

Molecular aspects of milk allergens and their role in clinical events

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Abstract Milk allergy is the most frequent food allergy in childhood. Even though cases of newly developed milk allergy in adulthood are known, this allergy is less frequent in adults since it is normally outgrown by children during the first years of life. One of the reasons why allergy to cow's milk shows its highest prevalence in children is its early introduction into the diets of babies when breast feeding is not possible. The major allergens are caseins and β -lactoglobulin, but allergies to other minor proteins (immunoglobulins, bovine serum albumin) have also been reported. Milk allergenicity can be reduced by various treatments (mainly hydrolysis), meaning that formulas based on cow's milk can often be safely fed to children allergic to milk proteins. Cross-reactivity has been described between different mammalian milks and between milk and meat or animal dander. Cross-contamination can result from inadequate cleaning of industrial equipment and constitutes a hidden danger for allergic subjects who unknowingly ingest milk proteins.

Keywords Milk allergy · Caseins · Whey proteins · Serum albumins · Cross-reactivity · Technological treatments

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Introduction

Cow's milk is a common cause of food allergy in childhood. Most children outgrow the disease during the first years of life, but cow's milk allergy does sometimes persist or can newly develop in adulthood.

Milk contains approximately 20 different proteins that can potentially be involved in allergic sensitization, but only a few milk proteins are recognized as being major allergens; some are considered to be minor allergens, while a tiny number have rarely been described as being responsible for clinical reactions.

Cow's milk protein composition and known allergens

The protein composition of cow's milk [1] and the names of allergens included in the official international list [2] are shown in Table 1. Milk proteins are classified as caseins or whey proteins. The casein fraction constitutes up to 80% of the total protein and contains α_{s1} -, α_{s2} -, β - and κ -caseins as independent milk protein components and three γ -caseins deriving from the hydrolysis of β -casein. γ_1 , γ_2 and γ_3 represent, respectively, the sequences 29–209, 106–209 and 108–209 of β -casein [3]. γ -Caseins are present in milk in minute quantities, while they are abundant in cheeses characterized by proteolytic ripening (such as Grana cheese). Whey proteins are less abundant (20% of the total protein) and β -lactoglobulin is the main component (up to 50% of whey proteins).

When appropriate, milk proteins are also classified using the allergen nomenclature, an international code containing the taxonomic name of the animal considered (*Bos d* is *Bos domesticus*), and a number indicating the chronological order in which the allergen was identified as such (*Bos d* 4–8). *Bos d* 1–3 are not involved in food allergies and so they will not be considered in this review.

Table 1 Chemical characteristics of cow's milk proteins and their inclusion in the official list of allergens

Fraction	Protein	Allergen name	g/L	% total protein	MW (kDa)	Number of aa	pI
Caseins			~30	80			
	alpha _{s1} -casein	<i>Bos d 8</i>	12-15	29	23.6	199	4.9-5.0
	alpha _{s2} -casein		3-4	8	25.2	207	5.2-5.4
	beta-casein		9-11	27	24.0	209	5.1-5.4
	gamma ₁ -casein		1-2	6	20.6	180	5.5
	gamma ₂ -casein				11.8	104	6.4
	gamma ₃ -casein				11.6	102	5.8
	kappa-casein		3-4	10	19.0	169	5.4-5.6
Whey proteins			~5.0	20			
	alpha-lactalbumin	<i>Bos d 4</i>	1-1.5	5	14.2	123	4.8
	beta-lactoglobulin	<i>Bos d 5</i>	3-4	10	18.3	162	5.3
	immunoglobulins	<i>Bos d 7</i>	0.6-1.0	3	160.0	-	-
	bovine serum albumin	<i>Bos d 6</i>	0.1-0.4	1	67.0	583	4.9-5.1
	lactoferrin		0.09	traces	800.0	703	8.7
Total proteins			36.0	100			

Data from [1–3]

Cow's milk proteins and clinical reactions

Since β -lactoglobulin is absent from human milk, this protein was formerly considered to be the most important cow's milk allergen, but it was later shown that other proteins, including caseins, are also critical milk allergens.

Figure 1 illustrates the involvement of the most abundant proteins in the sensitization of 80 children. These data are based on the results of three different diagnostic tests performed in different clinical studies including children/teenagers (0–16 years old) who were severely allergic to cow's milk, namely the skin prick test (SPT), which measures skin-specific IgEs, the CAP test (CAP), and immunoblotting (IMM); the latter two measure specific circulating IgEs.

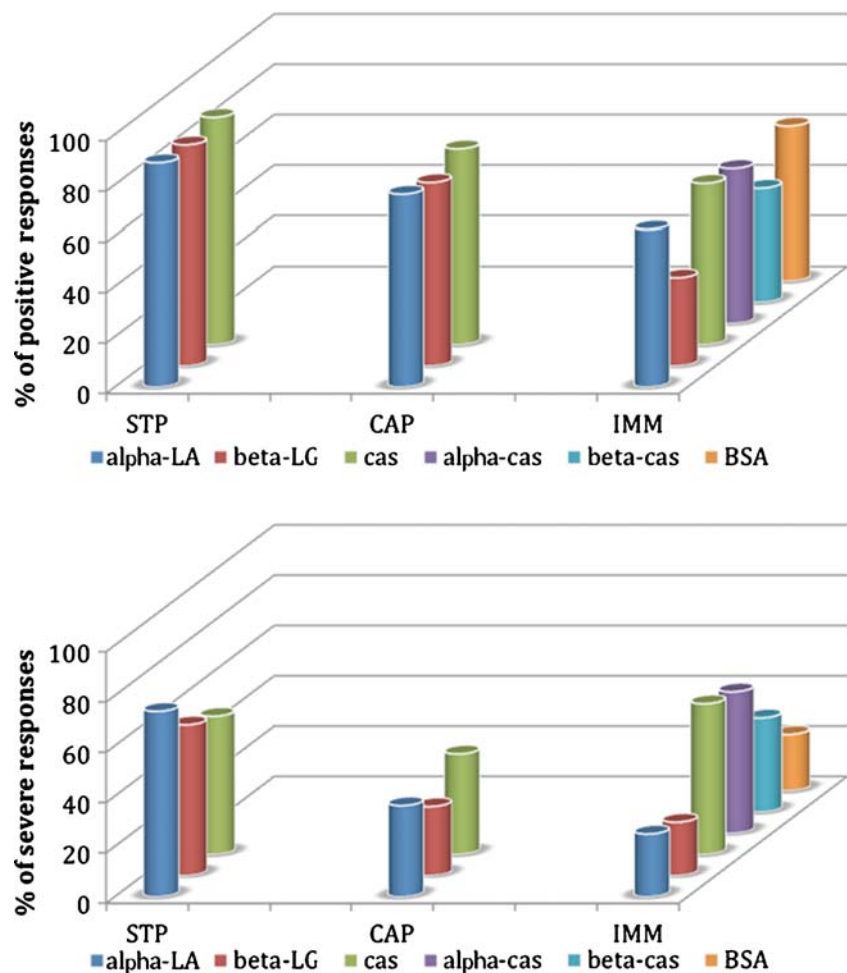
Eligibility for the considered clinical studies was limited to patients with immediate reactions confirmed by positive cow's milk double-blind, placebo-controlled food challenge or anaphylaxis in the two months prior to enrollment. Clinical symptoms were: 12.8% anaphylaxis, 25.6% urticaria, 20.8% asthma, 19.2% gastrointestinal symptoms, 8.8% angioedema; 7.2% rhinitis, 1% laryngospasm.

