Buffalo's milk allergy: Role of sensitization to caprine β-casein

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buffalo's milk allergy, caprine β-casein, cross-reactivity, mozzarella allergy, primary sensitization

Abbreviations

specific Immunoglobulin E (sIgE)

skin prick test (SPT)

To the Editor,

Water buffalo milk is one of the non-cow's milks increasingly produced and consumed. Looking at the global worldwide milk production, buffalo's milk proportion as nearly tripled over the last fifty years[1]. It currently accounts for about 15% of world milk production[1]. Mostly produced in India, Pakistan, and Nepal, it is also widely used to produced typical Italian cheese. Food allergy to buffalo's milk is exceptionally report, although its consumption is nowadays widespread even among children. Furthermore, its cross-reactivity with other milks needs to be further investigated. Herein, we report a rare case of buffalo's milk allergy in a goat's and sheep's milk allergic patient tolerant to cow's milk. We analyzed specific IgE sensitization to buffalo milk proteins and investigated the cross-reactivity with cow's and goat's milk to identify the initial sensitizer.

The patient was a 4-year-old French girl, with a history of severe food allergy to goat's and sheep's milks, allergic asthma and rhinoconjunctivitis to birch pollen with a pollen-food syndrome and atopic dermatitis. The child used to eat cow's milk from her first year of life without any reaction. One hour after eating buffalo's mozzarella, she developed abdominal pain, feeling weak and fainting leading to intramuscular injection of epinephrine. She had never consumed buffalo's milk before. Allergy workup to buffalo's milk was performed eight months later. Skin prick test (SPT) with buffalo's milk (1:10 dilution) and buffalo's mozzarella were positive after 20 minutes: wheal diameter of 10 mm with pseudopods and 5 mm respectively, both with a skin flare. SPT were positive for goat's milk (wheal diameter 8 mm with pseudopods, at 1:1000 dilution) and sheep's milk (wheal diameter 6 mm, at 1:1000 dilution). SPT to cow's milk was negative. Positive control with histamine at 10 mg/mL revealed a wheal of 5 mm diameter and control with saline solution was negative. Specific IgE (sIgE) assays by ImmunoCap® system (ThermoFisher Scientific, Uppsala, Sweden) provided the following results: goat's milk (10.30 kU/L), sheep's milk (8.18 kU/L), bovine casein (0.59 kU/L), bovine β-lactoglobulin (0.17 kU/L), bovine α-lactalbumin (<0.1 kU/L), bovine serum albumin (<0.1 kU/L).

Buffalo milk proteins were purified from raw milk. Caseins were isolated and characterized using a combination of isoelectric precipitation at pH 4.6 and reverse phase-high performance liquid chromatography as previously described[2]. Using indirect ELISA (see Supplementary methods), with purified caseins adsorbed on the solid phase, we confirmed that the patient was sensitized to all caseins. The patient had higher levels of sIgE to caprine and buffalo's αS1- and β-caseins than to the bovine homologs (Table 1). However, indirect ELISA does not differentiate low- from high- affinity IgE-binding to the different caseins. We then used a second immunoassay based on the capture of serum IgE antibodies by a monoclonal antibody immobilized on the solid phase and the binding of biotinylated β-caseins[3]. The IgE cross-reactivity between β-caseins was analyzed by performing competitive inhibitions of IgE-binding to caprine and buffalo's β-caseins, as previously described[4]. As shown in Figure 1, competitive inhibitions revealed a strong IgE cross-reactivity between buffalo's and caprine β-caseins. The IgE binding to caprine β-casein was partially inhibited by buffalo's β-casein (Figure 1A), whereas the IgE binding to buffalo's β-casein was totally inhibited by caprine β-casein (Figure 1B). Therefore, the IgE-reactivity of the buffalo's β-casein results from the primary sensitization to caprine β-casein for our patient. Moreover, no inhibitory capacity of the bovine β-casein was observed. This confirms that sIgE-binding to bovine caseins detected by ImmunoCap® system and by indirect ELISA is of low avidity without clinical relevance.

The parents of the child gave their informed consent for the investigations and the publication of this case.

Nowadays, there is a growing interest in non-cow's milks for their nutritional benefits as well as their hypoallergenic potential, although their allergenicity needs to be further investigated. Compared to cow's milk, buffalo milk has higher rate of proteins and fat[1,5]. Buffalo milk proteins are mostly caseins, with on average 32 to 40g/L of caseins[1,5].

So far, very few clinical cases of buffalo milk allergy have been published.

Broekaert et al. published the first clinical case of buffalo's milk allergy in a 70 years old man without any medical history of allergy[6]. He presented a severe anaphylaxis after eating Italian buffalo's mozzarella. No sensitization to cow's or goat's milk was revealed by SPT.

Specific IgE assay and molecular diagnosis of buffalo's milk allergy is not usually performed because no commercial assay is currently available. Seven potential molecular allergens have been described in the buffalo's milk allergen source[7]. However, none has been registered by the WHO/IUIS Allergen Nomenclature Subcommittee yet.

