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Cow's Milk Allergenicity

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Abstract: In this review, clinical and epidemiological aspects of milk allergy along with current data on the structure and function of the main cow's milk allergens, are presented. Milk allergy is the most frequent food allergy in childhood. One of the reasons why allergy to cow's milk shows its highest prevalence in children is its early introduction into the diets of infants when breast feeding is not possible. The major allergens are caseins, a-lactalbumin and β-lactoglobulin, but allergies to other minor proteins (immunoglobulins, bovine serum albumin) have also been reported. Milk allergenicity can be reduced by various processing methods (mainly hydrolysis), and processed formulas based on cow's milk can often be safely introduced to children allergic to milk proteins.

Cross reactivity has been described between different mammalian milks and between milk and meat or animal dander.

Keywords: Cow's milk allergenicity, cow's milk composition, cow's milk cross-reactivity, distribution of milk allergens, structure of milk allergens, stability of milk allergens.

INTRODUCTION

Cow's milk allergy (CMA) affects about 2.5% of infants during their first years of life and is the most common food allergy in early childhood [1] with symptoms ranging from relatively mild to severe and life-threatening [2]. Although most children outgrow CMA by 3 years of age, in a minority of children it can become persistent. On the other hand, CMA is related with an increased risk of development of other allergic disorders, such as allergic asthma, atopic eczema, rhinoconjunctivitis or egg allergy [3]. Patients suffering from CMA develop an IgE response toward different cow's milk proteins (CMPs).

COW'S MILK PROTEIN COMPOSITION

Cow's milk contains approximately 30 to 35 g of proteins per litre. The action of chymosin (rennin), or the acidification of the milk to pH 4.6 results in the segregation of proteins into 2 fractions: lactoserum (whey) which contains approximately 20% of the CMPs (*i.e. ca*. 6 g/L), and coagulum (curd) contains approximately 80% of the CMPs (*i.e. ca*. 28–30 g/L) in the form of casein. The protein composition of cow's milk [4] and the names of allergens included in the official international list [5] are shown in Table **1** [6]. Each fraction contains five major components [5, 6]. The only protein not present in human milk is b-lactoglobulin (b-Lg).

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.

In the coagulum, the whole casein (Cas) is consisted of 4 major proteins (as1-caseins, as2-caseins, b caseins and k-caseins, Bos d 8) where in the whey the major proteins are a-lactalbumin (a-Lac, Bos d 4) and b-lactoglobulin (b-Lg, Bos d 5) are the most important allergens [7, 8, 9]. Other whey proteins are bovine serum albumin (BSA, Bos d 6), lactoferrin (Lf), and immunoglobulins (Bos d 7) (9). The above are considered as minor allergens, although recently, Gaudin JC *et al.*, using a sensitive microarray assay, have shown that lactoferrin can be classed as one of the strong and frequent (41%) allergens in cow's milk [10].

Most of the patients are sensitized to several proteins. Cas, b-Lg and a-Lac are considered major allergens, i. e. more than 50% of the individuals with CMA are sensitized to those proteins [4, 11]. Multi sensitizations to the different kind of caseins usually occur in patients sensitized to the whole Cas [12].

Whey contains essentially globular proteins. The major ones, *i.e.*, b-Lg and a-Lac, are synthesized in the mammary gland, whereas others, such as BSA, Lf, or Igs, come from the blood. b-Lg is the main component (up to 50% of whey proteins) [4, 13-15]. Cas is comprised of 4 independent proteins coded by different genes carried on the same chromosome, *i.e.*, *as*1- , *aS2*-, *β*-, and *κ*-casein and three γ-caseins deriving from the hydrolysis of β-casein, namely γ1, γ2 and γ3. The latters represent the sequences 29–209, 106–209 and 108–209 of β-casein, respectively [16]. Each casein represents a well-defined chemical compound, however the different caseins readily cross-link with one another to form

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Table 1. 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	*Bos d 8*	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 $Gamma_{2}$.casein $Gamma_{3}$.casein		1-2	6	20.6 11.8 11.6	180 104 102	5.5 6.4 5.8
	Kappa-casein		3-4	10	19.0	169	5.4-5.6
Whey proteins			**~ 5.0**	**20**			
	Alpha-lactalbumin	*Bos d 4*	1-1.5	5	14.2	123	4.8
	Beta-lactoglobulin	*Bos d 5*	3-4	10	18.3	162	5.3
	Immunuglobulins	*Bos d 7*	0.6-1.0	3	160.0	-	-
	Bovine serum albumin	*Bos d 6*	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**			