Positive responses shown in the upper half of Fig. 1 include both mild and severe reactions, corresponding to wheal

diameter ≥ 3 mm in the SPT, $\text{kU/L} \geq 0.35$ in the CAP test, and classes 1–6 in IMM. All of the proteins assayed induced sensitization in the 80 children considered. Immunoblotting data for single caseins and serum albumin (BSA) are also shown. The percentage of children reacting in the SPT was 87.5% for β -LG, 88.8% for α -LA, and 90% for total caseins; in the CAP test 72.5% reacted to β -LG, 76.3% to α -LA, and 77.5% to total caseins; in immunoblotting 35% reacted to β -LG, 62.5% to α -LA, 63.8% to total caseins (61.3% to α -cas and 45% to β -cas), and 61.3% to BSA.

The lower half of Fig. 1 shows only the highest degrees of reactivity, i.e., wheal diameter > 5 mm in the SPT, $\text{kU/L} > 5$ in the CAP test, and classes 3–6 in immunoblotting. This further classification was adopted because a higher concentration of IgE antibodies and larger SPT wheal size correlate with an increasing risk of a clinical reaction [4]. The percentages of children reacting positively according to these criteria were of course lower: 55% for α -LA, 60% for β -LG and 73.8% for total caseins in SPT, while in the CAP test the percentages were very much lower: 36.3% for α -LA, 27.5% for β -LG and 40% for total caseins, and in immunoblotting the percentages decreased to 25% for α -LA, 21.3% for β -LG, 60% for total caseins (56.3% α -cas and 37.5% β -cas), and 22.5% for BSA. Data

Fig. 1 Involvement (expressed as percentage of total subjects) of the most abundant milk proteins in the sensitization of 80 children allergic to cow's milk. The *upper panel* includes all positive responses, even minor ones; data in the *lower panel* are restricted to the most severe positive responses (see text for details). *SPT*, skin prick test; *CAP*, CAP test; *IMM*, immunoblotting; *alpha-LA*, α -lactalbumin; *beta-LG*, β -lactoglobulin; *cas*, caseins; *BSA*, bovine serum albumin



from the CAP test and immunoblotting are similar since both assays measure specific circulating IgEs, while the percentage of positive subjects in the SPT (which measures skin-specific IgEs) remains higher. Discrepancies between different diagnostic tests are well known and are described in a recent review [5].

All of the proteins seem to be involved in skin reactions (SPT), but caseins seem to be responsible for the strongest systemic (circulating IgEs) sensitizations. Unfortunately, for practical and ethical reasons, these data cannot be confirmed through oral challenge with purified proteins.

Stability of cow's milk allergens to technological treatments and clinical aspects

Heating processes

Cow's milk is commonly available only after it has been subjected to some technological process, usually involving heating.

Pasteurization, for example, reduces the viability of microorganisms in milk, eliminating those with pathogenic

potential; temperatures below the boiling point are used, usually 70–80 °C for 15–20 s. The product has a short shelf-life, and must be consumed within 4–6 days of the treatment.

Ultra-high-temperature processing (UHT) completely sterilizes milk by eliminating not only microorganisms but also spores. The treatment, performed at temperatures exceeding 100 °C for a few seconds, provides a shelf-life of at least three months.

Evaporation for the production of powdered infant formula is achieved through either a dry blending process or a wet mixing–spray drying process. Some manufacturers use a combination of these processes. Relatively high temperatures are needed for spray drying operations, but the consequent heat modification of the proteins is only slightly greater than in the processing of liquid milk because there is an evaporative cooling effect during the critical drying period and the subsequent time of exposure of the dry material to high temperatures may be very short.

Heating has only a small effect on the antigenic/allergenic potential of cow's milk proteins, even though some authors have shown a reduction in whey protein antigenicity. Norgaard et al. [6] performed the SPT after milk

had been boiled for 10 and 2 min in eight adult subjects with positive responses to oral challenge. Boiling milk for 10 min (but not for 2) eliminated the SPT responses in subjects reactive to BSA and β -lactoglobulin, whereas wheal diameter remained positive in those sensitized to caseins. Other studies showed no difference in antigenicity when raw and heated milks were compared [7], and in some cases the production of new protein polymers capable of binding specific IgEs was clearly demonstrated. Restani et al. [8] showed that after boiling BSA at 100 °C for 10 min, the quantity of dimeric, trimeric and higher polymeric forms increased, all maintaining their IgE-binding properties.

