Anaphylaxis to goat's and sheep's milk in an adult tolerant to cow milk: A sensitization profile study

Hirsiger JR[1], Heijnen IAFM [2], Hartmann K[3,4], Berger CT [1,3]

[1]Translational Immunology, Department of Biomedicine, University of Basel, Basel, Switzerland

[2]Medical Immunology, Laboratory Medicine, University Hospital Basel, Switzerland

[3]Division of Allergy, Department of Dermatology, University Hospital Basel and University of Basel, Basel, Switzerland

[4]Department of Biomedicine, University Hospital Basel and University of Basel, Basel, Switzerland

Correspondence:
Christoph T. Berger
Translational Immunology; Department of Biomedicine, University Hospital Basel; 20 Hebelstrasse, 4031 Basel, Switzerland.
Email: christoph.berger@usb.ch

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.18176/jiaci.0721

Key words: Milk allergy, Sheep, Goat, Allergen, Anaphylaxis.

Palabras clave: Alergia a la leche, Oveja, Cabra, Alérgeno, Anafilaxia.

Allergy to cow's milk (CM) is a common food allergy in children which 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]. The majority of CM-allergic subjects 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 subjects. 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]. Here we describe an adult patient in whom evaluation for sensitization to milk proteins unravelled a very uncommon GSM-specific allergy.

A 39-year-old male presented with recurrent episodes of anaphylaxis over 20 years, with one 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. In all instances, symptoms resolved after treatment with an epinephrine auto-injector, whereas antihistamines alone were insufficient. He reports on episodes after eating feta cheese, traces of GM cheese, or other GSM cheese-containing foods, but tolerates CM's cheese and large quantities of pasteurized CM without symptoms. Notwithstanding this tolerance, the patient recalls reactions after ingestion of concentrated CM-derived whey protein nutritional supplements. He has a 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, ThermoFisher) showed sIgE against whole GM (0.53 kUA/l) and sheep's milk (SM) (0.76 kUA/l), as well as SM whey proteins (0.74 kUA/l) (***Table S1***). Commercial tests for sIgE against GM whey proteins, and GM and SM caseins were not available. Levels of sIgE against whole CM (0.19 kUA/l) and CM whey proteins (α-lactalbumin (0.16 kUA/l); β-lactoglobulin (0.11 kUA/l)) were just above detection limit, while sIgE to CM caseins could not be detected (***Table S1***). We excluded a sIgE sensitization to LTP, omega-5-gliadin, and galactose-alpha-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

doi: 10.18176/jiaci.0721

(WB) analyses with CM, GM, and SM. We included control sera from subjects with distinct molecular sensitization profiles to milk, i.e., isolated sensitization to casein with anaphylaxis to milk from all three species (*subject #2*), sensitization to CM casein, α-lactalbumin, and β-lactoglobulin with mild CM allergy (*subject #3*), or isolated sensitization to BSA with tolerance to milk (*subject #4*) (***Table S1)***. The serum of *subject #1* showed IgE binding to a band at 19 kDa both in GM and SM (***Fig. 1A***). This band was at the same molecular weight as the lowest band in subject #2. The known sIgE sensitization profile in subject #2 (isolated sensitization to caseins) suggests that the lowest band represents κ-casein, and the remaining α- and β-casein. Thus, we concluded that the band in our patient was compatible with κ-casein. The specific binding of IgE was not inhibited following serum pre-adsorption with cow casein-loaded beads (maximal signal suppression 21%) (***Fig. 1B***). In contrast, 80% inhibition was observed in subject #2, indicating cross-reactivity of IgE to CM and GSM caseins in this subject.

Notably, sIgE to whey proteins in *subject #1* were only detected by WB at high protein concentrations when using pasteurized GSM (*data not shown*). In contrast, WB analyses using fresh unpasteurized SM, and separated casein and whey fractions of SM confirmed the sensitization against SM whey proteins, showing bands at around 15 kDa and 12 kDa, comparable to the bands in subject #3 with CM allergy and sIgE against CM α-lactalbumin and β-lactoglobulin (***Fig. 1C***). We confirmed the observation that the milk processing temperature affects the IgE detection in WB also for CM, underpinning the relevance of the milk treatment temperatures used in milk-specific IgE sensitization profile studies (***Fig. S2***). Combined, our data indicate that sIgE in our patient targeted whey proteins and κ-casein in GSM, without 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 a 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 (***Fig. 1D***). The whey proteins showed a higher degree of interspecies structural similarity.

