

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).

References from the literature review:

1. Tavares B, Pereira C, Rodrigues F, Loureiro G, Chieira C. Goat's milk allergy. *Allergol Immunopathol (Madr)*. 2007;35(3):113-6.
2. Wuthrich B, Johansson SG. Allergy to cheese produced from sheep's and goat's milk but not to cheese produced from cow's milk. *J Allergy Clin Immunol*. 1995;96(2):270-3.
3. Alvarez MJ, Lombardero M. IgE-mediated anaphylaxis to sheep's and goat's milk. *Allergy*. 2002;57(11):1091-2.
4. Voskamp AL, Zubrinich CM, Abramovitch JB, Rolland JM, O'Hehir RE. Goat's cheese anaphylaxis after cutaneous sensitization by moisturizer that contained goat's milk. *J Allergy Clin Immunol Pract*. 2014;2(5):629-30.
5. Suzuki K, Futamura K, Hasegawa E, Aoki Y, Nakamura M, Matsunaga K, et al. Adult onset sheep's milk allergy in a patient without cow's milk allergy. *J Investig Allergol Clin Immunol*. 2020.