The persistence of allergenicity in heat-treated milk is confirmed by the fact that sensitization to milk develops after consumption of heat-treated milk, the only kind that is available to most consumers. Furthermore, heating processes can only modify conformational epitopes, which might lose their binding capacity to specific IgEs, but linear epitopes are not affected by structural changes and maintain their allergenic potential after heating. Milk proteins contain both types of epitopes and, even though a slight reduction of antigenicity can be observed with whey proteins, insignificant alterations in binding properties are reported with caseins.

Some authors have shown [10] that vigorous heating, such as that used for certain sterilization processes (121 °C for 20 min) but also the less drastic pasteurization process, may actually enhance some allergenic characteristics. Moreover, Fenaille et al. [11] demonstrated that milk proteins could be oxidized during industrial treatments, resulting in the formation of modified/oxidized amino acid residues, particularly in β -lactoglobulin, which may be responsible for the development of new immunologically reactive structures.

Microfiltration

A relatively new technological approach has been included among industrial processes applied to milk: microfiltration. The most common application is the removal of bacteria and spores from pasteurized milk to yield an extended shelf-life (10 instead of 4–6 days). Different microfiltration membranes can also be used to separate casein from whey proteins in order to obtain casein-rich milk concentrates, which can be used in cheese-making.

The process has no effect on allergenicity. Although fractionation of proteins by microfiltration could permit the formulation of products for subjects specifically sensitized to one type of protein fraction (casein or whey protein), the approach has little chance of success, as patients allergic to cow's milk normally show polysensitivity to all of the main milk proteins, and in any case the presence of a residual quantity of casein in whey proteins, and vice versa, would

be risky for the most sensitive subjects. Complete separation by this method is too complicated and expensive.

Hydrolysis

Two kinds of hydrolytic processes can be applied to milk, those involving the carbohydrate (lactose) or the protein fraction. The lactose-free product has been studied for subjects suffering from lactose intolerance and cannot be consumed by allergic subjects, but protein hydrolysis has been successfully used to develop hypoallergenic formulas.

Several cow's milk protein hydrolysates produced by the action of various food-grade enzymes on isolated caseins or whey proteins are present on the international market. These formulas are classified as partial or extensive hydrolysates according to the molecular weight of the peptides derived. Only extensively hydrolyzed products can be considered sufficiently safe for allergic patients, and their use is often recommended as a first alternative in children with a confirmed diagnosis of cow's milk allergy. Extensive hydrolysates of both casein and whey protein show a similar clinical tolerance [13, 14].

As a small amount of undigested protein can remain after the proteolytic process [15], a series of combined treatments is normally used to improve the safety of the final products: heat treatment and ultrafiltration are the most widely applied of these. Ultrafiltration is normally capable of removing all undigested proteins from the filtrate [16], which is then used to prepare hypoallergenic infant formulas. High pressure can also be applied during the proteolytic processes: different authors [16–20] have shown that the antigenic potential of β -lactoglobulin is strongly reduced when proteolysis is performed under high pressure (~300 MPa). Less satisfactory results are obtained when high pressure is applied before starting the enzymatic attack.

Figure 2 shows the protein profiles of different commercial milk-based products (panel A) and the corresponding binding capacity to anti- β -lactoglobulin monoclonal antibodies (panel B) or IgEs contained in the serum of a child severely allergic to cow's milk (panel C). No significant reduction in antigenicity is shown in cow's milk after pasteurization, sterilization, microfiltration or lactose hydrolysis, while immunoreactivity was reduced or eliminated in partially and extensively hydrolyzed formulas.

Cross-reactivity and associated clinical aspects

There are three possible types of cross-reaction in subjects suffering from cow's milk allergy: 1) between milks from different animal species; 2) between milk and other foods; 3) between milk and other allergenic sources.