To date, only a few adults with GSM allergy tolerating CM have been reported (***Table S2***). Molecular investigations identified sIgE against whey proteins or caseins [4, 6]. GSM κ-casein was likely the main allergen in our patient since he had clinical, skin-prick-test, and IgE reactions to GSM cheese, which contains caseins primarily. The lower interspecies cross-reactivity between κ-casein compared to other casein proteins [7] may explain the tolerance to CM in our patient.

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 food trends. Moreover, casein-containing GM or SM powders are being used as nutritional supplements. In

doi: 10.18176/jiaci.0721

the presented case, severe allergic reactions occurred to traces of GSM products, while CM was tolerated in high doses. Larger cohorts are needed to assess whether GSM-specific κ-casein or whey protein sensitization are associated with severe GSM anaphylaxis in general.

Conflict of interest statement

The authors declare no conflict of interest.

Financial Source

The study was supported by independent research funds of CTB

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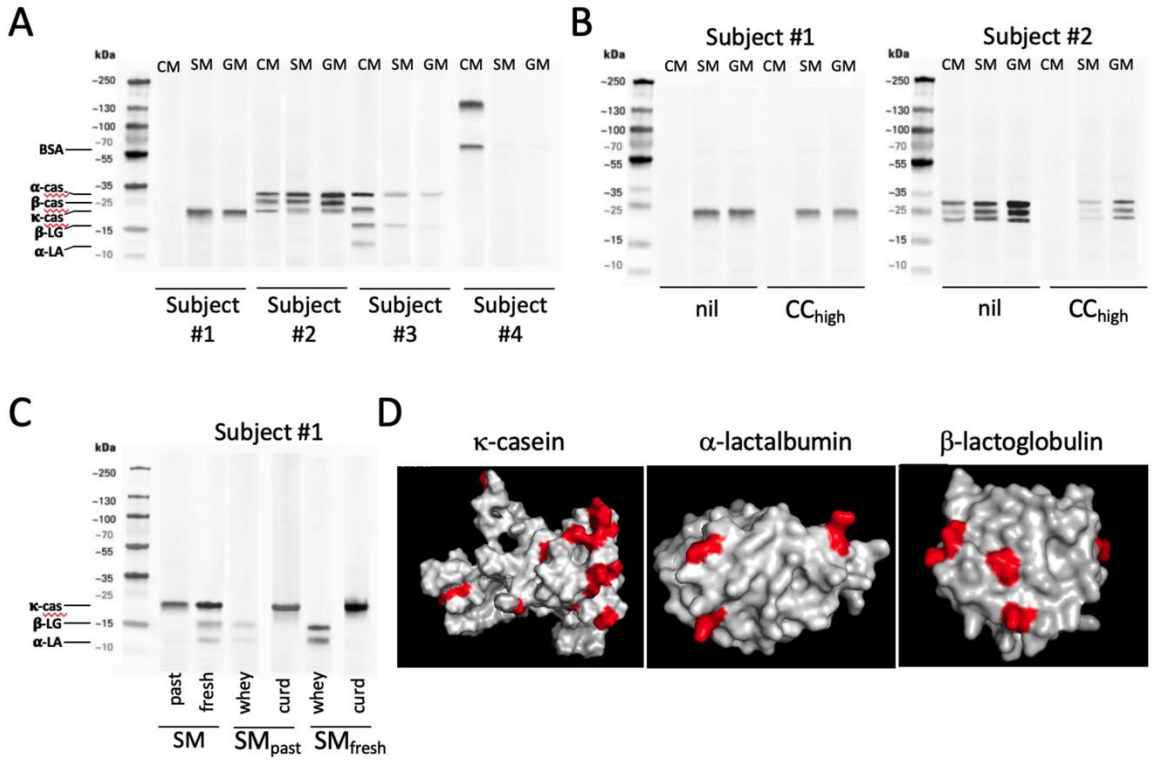
doi: 10.18176/jiaci.0721

Figure legend

Figure 1. Milk sensitization profile. (A) Western blot (WB) of pasteurized CM, SM, and GM using patient sera (***Table 1)***. Arrows indicate main milk protein. (B) CM pre-adsorption experiments with CM casein (CC). (C) WB 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.



doi: 10.18176/jiaci.0721