactalbumin (cow: NP_776803; sheep: NP_001272564, goat: NP_001009797), and β-lactoglobulin (cow: NP_776354; sheep: NP_001009366, goat: NP_001272468) were aligned with Clustal Omega

(European Bioinformatics Institute). Amino acids that are different in goat and sheep compared to cow casein were identified and visualized with PyMOL (v. 1.7, Schrödinger LLC). For visualization of possible antibody binding sites, the online tool Raptor-X (<http://raptorx.uchicago.edu>) was used to generate a three-dimensional structure model of κ -casein. RCSB PDB database was retrieved for three-dimensional structures of α -lactalbumin (PDB 1F6S) and β -lactoglobulin (PDB 5IO6).

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SUPPLEMENTARY MATERIAL

Supplementary Figure

Figure S1. (A) Skin prick testing with different types of milk and cheese. Numbers indicate wheal and erythema size in mm: CM= cow milk, Past=pasteurized, UHT= ultrahigh temperature treatment; GM= goat milk; CC I= fresh cow cheese; CC II= hard cow cheese; SC I= hard sheep cheese; GC I= fresh goat cheese; GC II= hard goat cheese; SC II = soft sheep cheese; SM= sheep milk (pasteurized); Histamine = positive control; NaCl= negative control. (B) WB with pure CM casein incubated with sera from subject 1 and 2. (C) WB with fresh goat cheese incubated with sera from subject 1 and 2. (D) Visualization of the whole proteins in the whey and curd fractions are shown as loading controls for the WB in Fig. 1C). (E) Sequence alignment of cow, sheep and goat κ -casein (top), α -lactalbumin (middle), and β -lactoglobulin (bottom) are shown. Dashes indicate conserved amino acids between the three species.