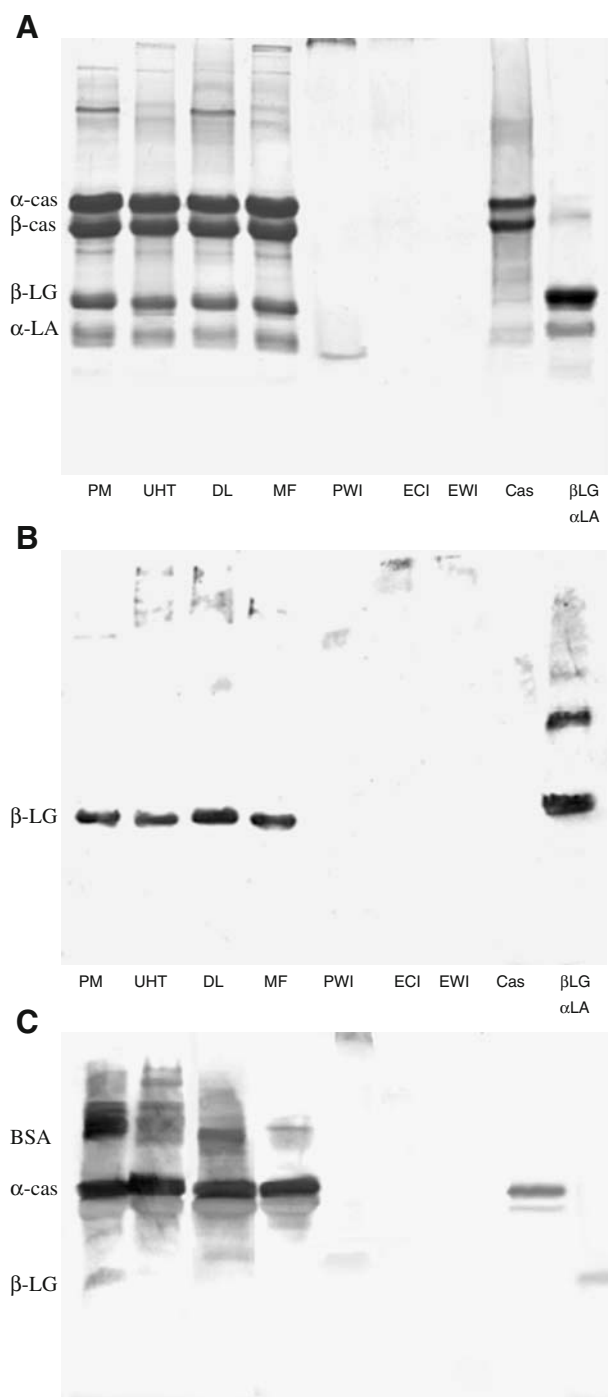


Fig. 2 SDS-PAGE of cow's milk subjected to different technological treatments (**A**), and corresponding immunoblottings obtained by incubation with anti- β -LG monoclonal antibodies (**B**) and with the serum of a child severely allergic to cow's milk (**C**). *PM*, pasteurized milk; *UHT*, sterilized milk; *DL*, delactosed milk; *MF*, microfiltrated milk; *PWI*, partially hydrolyzed whey protein formula; *ECI*, extensively hydrolyzed casein formula; *EWI*, extensively hydrolyzed whey protein formula; *Cas*, total caseins; α -*LA*, α -lactalbumin; β -*LG*, β -lactoglobulin; *BSA*, bovine serum albumin

Cross-reactivity between milks from different animal species

Cross-reactivity is associated with similarity of three-dimensional protein conformations (conformational epitopes) or similarity of critical amino acid sequences (linear/sequential epitopes). Cross-reactivity between proteins from different species (both animals and vegetables) depends in general on phylogenetic relationships. Vertebrate homologous proteins are often cross-reactive [21]. The complete taxonomy of the cow (*Bos domesticus*) is illustrated in Table 2 [22–24], while the simplified taxonomies of the most common domestic mammalian species are compared in Table 3. All species illustrated have a common taxonomy up to the class of Mammalia or the superorder of Laurasiatheria, depending on whether one includes human beings or not. A further taxonomic difference can be observed in the order: horse and donkey are Perissodactyla rather than Artiodactyla, and only four species have the same family classification (cow, buffalo, sheep and goat).

The taxonomic classification leads to similarities and differences in the corresponding milks:

- The total protein content in milk from Artiodactyla is higher than it is in milk from Perissodactyla
- The ratio of casein to whey protein is very similar among Bovidae, with values close to 80:20
- The milk from Equidae has a lower total protein content (similar to human milk) and a lower ratio of casein to whey protein.

Table 3 also shows the percentage homology between each cow milk protein and the milk proteins from other animal species, including humans. Data were obtained from the ExPASy website, using the SIM alignment tool for protein sequences [32].

Table 2 Taxonomy of *Bos taurus/domesticus*

Taxonomy	Classification
Superkingdom	Eukaryota
Kingdom	Metazoa
Phylum	Chordata
Subphylum	Craniata
Class	Mammalia
Superorder	Laurasiatheria
Order	Artiodactyla
Suborder	Ruminantia
Infraorder	Pecora
Family	Bovidae
Subfamily	Bovinae
Genus	<i>Bos</i>
Species	<i>B. taurus/domesticus</i>

Table 3 Mammalian taxonomy: milk protein composition and homology

	Cow	Water buffalo	Sheep	Goat	Pig	Dromedary	Horse	Donkey	Human
Class	Mammalia	Mammalia	Mammalia	Mammalia	Mammalia	Mammalia	Mammalia	Mammalia	Mammalia
Superorder	Laurasiatheria	Laurasiatheria	Laurasiatheria	Laurasiatheria	Laurasiatheria	Laurasiatheria	Laurasiatheria	Laurasiatheria	Euarchontoglires
Order	Artiodactyla	Artiodactyla	Artiodactyla	Artiodactyla	Artiodactyla	Artiodactyla	Perissodactyla	Perissodactyla	Primates
Family	Bovidae	Bovidae	Bovidae	Bovidae	Suidae	Camelidae	Equidae	Equidae	Hominidae
Subfamily	Bovinae	Bovinae	Caprinae	Caprinae	Suinae				Homininae
Genus	<i>Bos</i>	<i>Bubalus</i>	<i>Ovis</i>	<i>Capra</i>	<i>Sus</i>	<i>Camelus</i>	<i>Equus</i>	<i>Equus</i>	<i>Homo</i>
Species	<i>B. domesticus</i>	<i>B. bubalis</i>	<i>O. aries</i>	<i>C. aegagrus</i>	<i>S. domestica</i>	<i>C. dromedarius</i>	<i>E. f. caballus</i>	<i>E. asinus</i>	<i>H. sapiens</i>
Protein (g %)	3.2	4.5	4.9	4.3	4.8	3.6	2.14	2.2	1.25
Caseins (%)	80	82	84	84	58	74	56	58	40
Whey proteins (%)	20	18	16	16	42	26	44	42	60
Homology									
α_{s1} -Casein	100	95.3	88.3	87.9	47.2	44.2	43.3	–	31.9
α_{s2} -Casein	100	95.0	89.2	88.3	62.8	58.3	–	60.0	–
β -Casein	100	97.8	92.0	91.1	67.0	69.2	60.5	–	56.5
κ -Casein	100	92.6	84.9	84.9	54.3	58.4	57.4	–	53.2
α -Lactalbumin	100	99.3	97.2	95.1	74.6	69.7	72.4 (A), 69.1 (B/C)	71.5	73.9
β -Lactoglobulin	100	96.7	93.9	94.4	63.9	absent	59.4 (1)	56.9 (1), 51.6 (2)	absent
Serum albumin	100	–	92.4	71.2	79.9	–	74.5	74.1	76.6
Average	100	96.1	91.1	87.6	64.2	60.0	62.4	62.8	58.4

– Not found in databank

The protein variants of α -lactalbumin and β -lactoglobulin are shown in parentheses

Data from [1, 25–31]

Homology calculated using the ExPASy website [32]