The main buffalo's milk caseins, β-casein and αS1-casein, are highly similar to bovine caseins with 95 to 97 % sequence homology[8]. Thus, cross-reactivity with other mammalian milks raise a significant issue.

Two cases of buffalo's milk allergy were previously reported in goat's and sheep's milk allergic patient who tolerated cow's milk [9,10]. Although, co-sensitization between cow's, buffalo's, sheep's, and goat's milks has been described both in vitro and in vivo, the cross-reactive molecular allergen and the initial sensitizer has never been demonstrated experimentally [8]. To the best of our knowledge, we provide evidence for the first time that allergy to buffalo's milk was triggered by a primary sensitization to goat's milk.

Currently, the use of buffalo's milk in cow's milk allergic patient is usually not recommended because of the high similarity degree and the cross-reactivity between cow's and buffalo's milk proteins[8,11]. Furthermore, in vivo cross-sensitization to buffalo's milk has been also described in patients with IgE-mediated cow's milk allergy. Indeed, in two cohort studies, all cow's milk allergic patients with positive SPT to cow's milk had a positive SPT to buffalo's milk[12,13]. However, the clinical relevance of this skin sensitization has not been evaluated.

Conversely, a rare clinical case of a young boy allergic to cow's milk and clinically tolerant to buffalo's milk was reported[14].

Thus, further study on larger cohort should be interesting to evaluate the clinical relevance of buffalo's milk allergens and their cross-reactivity other mammalian milks.

In conclusion, we investigated a rare case of buffalo's milk allergy in a young girl allergic to goat's and sheep's milk, and tolerant to cow's milk. Our study showed that buffalo's milk allergy was due to primary sensitization to goat's milk because of an IgE cross-sensitization to caprine β-casein. Of note, the patient was not sensitized to buffalo's β-lactoglobulin (data not shown). Nevertheless, several clinical phenotypes of buffalo's milk allergy may exist with or without concurrent cow's milk allergy, suggesting that different allergenic sensitization pathways should be involved. Further investigations on molecular allergen sensitization, IgE cross-reactivity with other ruminants' milks and allergen epitope identification should improve our knowledge of buffalo's milk allergy.

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References

1. Roy D, Ye A, Moughan PJ, Singh H. Composition, Structure, and Digestive Dynamics of Milk from Different Species-A Review. Front Nutr. 2020;7:577759.

2. Ah-Leung S, Bernard H, Bidat E, Paty E, Rancé F, Scheinmann P, Wal JM. Allergy to goat and sheep milk without allergy to cow's milk. Allergy. 2006;61(11):1358-65.

3. Bernard H, Drumare MF, Guillon B, Paty E, Scheinmann P, Wal JM. Immunochemical characterisation of structure and allergenicity of peanut 2S albumins using different formats of immunoassays. Anal Bioanal Chem. 2009;395(1):139-146.

4. Bernard H, Ah-Leung S, Tilleul S, Drumare MF, Paty E, Bidat E, Wal JM, Hazebrouck S. Specificity of IgE antibodies from patients allergic to goat's milk and tolerant to cow's milk determined with plasmin-derived peptides of bovine and caprine β-caseins. Mol Nutr Food Res. 2012;56(10):1532-40.

5. Ahmad S, Anjum FM, Huma N, Sameen A, Zahoor T. (2013). Composition and physico-chemical characteristics of buffalo milk with particular emphasis on lipids, proteins, minerals, enzymes and vitamins. J Anim Plant Sci. 2013;23 Suppl 1:62-74.

6. Broekaert SM, Darsow U, Ollert M, et al. Anaphylactic shock caused by buffalo's mozzarella cheese. Ann Allergy Asthma Immunol. 2008;101(1):105-107.

7. Allergome. http://www.allergome.org/. Accessed April 7, 2023.

8. Restani P, Ballabio C, Di Lorenzo C, Tripodi S, Fiocchi A. Molecular aspects of milk allergens and their role in clinical events. Anal Bioanal Chem. 2009;395(1):47-56.

9. Pétrus M, Rayssac M, Cossarizza G, Lasserre J, Dutau G. Allergy to buffalo milk mozzarella. Case report concerning a 16-year-old adolescent. Revue Française d'Allergologie. 2011; 51(6): 553-555.

10. Mori F, Restani P, Pucci N, et al. Allergy to all mammalian Bovidae proteins but cow's milk in a child. Allergol Immunopathol (Madr). 2013;41(5):349-350.

11. Restani P, Gaiaschi A, Plebani A, et al. Cross-reactivity between milk proteins from different animal species. Clin Exp Allergy. 1999;29(7):997-1004.

12. Sheehan WJ, Gardynski A, Phipatanakul W. Skin Testing With Water Buffalo's Milk in Children With Cow's Milk Allergy. Pediatr Asthma Allergy Immunol. 2009;22(3):121-125.