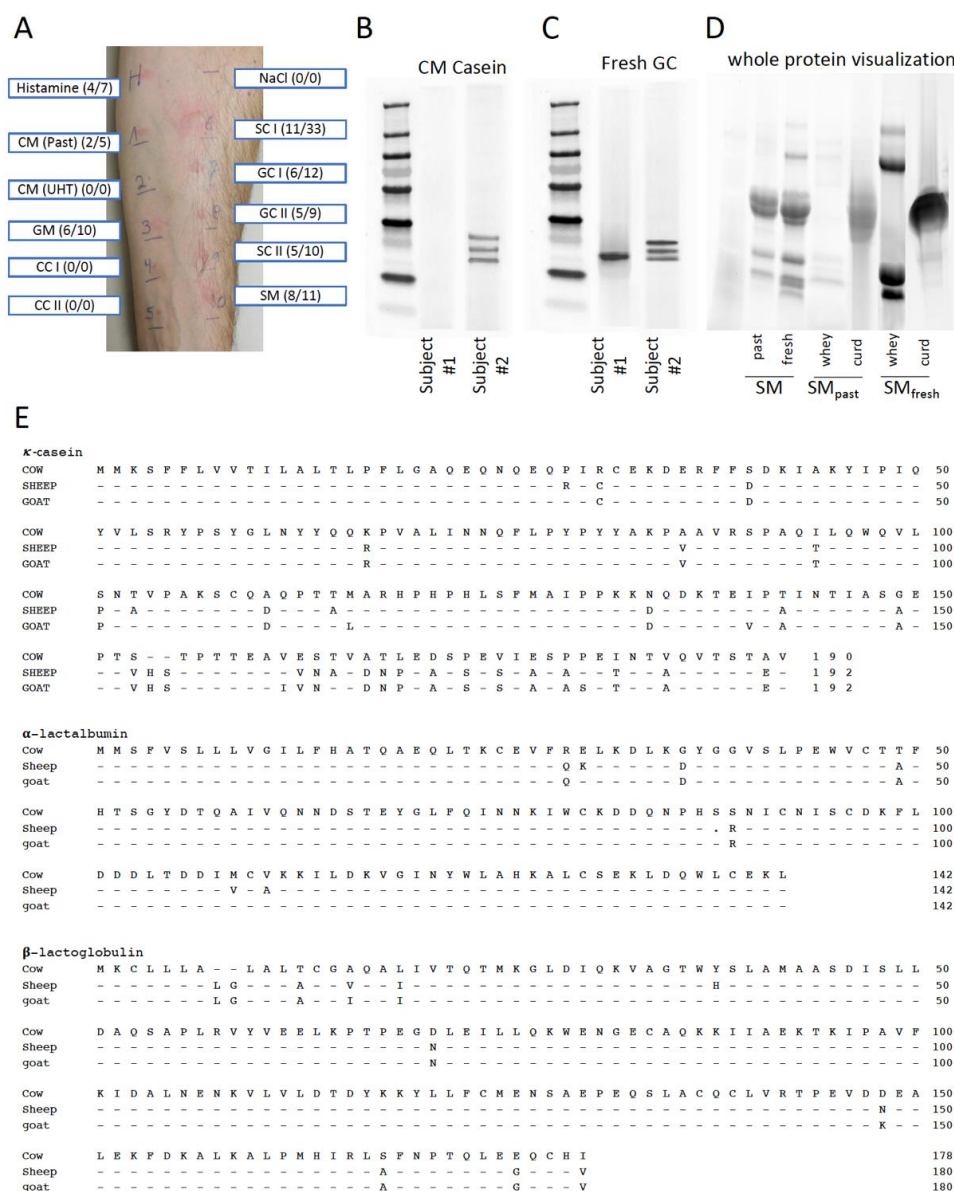
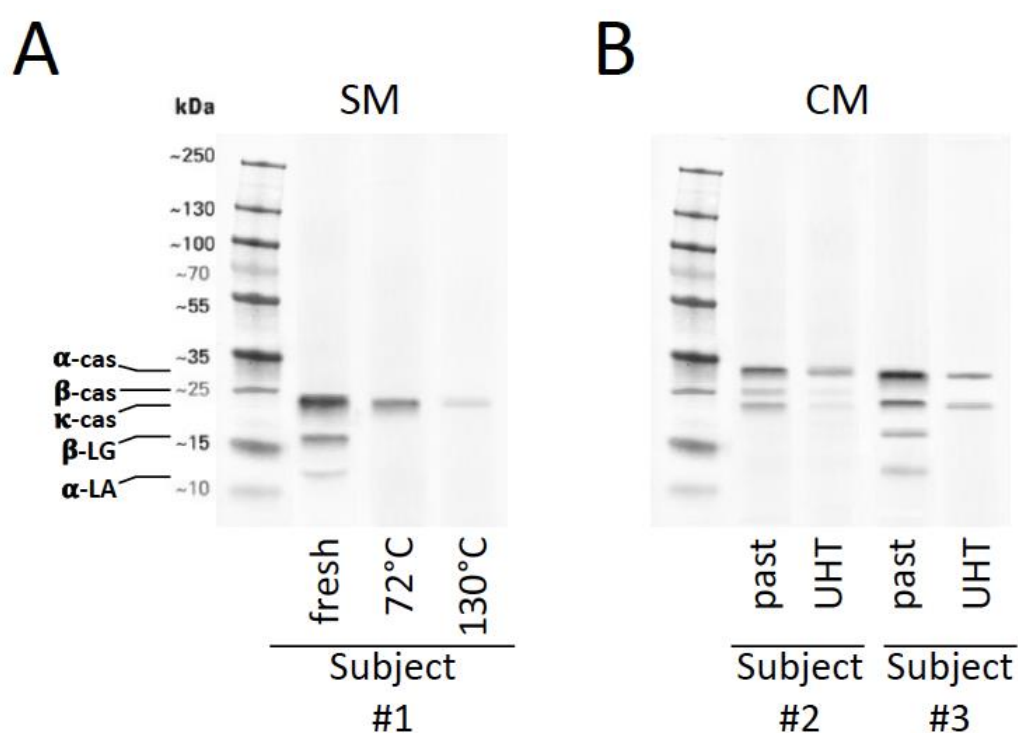