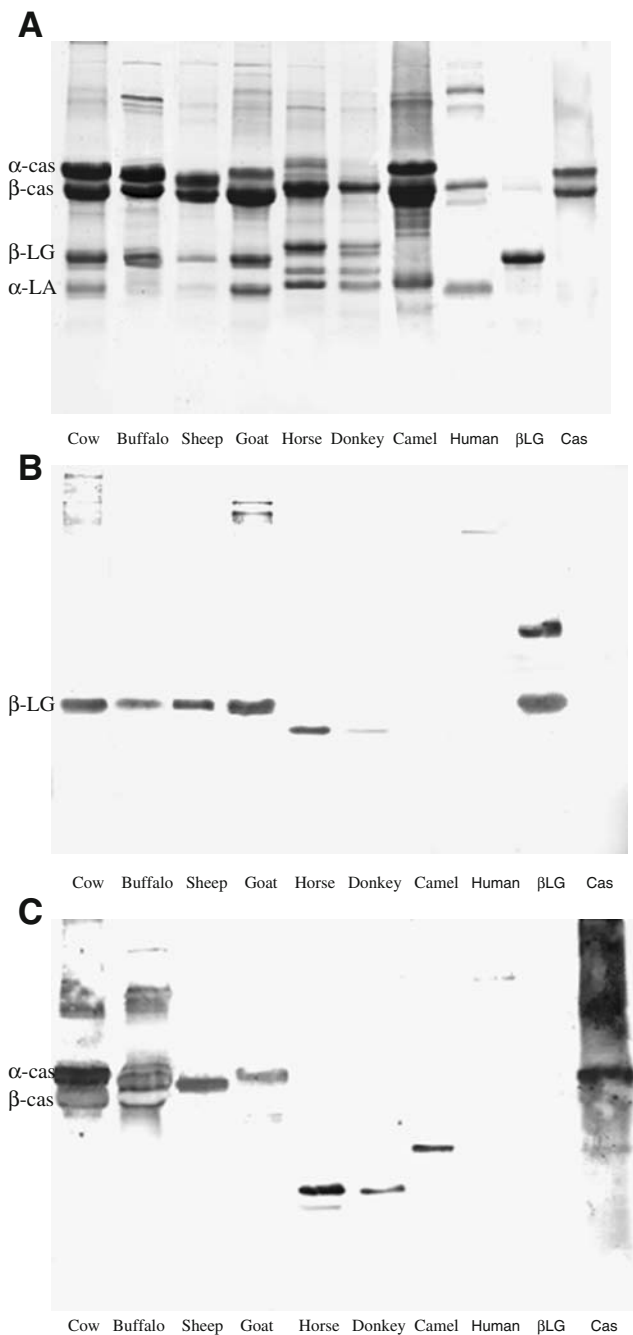


Fig. 3 SDS-PAGE of milk from different mammalian species (**A**), and corresponding immunoblottings obtained by incubation with an anti- β -lactoglobulin monoclonal antibodies (**B**) and anti-total casein polyclonal antibodies (**C**). α -cas, α -casein; β -cas, β -casein; α -LA, α -lactalbumin; β -LG, β -lactoglobulin

The highest homologies are observed between the milk proteins of cows and other Bovidae, being on average 96.1% for buffalo, 91.1% for sheep and 87.6% for goat.

Lower sequence similarities are associated with the milks from Suidae (pigs and boars, 64.2%), Equidae (horse 62.4% and donkey 62.8%), Camelidae (dromedary 60.0%) and humans (58.4%).

These data perfectly reflect the results of *in vitro* tests in which milks from different animal species are incubated with polyclonal or monoclonal antibodies produced against cow's milk proteins. The protein electrophoretic profiles of milks from different mammalian species are shown in Fig. 3 (panel A). All milks from Bovidae show similar protein patterns but different relative distributions. In particular, goat's milk shows the well-known relative deficiency in α -casein. Camel/dromedary's milk is characterized by a very high proportion of β -casein as well as an absence of β -lactoglobulin (in common with human milk).

Horse (mare) and donkey milks have different protein distributions from cow's milk, with a lower proportion of caseins and a higher number of electrophoretic bands of whey proteins. Figure 3 (panels B and C) illustrates the result of incubating two membranes containing the same protein pattern as shown in panel A with an anti- β -LG monoclonal antibody (panel B) and a polyclonal antibody specifically developed against total caseins (panel C). Both antibodies strongly react with proteins from buffalo, goat and sheep milks, while very mild immunoreactivity is observed with camel, horse and donkey milks. Similar results were obtained with other anti-cow's milk protein antibodies and human sera from allergic subjects [33, 34].

An analogous pattern of cross-reactivity has been reported in clinical studies where milks from different animal species were tested for significant allergic reactions or for their tolerance in children allergic to cow's milk proteins.

No clinical study has been published on the tolerance or intolerance to buffalo milk of subjects who are allergic to cow's milk, probably because of a general awareness of the high homology between these two animal species and the usual avoidance of buffalo milk derivatives (the most common being mozzarella) by allergic subjects. On the other hand, when an anaphylactic shock developed in a 70-year-old German eating buffalo mozzarella cheese in Italy [35], the subject was found to be specifically allergic to buffalo milk without sensitization to cow's milk (measurement of circulating IgEs against caseins and whey proteins, SPT and labial provocation).

Goat's milk, suggested as a safe alternative to cow's milk since it contains a lower quantity of α -casein, is the subject of intense discussion and the clinical results are controversial. A study in France showed 93% of 55 children allergic to cow's milk tolerated goat's milk for periods ranging from 8 days to 1 year [34], but several authors showed the opposite, with a low percentage (20–25%) of tolerance [36–40]. Sheep's milk has rarely been assayed for tolerance by subjects allergic to cow's milk; its use should be limited, because of its high homology with goat's milk, to well-defined clinical cases who are being monitored by allergologists.

Several case reports indicate that IgE-mediated sensitization and allergic reactions to sheep's and goat's milks can occur in children and adults who are tolerant of or not significantly sensitized to cow's milk [41–43]. A further important clinical consideration is a possible reaction to goat or sheep milks during the period of specific oral tolerance induction (SOTI) to cow's milk, which is often undertaken when an allergy to cow's milk does not evolve towards tolerance from the age of four—in other words, when this allergy is considered persistent [44, 45]. Although this practice is still discussed and not universally accepted, the results of this procedure are often described as being good, and patients are eventually able to follow a diet without any cow's milk restrictions. Although it seems logical to suppose that induced artificial tolerance to cow's milk proteins could extend to all dairy proteins regardless of their animal origin, some patients treated in this way and tolerating up to 200 mL of cow's milk suffered severe allergic reactions after having eaten goat [46] and sheep cheese (unpublished data from the authors).