aa= number of amino-acids residues per molecule
MW= molecular weight
Data from 4,6,16

large aggregates (micelles) that are in suspension in the aqueous phase of lactoserum. The proportion by weight of the *as1- β-*, *aS2-*, and *κ*-casein in the micelles is relatively constant, approximately 37, 37, 13, and 13%, respectively of the protein content of the Cas [17]. γ-caseins are present in milk in minute quantities, while they are abundant in cheeses characterized by proteolytic ripening (such as Grana cheese). It is noteworthy that whey may contain casein-derived fragments. Limited hydrolysis of *β*-casein by endogenous proteolytic enzymes, such as plasmin, normally present in milk gives rise to *γ*-caseins and to smaller fragments called proteose peptones. These peptides correspond to the N-terminal part of the *β*-casein molecule; they are soluble and remain in the lactoserum. Similarly, the limited proteolysis attributable to the action of chymosin during clotting of milk splits *κ*-casein into two peptides: hydrophobic para κ casein, f (1-105) and the highly polar caseino-macropeptide, f (106-169), which is soluble and remains in the whey, and not in the coagulum.

DISTRIBUTION OF ALLERGENS IN MILK

In vitro tests, such as RAST and derived tests, and *in vivo* tests such as skin prick tests (SPTs), combined and confirmed sometimes by oral challenges, have allowed us to outline the incidences of sensitization to the main CMPs [18, 19]. Quantitative enzyme-linked immunoadsorbent assay (ELISA) tests, specifically enzyme allergosorbent test (EAST) for determination of specific IgE to highly purified isoforms of milk proteins, have been used to study the variability of the affinity, specificity, and magnitude of the human IgE response [11]. Sensitivities to various CMPs have now been proven to be widely distributed and not limited to a single protein [20, 21]. Most proteins are involved at different extents, and some patients may even be only sensitized to minor proteins, present at very low concentration in milk, such as BSA, and especially Lf. Only 26% were monosensitized; 17, 22, 20, and 15% of the patients were sensitized to 2, 3, 4, and 5 allergens, respectively. Almost all possible combinations of allergens were observed. Although 65, 61, and 51% of patients were sensitized to the main proteins by weight, namely Cas, b-Lg, and a-Lac, those proteins present in very low quantities also appeared to be of great importance. More specifically, 43, 36, and 35% of patients were sensitized to BSA, Igs, and Lf, respectively. Sensitivities to Cas, a-Lac, and b-Lg appeared to be closely related, whereas sensitivity to BSA was completely independent and therefore cannot be considered a reliable marker of milk allergy [22].

In another more recently study, different patterns of sensitisation were observed. Most of the patients were sensitized to one or two proteins (33 and 41%, respectively), and only 26% to more than two proteins. Further, the importance of whey proteins was much decreased, with frequencies of sensitization to b-Lg, a-Lac, and Lf being 51, 19, and 21%, respectively. Prevalence of sensitization to BSA was increased from 43% to 54%, although, as already has been noted, BSA sensitization is not correlated with milk allergy. In parallel with the striking decrease of whey proteins, an increase of caseins in terms of importance as milk allergens (72% *vs* 65%) was observed [23]. Although

no definite explanation can be given for this observed evolution, a plausible hypothesis could be that the modifications in technologic processes applied to milk and dairy products and/or the changing consuming habits of allergic individuals may have resulted in a shift of the allergenicity pattern of different milk proteins [4]. In a recent study it was shown that the percentage of children reacting in the SPTs was 87.5% for b-Lg, 88.8% for α-Lac, and 90% for total caseins; in the CAP test 72.5% reacted to b-Lg, 76.3% to a-Lac, and 77.5% to total caseins; in immunoblotting 35% reacted to b- Lg, 62.5% to α-Lac, 63.8% to total caseins (61.3% to α-Cas and 45% to β-Cas), and 61.3% to BSA [24]. Hence, considering all the different diagnostic procedures applied for characterising and identifying the allergenic properties of milk proteins, the involvement of milk allergens in cow's milk sensitised individuals varies. Either way, the more prevalent proteins are the caseins.

STRUCTURE AND FUNCTION OF MILK PROTEINS

Milk proteins are very heterogeneous and share very few structural and functional features. Furthermore, because of their genetic polymorphism, each protein has several variants. Despite their primary structure which is fully elucidated their secondary and tertiary structures are only partially known. Caseins cannot be crystallized, and their tertiary structure is approached and studied with molecular modelling.