Figure S2. Effect of milk-processing temperature on IgE profiles in western blot analyses. (A) Fresh, unpasteurized sheep milk was heated to 72°C or 130°C for 15s and incubated with sera from subject #1. At 72°C, the whey protein specific IgE became undetectable (see also Figure 1C) and at 130°C the casein-specific IgE band showed substantially reduced intensity. (B) The temperature effect on the detection of IgE against casein was also observable in subjects with CM sensitization. WB using CM and sera of control subjects #2 (IgE against CM caseins) and #3 (IgE against CM caseins and whey proteins). When using UHT processed CM, the IgE against the caseins were reduced (subject #2 and #3) and the whey protein fraction became undetectable (subject #3). Western blot was performed as described in the supplementary methods using sera of subjects characterized in Table S1.



SUPPLEMENTARY MATERIAL

Supplementary Tables

Supplementary Table S1. Clinical presentation and sensitization profiles of the patient and the controls.

			Cow's milk slgE					Sheep's milk slgE		Goat's milk slgE
Subj.	Clinical presentation	Total IgE (IU/ml)	Cow milk (kUA/l)	BSA (kUA/l)	α - lactalbumin (kUA/l)	β - lactoglobulin (kUA/l)	Casein (kUA/l)	Sheep's milk (kUA/l)	Sheep whey (kUA/l)	Goat's milk (kUA/l)
#1	Severe anaphylaxis to GSM, no CM allergy	100	0.19	<0.10	0.16	0.11	<0.10	0.76	0.74	0.53
#2	Severe anaphylaxis to CM and GSM	161	0.73	<0.10	<0.10	<0.10	1.47	27.7	ND	39.6
#3	CM allergy, GSM allergy not reported	302	7.1	<0.10	2.8	4.07	1.78	2.18	ND	1.37
#4	No milk allergy, but sensitization to BSA	124	0.83	1.74	<0.10	<0.10	<0.10	<0.10	ND	<0.10

Supplementary Table S2. Literature Review: Adult patients (>18 years) with isolated GSM allergy.

Age, Sex	Allergen	Tolerated	SPT	IgE (or RAST)	WB using GM and/or SM	Reaction	Atopy	Ref
39, m	GC, SC	CM	GM+, GC+, SM+, SC+, CM-, CC-	GM+, SM+	κ -casein (18kD) whey protein	U, A, H	ARC	*
27, f	GC	CM, CC, SC	GM+, GC+, CM-	GM-, CM-	α -Lactalbumin (14kD)	U	AA, ARC	[1]
25, m	GC, SC	CC	CM-, GM+	GC+ SC +	n/a #	U, A, R	no	[2]
43, m	SM, SC	CM	SM+, SC+, GM+, CM-	SM+, GM+, CM-	α - or β -casein (25kD)	G, R, H	no	[3]
55, f	GC	n/a	n/a	GM+	β - or γ -casein (20-28kD)	U, A, R	AD, AA	[4]
25, f	SC	CM, CC	SM+, SC+, GM+, CM-	SM+, CM-	α -S2 casein	A, G	AA, AD	[5]

*= this report. A= angioedema; AA= allergic asthma; AD= atopic dermatitis; ARC= allergic rhino-conjunctivitis; CC= cow cheese; CM= cow milk; f= female; G= gastrointestinal; GC= goat cheese; GM= goat milk; H= hypotension; m= male; n/a= not available/reported; neg.= negative; R= respiratory; SC= sheep cheese; SM= sheep milk; U= urticaria; WB= Western Blot/Immunoblot. #= goat and sheep casein positive in RAST