Better clinical results were obtained using milks from mammals that are more phylogenetically distant from Bovidae, such as horse, donkey and camel. Businko et al. [47] showed that mare's milk was a good dietetic substitute in 24/25 children with severe cow's milk allergy. However, a supervised oral challenge is strongly recommended during such a trial in order to confirm the tolerance, since there was one case of a reaction to mare's milk during oral challenge in the same study, and other clinical cases have been reported when there was no cow's milk allergy [48].

Monti et al. [49] showed that 38 out of 46 children allergic to cow's milk tolerated donkey's milk at oral challenge and for the entire period of follow-up.

Finally, camel milk has been suggested as a possible alternative in allergic subjects on the basis of *in vitro* [33, 34] and *in vivo* [50] studies. Shabo et al. [50] showed good tolerance and fast recovery in eight children suffering from food (mainly milk) allergy who had not benefited from more conventional dietetic approaches. The homology with proteins from cow's milk is only 60%, the lowest level of similarity among the species considered. Camel and dromedary ruminant, but they are not as ruminant as Bovidae; their milk compositions are affected by these physiological differences, and in particular they are free from β -lactoglobulin. Although it would be interesting from a biochemical point of view, no study has been performed on the cow's milk tolerance of patients allergic to cow's milk.

Cross-reactivity between milks and other foods

Subjects allergic to cow's milk cannot consume milk derivatives (cheese, yoghurt, milk-based ice cream, etc.);

even in the case of cheeses such as Grana cheese, where significant proteolysis develops during the ripening period, the tolerance is negligible. The risk associated with the consumption of milk derivatives is well known to allergic subjects who spontaneously avoid these foods.

However, because there are several specific sensitizations to milk from species other than cow, and there are hundreds of different types of cheese on the international market, it is essential to know the animal from which the cheese is derived [51].

Greek feta cheese can be made from cow, ewe, or goat milk; pecorino comes from ewe milk; ricotta from cow or ewe milk; and mozzarella from cow, ewe, or buffalo milk. Less common sources of milk used in cheese production include camel, mare, reindeer, and yak.

Minute amount of caseins and whey proteins can be present in butter and cream, and their inclusion in or exclusion from hypoallergenic diets depends on the specific reactivity of each patient.

The prevalence of beef allergy in patients suffering from cow's milk allergy has been considered in a few studies; the number of subjects included is usually limited, and this double reactivity is rarely evaluated by oral challenge. The prevalence in two papers was 13.35% [52] and 20% [53]. An accurate clinical study by Martelli et al. [54] showed that among 28 children allergic to beef, 26 were allergic to cow's milk and presented a positive STP response to BSA. The two children allergic to beef but tolerant of cow's milk did not react positively to BSA, which indicates that this allergen is the main predictive marker of sensitization to both cow's milk and beef.

Cross-reactivity between milk and corresponding animal dander

BSA is also involved in the cross-reactivity between cow's milk and cow's dander [55, 56]. Native serum albumin in epithelia is an important allergen in patients with persistent cow's milk allergy and circulating IgEs to BSA. These patients should avoid meat and furry pets in order to reduce the risk of allergic and respiratory symptoms [56].

A clinical reaction to mare's milk observed in a 30-year-old woman with no history of cow's milk allergy was associated with sensitization to the main horse-dander allergen corresponding to serum albumin, known as Equ c 3 [48].

Cross-contamination and unexpected presence of milk in foods

Cross-contamination is well known in the food industry and several guidelines have been published aimed at minimiz-

ing the problem when the same equipment is used to process different foods. Particular attention has been paid to milk, as it is an ingredient that is widely used in food preparations: traces of milk have been detected in foods where milk is not included in the list of ingredients. Since no threshold amount has been established that can be considered safe for the most sensitive patients, the role of these minute amounts in clinical events is still under debate. A common example of cross-contamination is the presence of traces of cow's milk in dark chocolate.

There are other foods where the presence of milk proteins is often declared but not suspected by consumers. Milk can be found in hamburger steak, hot dogs, sausages, and natural flavorings (caramel flavoring, coconut cream flavoring, etc.). Preparations of probiotics should be consumed by allergic subjects only where the absence of milk is certified, since various authors have shown that allergic children can worsen their symptoms after the consumption of commercial probiotic preparations [57–59].

Finally, aids to the processing of food and drink must be maintained under strict control. The labeling of processing aids is not usually compulsory since they are not supposed to remain in the final product. As an example, residual caseinates used in wine clarification can produce clinical reactions to allergens that are difficult to identify. This problem is now the subject of international research aimed at protecting the most sensitive allergic patients.

Concluding remarks and future approaches

Milk allergy is considered to be an increasing problem in infancy. The acquisition of new biochemical knowledge is crucial to addressing both the clinical and nutritional problems of allergic subjects. It is desirable to develop new food technologies or to combine known chemical or physical processes in order to obtain products that are free from residual antigenic activity, and to study milk from less familiar mammals (mare, camel, etc.) in clinical studies aimed at evaluating their tolerance.

Finally, allergologists must take into account different patterns of sensitization and cross-reactivity when defining the best dietetic approach for each subject.