Whey Proteins

The major allergens of lactoserum are b-Lg and a-Lac. b-Lg occurs naturally as a 36-kDa dimer. Each subunit is a 162-residue polypeptide and the whole molecule possesses two disulphide bridges and one free cysteine. [24]. The tertiary structure of b-Lg is known (Fig. **1**). There are two main genetic variants (isoforms) of b-Lg, named A and B. The A variant carries residues of aspartic acid and valine whereas the B glycine and alanine at positions 64 and 118, respectively. With the use of tryptic and synthetic peptides, it has been shown that sensitization involves many epitopes along the b-Lg molecule. Some of them are short linear sequences, while others are more complex consisting of large fragments that may contain conformational epitopes or parts of epitopes [25]. Due to the relative resistance of b-Lg to acid hydrolysis and to proteases, some of the protein may apparently remain and absorbed intact during digestion. As a consequence, b-Lg from ingested cow's milk, can be detected in human milk and has it been suggested that it may be responsible for colic in breast-fed infants [26]. b-Lg's immunoreactivity has been largely attributed to peptide fragments (41–60), (102–124) and (149–162), each one accounting for 10-15% of whole b-Lg [26, 27]. b-Lg belongs to the lipocalin family which are retinol-binding proteins [28]. Lipocalins bind and carry hydrophobic ligands. They share well conserved sequences in their N-terminal parts, with tryptophan always present at position 19. Crystallography studies revealed a very similar conical-shaped tertiary structure called *β*-barrel (or calyx), with the same arrangements of 8 (or 10) anti parallel *β*-strands [29]. These kinds of molecules have a high allergenic potential. The interior of the calyx contains a hydrophobic pocket, allowing the binding of small hydrophobic molecules such as retinoids, fatty acids, vitamins, and cholesterol [30-32].

a-Lac is a monomeric globular protein of 123 amino acid residues with four disulfide bridges and molecular weight 14.4-kDa. It constitutes a regulatory element of the enzymatic system of galactosyl transferase which synthesises lactose. It contains a high-affinity binding site for calcium, which is very important for the stability of its secondary structure. a-Lac and hen's egg white lysozyme have quite similar linear and also secondary and tertiary structures, having evolved from a common gene. The amino acid sequence of bovine a-Lac shows extensive homology with human a-Lac, since 74% of the amino acid residues are

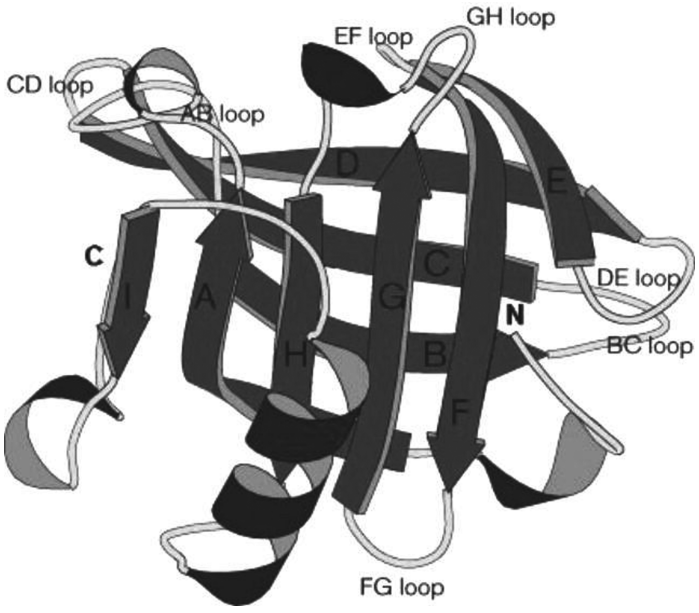

Fig. (1). Schematic representation of a single subunit of β -lactoglobulin lattice X. The β strands and joining loops are labelled [28].

identical and another 6% chemically similar [33]. However, despite the obvious similarity, bovine a-Lac is one of the major cow's milk allergen [34].