13. Katz Y, Goldberg MR, Zadik-Mnuhin G, Leshno M, Heyman E. Cross-sensitization between milk proteins: reactivity to a "kosher" epitope?. Isr Med Assoc J. 2008;10(1):85-88.

14. Sheehan WJ, Phipatanakul W. Tolerance to water buffalo milk in a child with cow milk allergy. Ann Allergy Asthma Immunol. 2009;102(4):349.

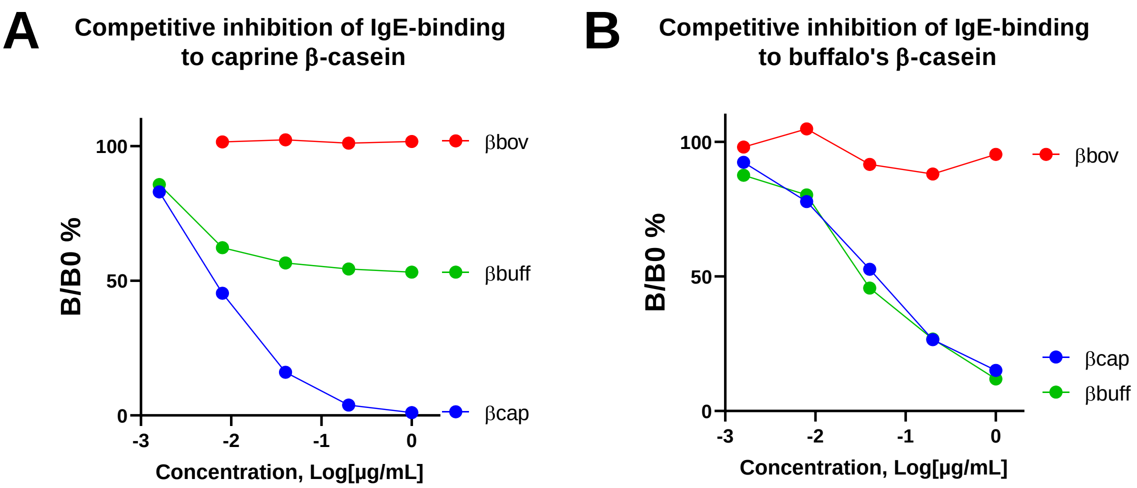
Table

Table 1. Specific IgE (sIgE) levels to cow's, buffalo's, and goat's milks caseins.

sIgE levels	Cow's milk	Buffalo's milk	Goat's milk
sIgE to αS1-casein (UI/mL)	0.35	1.30	1.8
sIgE to β-casein (UI/mL)	0.48	1.13	3.36

Figure

Figure 1. A: Competitive inhibition of the IgE binding to caprine β-casein by caprine β-casein (blue), buffalo's β-casein (green), and bovine β-casein (red). B: Competitive inhibition of the IgE binding to buffalo's β-casein by caprine β-casein (blue), buffalo's β-casein (green), and bovine β-casein (red). Results were expressed as B/B0, B0 and B representing the amount of labeled β-casein bound to immobilize IgE antibodies in the absence or presence of a known concentration of inhibitor, respectively.



Supplementary methods

Indirect ELISA

Microtiter plates were coated with purified milk proteins (5 µg/mL) and then saturated with EIA buffer (0.1 M phosphate buffer, 0.1% bovine serum albumin, 0.15 M NaCl, 0.01% sodium azide, pH 7.4). After ON incubation with sera diluted 1:5 and 1:20, plates were washed and IgE-binding was revealed by addition of a mouse anti-human IgE mAb (clone BS17) labeled with acetylcholinesterase (AchE, 2 Ellman Unit (EU)/mL). AChE activity was revealed after addition of Ellman’s reagent and absorbance was measured at 414 nm.

IgE-capture ELISA

sIgE levels were also evaluated by measuring the binding of biotinylated allergens to serum IgE antibodies captured by a monoclonal antibody (mAb) immobilized on the solid phase.[27,31] Briefly, mouse anti-human IgE mAb (Clone LE27) was adsorbed on microtiter plates (2.5 µg/mL).[30] After ON incubation with diluted sera (1:5), plates were washed and biotinylated β-casein was added (0.05 nmol/mL) for 4h at RT. After washing, AChE-labeled neutravidin was added before revelation with Ellman’s reagent and absorbance was measured at 414 nm and expressed in Absorbance Unit (AU_{414nm}).

For competitive inhibition of IgE-binding, after ON incubation with diluted sera (1:5), 25 µL of inhibitors (i.e. increasing concentration of unlabeled milk protein) were mixed with 25 µL of biotinylated β-casein protein (0.05 µg/mL), and incubated at RT for 4h. IgE-binding was revealed as described above. Results were expressed as B/B0, B0 and B representing the amount of labeled SFS protein bound to immobilize IgE antibodies in the absence or presence of a known concentration of inhibitor, respectively.