References

- Wal J-M (2002) Cow's milk proteins/allergens. *Ann Allergy Asthma Clin Immunol* 89(Suppl 9):3–10
- International Union of Immunological Societies Allergen Nomenclature Sub-Committee (2009) Allergen nomenclature. <http://www.allergen.org/Allergen.aspx>
- Somma A, Ferranti P, Addeo F, Mauriello R, Chianese L (2008) Peptidomic approach based on combined capillary isoelectric focusing and mass spectrometry for the characterization of the plasmin primary products from bovine and water buffalo β -casein. *J Chromatogr A* 1192:294–300
- AAAAI Work Group (2003) Current approach to the diagnosis and management of adverse reactions to foods (report). http://www.aaaai.org/members/academy_statements/practice_papers/adverse_reactions_to_foods.pdf
- Asero R, Ballmer-Weber BK, Beyer K, Conti A, Dubakiene R et al (2007) IgE-mediated food allergy diagnosis: current status and new perspectives. *Mol Nutr Food Res* 51:135–147
- Norgaard A, Bernard H, Wal JM, Peltre G, Skov PS, Poulsen LK, Bindslev-Jensen C (1996) Allergenicity of individual cow milk proteins in DBPCFC-positive milk allergic adults. *J Allergy Clin Immunol* 97(1Pt3):237
- Werfel SJ, Cooke SK, Sampson HA (1997) Clinical reactivity to beef in children allergic to cow's milk. *J Allergy Clin Immunol* 99:293–300
- Restani P, Ballabio C, Cattaneo A, Isoardi P, Terracciano L, Fiocchi A (2004) Characterization of bovine serum albumin epitopes and their role in allergic reactions. *Allergy* 59(Suppl 78):21–24
- European Commission (1997) Effects of processing and preparation of food. In: Ortolani C (ed) Study of nutritional factors in food allergies and food intolerances (EUR 16893 EN). European Commission, Directorate-General XII (Science, Research and Development), Brussels
- Roth-Walter F, Berin MC, Arnaboldi P, Escalante CR, Dahan S, Rauch J, Jensen-Jarolim E, Mayer L (2008) Pasteurization of milk proteins promotes allergic sensitization by enhancing uptake through Peyer's patches. *Allergy* 63:882–890
- Fenaile F, Parisod V, Tabet J-C, Guy PA (2005) Carbonylation of milk powder proteins as a consequence of processing conditions. *Proteomics* 5:3097–3104
- Terracciano L, Isoardi P, Arrigoni S, Zoja A, Martelli A (2002) Use of hydrolysates in the treatment of cow's milk allergy. *Ann Allergy Asthma Immunol* 89(6 Suppl 1):86–90
- Martin-Esteban M, Garcia-Ara MC, Banque-Molas M, Boyano-Martinez MT, Martin-Munoz F, Diaz-Pena JM (1999) Evaluation of an extensively hydrolyzed casein-whey protein formula in immediate cow's milk protein hypersensitivity. *J Pediatr Gastroenterol Nutr* 26:398–401
- El-Agamy EI (2007) The challenge of cow milk protein allergy. *Small Ruminant Res* 68:64–72
- Restani P, Velonà T, Plebani A, Ugazio AG, Poiesi C, Muraro A, Galli CL (1995) Evaluation by SDS-PAGE and immunoblotting of residual antigenicity in hydrolysed protein formulas. *Clin Exp Allergy* 25:651–658
- Restani P, Ballabio C, Fiocchi A (2006) Milk allergens: chemical characterization, structure modifications and associated clinical aspects. In: Pizzano R (ed) *Immunochemistry in dairy research*. Research Signpost, Kerala, pp 61–76
- Bonomi F, Fiocchi A, Frokiaer H, Gaiaschi A, Iametti S, Poiesi C, Rasmussen P, Restani P, Rovere P (2003) Reduction of immuno-reactivity of bovine β -lactoglobulin upon combined physical and proteolytic treatment. *J Dairy Res* 70:51–59
- Peñas E, Restani P, Ballabio C, Préstamo G, Fiocchi A, Gomez R (2006) Evaluation of the residual antigenicity of dairy whey hydrolysates obtained by combination of enzymatic hydrolysis and high-pressure treatment. *J Food Protection* 69:1707–1712
- Chicón R, Belloque J, Alonso E, Martin-Alvarez PJ, Lopez-Pandiño R (2008) Hydrolysis under high hydrostatic pressure as a means to reduce the binding of beta-lactoglobulin to immunoglobulin E from human sera. *J Food Protection* 71:1453–1459
- Chicón R, Lopez-Pandiño R, Alonso E, Belloque J (2007) Proteolytic pattern, antigenicity, and serum immunoglobulin E