CASEINS

As already mentioned, Cas is comprised of four different proteins, namely, *αS1-*, *aS2-*, *β-*, and *κ*-casein, which have little structural homology [35-37]. However, the caseins are quite similar in their characteristics and differ significantly from those of whey proteins. Despite the structural difference, polysensitization to many casein fractions is not unusual, and it is more likely due to cross-sensitization of some common or closely related epitopes. The region of the major site of phosphorylation is well conserved in bovine as1- as2- and b-caseins, and in the caseins of other species and it is suggested that it plays a pivotal role in cross-reactivity. In corroboration of the above is the fact that this region is strongly immunoreactive and resistant to digestive degradation [38]. Sensitization is very frequent against a-casein (100%) and k-casein (91.7%) [39]. *a-* and *β*-caseins constitute the major part of Cas and are calcium-sensitive caseins, in contrast with *κ*-casein which is not. Caseins do not have a rigid tertiary structure but rather develop a "random coil" conformation stabilized by hydrophobic interactions. Because of this rather flexible structure, caseins are often considered weakly immunogenic. The four caseins constituting Cas are present in the milk of other ruminant species and they display a high degree of sequence homologies ranging from 80% to more than 90%. This could result in allergic reactions in patients with CMA in case they consume ewe's or goat's milk as a substitute [40]. One very important common characteristic is the homology in the acidic peptide sequence containing the cluster of phosphoseryl residues: f (13-21) in *β*-casein, f (62-70) in *α*S-casein, f (7-12) and f (55-60) in *α*S-2-casein variant A. Several studies have suggested as1-casein to be a major allergen causing strong allergic reactions [41]. Although as1-casein represents a class I food allergen, it was found to contain both conformational and sequential IgE epitopes [42]. A study group [40] has described three sequences, peptides (19–30), (86–103) and (141–150), which were recognized by the sera of 15 allergic patients, as the most immunoreactive epitopes. The most immunoreactive of the 3 was peptide (86–103). These three major epitopes are located in the hydrophobic regions of the molecule, where they cannot be reached by antibodies unless, as is the case during digestion, casein is denatured or degraded. Moreover, it has been recently shown that the IgE response to caseins is much decreased through modification of the major phosphorylation sites. Using purified and dephosphorylated variants A1 and A from b- and as2-caseins, respectively, a purified rare variant D of as2-casein was formed. The latter lacks one major phosphorylation site, and the native and dephosphorylated tryptic fragment (1–25) from b-casein [43]. Interestingly, b-casein, which induced a high IgE response, is also abundant in human milk. It has recently been shown that regions shared by the two b-caseins, and especially the C-terminus moiety, that constitute the major sites of phosphorylation are responsible for the cross-reactivity of IgE. As a consequence, some of the polyclonal anti bovine b-casein IgE antibodies, react with human b-casein, although with a lower apparent affinity [44].

STABILITY OF COW'S MILK ALLERGENS TO TECHNOLOGICAL AND CLINICAL ASPECTS

Although the underlying pathophysiology of food allergy development is not fully understood, an allergen has first to survive the acidic and proteolytic environment of the stomach in order to reach the immune system of the intestine, or to share its epitopes with aeroallergens [45]. Many of the food allergens are stable and resistant to digestion by gastrointestinal enzymes proteins [46, 47] or they are digested into high molecular weight (MW) peptides which retain the IgE binding and T-cell stimulating properties [48, 49 50]. As far as it concerns milk, various methods of processing may increase, decrease, or retain unchanged the allergenic properties of its proteins (Table **2**). The molecular basis of alterations in allergenicity is the inactivation or destruction of epitopes, formation of new epitopes (neotopes), or greater access of hidden epitopes by denaturation of the native allergen (cryptotopes) [51, 52].

Glycation

Glycation (non-enzymatic glycosylation) is one of the most frequent chemical interventions during industrial processing [53, 54], used to better the quality of dairy products and increase the shelf life of canned foods, like baby milk formulas.

Since milk naturally contains large quantities of lactose, and milk proteins are present in various food preparations containing free reducing sugars, milk proteins, are very often prone to glycations. More specifically, they are modified by the so-called Maillard reaction or glycation which ensues during the heating of proteins in the presence of reducing sugars [55]. This is a quite complex reaction, leading to the formation of advanced Maillard reaction products (AMPs) or advanced glycated end products (AGEs). Many studies have tried to estimate the IgE-binding ability of modified allergens after a Maillard reaction [56-61]. In some cases, allergen glycation increases their recognition by IgE [56, 59], whereas in other cases, it may reduce IgE binding [57, 60] or have no real effect [57, 58]. As it is obvious from the above, studies were unable to come to a legitimate general conclusion; the effect seemed to be allergen- and sugar-dependent. In a recent study, it has been demonstrated that low and moderate glycation of b-Lg have no effect on the recognition of the protein by IgE, and that the strongest lysyl substitution rates are related with decreased recognition of b-Lg by IgE [62]. This result can be due to a "masking" effect of sugars. In addition, all major and minor b-Lg epitopes [15] contain at least one lysyl residue, and some of them are well known critical binding sites for IgE [63]. Other studies [64, 65] investigated the effects of Maillard reaction conditions on the antigenicity of b-Lg and a-Lac in the conjugates of whey protein isolate (WPI) with glucose using response surface methodology and it was shown that the conjugation of WPI with glucose reduced the antigenicity of b-Lg and α-Lac. More specifically, it was observed that

Table 2. Effect of common processing modes on cow's milk proteins' allergenicity.