- binding of β -lactoglobulin hydrolysates obtained by pepsin and high-pressure treatments. *J Dairy Sci* 91:928–938
21. Spitzauer S (1999) Allergy to mammalian proteins: at the borderline between foreign and self? *Int Arch Allergy Immunol* 120:259–269
 22. UniProt Consortium (2009) UniProt website (used to search for the taxonomy of *Bos*). <http://www.uniprot.org/>
 23. National Center for Biotechnology Information (NCBI) (2009) NCBI taxonomy browser. <http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi>
 24. Urbisinov ZhK, Servetnik-Chalaia GK, Izatullaev EA (1981) Protein composition of camel's milk (abstract). <http://www.ncbi.nlm.nih.gov/pubmed/7324429>
 25. Jenness R (1974) Biosynthesis and composition of milk. *J Invest Dermatol* 63:109–118
 26. van Enkevort LCM, Gobius du Sart A (2009) Looking at dairy products for young piglets. http://www.denkavit.nl/login/upload/export/pressreleases/looking_at_dairy_products_for_young_piglets.pdf
 27. Oftedal OT, Hintz HF, Schryver HF (1983) Lactation in the horse: milk composition and intake by foals. *J Nutrition* 113:2096–106
 28. Malacarne M, Martuzzi F, Summer A, Mariani P (2002) Protein and fat composition of mare's milk: some nutritional remarks with reference to human and cow's milk. *Int Dairy J* 12:869–877
 29. Guo HY, Pang K, Zhang XY, Zhao L, Chen SW, Dong ML, Ren FZ (2007) Composition, physicochemical properties, nitrogen fraction distribution, and amino acid profile of donkey milk. *J Dairy Sci* 90:1635–1643
 30. FAO (1982) FAO Corporate Document Repository: Camels and camel milk, III: Composition of camel milk. <http://www.fao.org/docrep/003/X6528E/X6528E03.htm>
 31. Urbisinov ZhK, Servetnik-Chalaia GK, Izatullaev EA (1981) Protein composition of camel's milk. *Vopr Pitan* 41–42
 32. Swiss Institute of Bioinformatics (2009) ExPASy Proteomics Server, binary alignment (SIM + LANVIEW). <http://www.expasy.org/>
 33. Restani P, Gaiaschi A, Plebani A, Beretta B, Cavagni G, Fiocchi A, Poesi C, Velonà T, Ugazio AG, Galli CL (1999) Cross-reactivity between milk proteins from different animal species. *Clin Exp Allergy* 29:997–1004
 34. Restani P, Beretta B, Fiocchi A, Ballabio C, Galli CL (2002) Cross-reactivity between mammalian proteins. *Ann Allergy Asthma Immunol* 89(Suppl 1):11–15
 35. Broekaert SMC, Darsow U, Ollert M, Ring J, Krause I, Schulmeister U, Spitzauer S, Valenta R (2008) Anaphylactic shock caused by buffalo's mozzarella cheese. *Ann Allergy Asthma Immunol* 101:105–107
 36. Reinert P, Fabre A (1996) Use of goat milk for infant feeding: experimental work at Creteil (France). In: Freund G (ed) *Intérêts nutritionnel et diététique du lait de chèvre*. Niort, France, pp 119–21
 37. Restani P (2004) Goat milk allergenicity. *J Ped Gastroenterol Nutr* 39:323–324
 38. Bellioni-Businco B, Paganelli R, Lucenti P, Giampietro PG, Perborn H, Businco L (1999) Allergenicity of goat's milk in children with cow's milk allergy. *J Allergy Clin Immunol* 103:1191–1194
 39. Juntunen K, Ali-Yrkko S (1983) Goat's milk for children allergic to cow's milk. *Kiel Milchwirtschaft Forschungsber* 35:439–40
 40. Infante Pina D, Tormo Carnice R, Conde Zanduetta M (2003) Use of goat's milk in patients with cow's milk allergy. *An Pediatr (Barcelona)* 60:385–386
 41. Alvarez MJ, Lombardero M (2002) IgE-mediated anaphylaxis to sheep's and goat's milk. *Allergy* 57:1091–1092
 42. Bidat E, Rancé F, Baranès T, Goulamhousen S (2003) Goat's milk and sheep's milk allergies in children in the absence of cow's milk allergy. *Rev Fr Allergol Immunol Clin* 43:273–277
 43. Ah-Leung S, Bernard H, Bidat E, Paty E, Rancé F, Scheinmann P, Wal JM (2006) Allergy to goat milk and sheep milk without allergy to cow's milk. *Allergy* 61:1358–1365
 44. Meglio P, Bartone E, Plantamura M, Arabito E, Giampietro PG (2004) A protocol for oral desensitization in children with IgE-mediated cow's milk allergy. *Allergy* 59:980–7
 45. Patriarca G, Schiavino D, Nucera E, Schinco G, Milani A, Gasbarrini GB (1998) Food allergy in children: results of a standardized protocol for oral desensitization. *Hepatogastroenterology* 45:52–8
 46. Alonso-Lebreroa E, Fuentes V, Zapatero L, Pérez-Bustamantea S, Pineda F, Martínez-Moleroa MI (2008) Goat's milk allergies in children following specific oral tolerance induction to cow's milk. *Allergol Immunopathol* 36:180–181
 47. Businco L, Giampietro PG, Lucenti P, Lucaroni F, Pini C, Di Felice G, Iacovacci P, Curadi C, Orlandi M (2000) Allergenicity of mare's milk in children with cow's milk allergy. *J Allergy Clin Immunol* 105:1031–1034
 48. Fanta C, Ebner C (1998) Allergy to mare's milk. *Allergy* 53:539–540
 49. Monti G, Bertino E, Muratore MC, Coscia A, Cresi F, Silvestro L, Fabris C, Fortunato D, Giuffrida MG, Conti A (2007) Efficacy of donkey's milk in treating highly problematic allergic children: an in vivo and in vitro study. *Pediatr Allergy Immunol* 18:258–264
 50. Shabo Y, Barzel R, Margoulis M, Yaghi R (2005) Camel milk for food allergies in children. *IMAJ* 7:796–798
 51. Calvani M Jr, Alessandri C (1998) Anaphylaxis to sheep's milk cheese in a child unaffected by cow's milk protein allergy. *Eur J Pediatr* 157:17–19
 52. Sampson HA (1986) Food hypersensitivity as a pathogenetic factor in atopic dermatitis. *N Engl J Allergy Proc* 7:511–519
 53. Werfel SJ, Cooke SK, Sampson HA (1997) Clinical reactivity to beef in children allergic to cow's milk. *J Allergy Clin Immunol* 99:293–300
 54. Martelli A, De Chiara A, Corvo M, Restani P, Fiocchi A (2002) Beef allergy in children with cow's milk allergy; cow's milk allergy in children with beef allergy. *Ann Allergy Asthma Immunol* 89(Suppl):38–43
 55. Szepefalusi Z, Ebner C, Urbanek R, Ebner H, Scheiner O, Boltz-Nitulescu G, Kraft D (1993) Detection of IgE antibodies specific for allergens in cow milk and cow dander. *Int Arch Allergy Immunol* 102:288–294
 56. Vicente-Serrano J, Caballero ML, Rodríguez-Pérez R, Carretero P, Pérez R, Blanco JG, Juste S, Moneo I (2007) Sensitization to serum albumins in children allergic to cow's milk and epithelia. *Pediatr Allergy Immunol* 18:503–507
 57. Moneret-Vautrin DA, Morisset M, Cordebar V, Codreanu F, Kanny G (2006) Probiotics may be unsafe in infants allergic to cow's milk. *Allergy* 61:507–8
 58. Tiger Lee T, Morisset M, Astier C, Moneret-Vautrin DA, Cordé V, Beaudouin E, Codreanu F, Bihain B, Kanny G (2007) Contamination of probiotic preparations with milk allergens can cause anaphylaxis in children with cow's milk allergy. *J Allergy Clin Immunol* 119:746–747
 59. Bruni FM, Piacentini GL, Peroni DG, Bodini A, Fasoli E, Boner AL (2009) Cow's milk allergic children can present sensitization to probiotics. *Acta Paediatrica* 98:321–327