Cow's Milk Proteins	Effect on Allerginicity			
	Heating	Glycation	Enzymic Hydrolysis	Lactic Acid Fermentatiom
Caseins	In part	NA	NA	NA
β-casein	↓			
α_{s1}-casein	↓			
α_{s2}-casein	↓			
κ-casein	↓			
Whey				
α-lactalbumin	↓	↓	NA	↓
β-lactoglobulin	↓	↓	↓	↓
Bovine serum albumin	↓	NA	NA	NA
Immunuglobulins	↓	NA	NA	NA

NA = not available

inhibition rates of the antigenicity of both proteins were more than 90% under optimal reaction condition.

Heating

In our time, milk is very exceptionally consumed in its raw state. Heating (pasteurization: heating at 72°C–150°C within 1 to 15 seconds), maybe the most common industrial process of milk, induces significant changes in protein structure and may influence this way the IgE binding and allergenicity [66]. The globular whey proteins are heat sensitive in the following order: immunoglobulins (Ig) < bovine serum albumin (BSA) < b-Lg < α-Lac [67]. Heating to >60° C causes destabilization of β-sheets, unfolding of the β-barrel, and exposes the disulphide bonds and free cysteine to the solvent. If the temperature rises to more than 65° C, denaturation becomes irreversible; the loss of secondary and tertiary structures results in new hydrophobic interactions and disulphide bond exchanges, leading to irreversible aggregation [68-71]. All these structural modifications can potentially alter the antigenic attributes of proteins [72]. The antigenicity of b-Lg enhances at temperatures in the range of 80-90° C and reduces after heating above 100° C [67]. The denatured structure of the molecule has been shown to amend the immunologic response of rat and mouse models of allergy [73, 74]. In a recent study [62], it was observed that nearly 70% of CMA patients sensitized to b-Lg, processed at 75° C, had reduced recognition by IgE. The results are in accordance with data from animal experimental models [73]. The maximal effect was observed between 85 and 95° C, and that was in line with the results obtained by other authors [75]. Because the denaturation (modification of secondary and tertiary structures) of b-Lg begins at 70-72° C, it seems that responsible for the disappearance of conformational epitopes and the resulting reduced binding is the aggregation of proteins. None of the patients sera showed a stronger IgE response against denatured b-Lg, implying that the protein does not contain masked linear epitopes. Reorganization of proteinic structure and/or aggregation of b-Lg may have a masking effect on the recognition of sequential (or linear) epitopes. This could explain, at least partially, why some CMA patients are more tolerant to boiled milk than to raw milk. Processing in high temperatures (baking) reduces the allergenicity of many food proteins, most likely by altering the conformation structure of heat-labile proteins and consequently destroying the allergenic epitopes [76]. Nowak-Wegrzyn *et al*., [77] demonstrated that approximately 75% of children with milk allergy are able to tolerate baked milk products (heated to 350 °F/177° C for 30 minutes).

The caseins and a-lactalbumin are more heat stable than the whey proteins b-Lg and BSA [78]. Casein is the most heat stable from cow's milk proteins. Heating milk at 120 °C for 15 min did not alter the antigenicity of bovine casein, but BSA and Igs lost their antigenicity at 70 to 80 or 100 °C [79]. Furthermore, the heating of skim milk, showed that a-Lac and casein lost a great part of their allergenic activity and retainined only 40 to 50% [80]. The explanation for this discrepancy was attributed to redirected intestinal epithelial uptake of whey proteins, particularly b-lactoglobulin. Pasteurization induces aggregation and so hinders absorption of whey proteins by enterocytes. As it is known, after passing through intestinal epithelial cells, antigens move to Peyer patches, where they enhance sensitization and promote food allergy induction [81]. In contrast, casein exists in an aggregated form (micelles), which inhibits its crossing of the epithelial barrier, and is thus taken up by Peyer patches. The above characteristics do not change with heat treatment [82] (Fig. **2**). The route of sensitization is very important in anaphylaxis induction as it was demonstrated in a study where allergic responses in mice could not be triggered by aggregates of milk proteins when administered orally. Only soluble milk allergens could interact with the intestinal mast cells after they were transcytosed from the intestinal epithelium. Systemically introduced aggregates could,

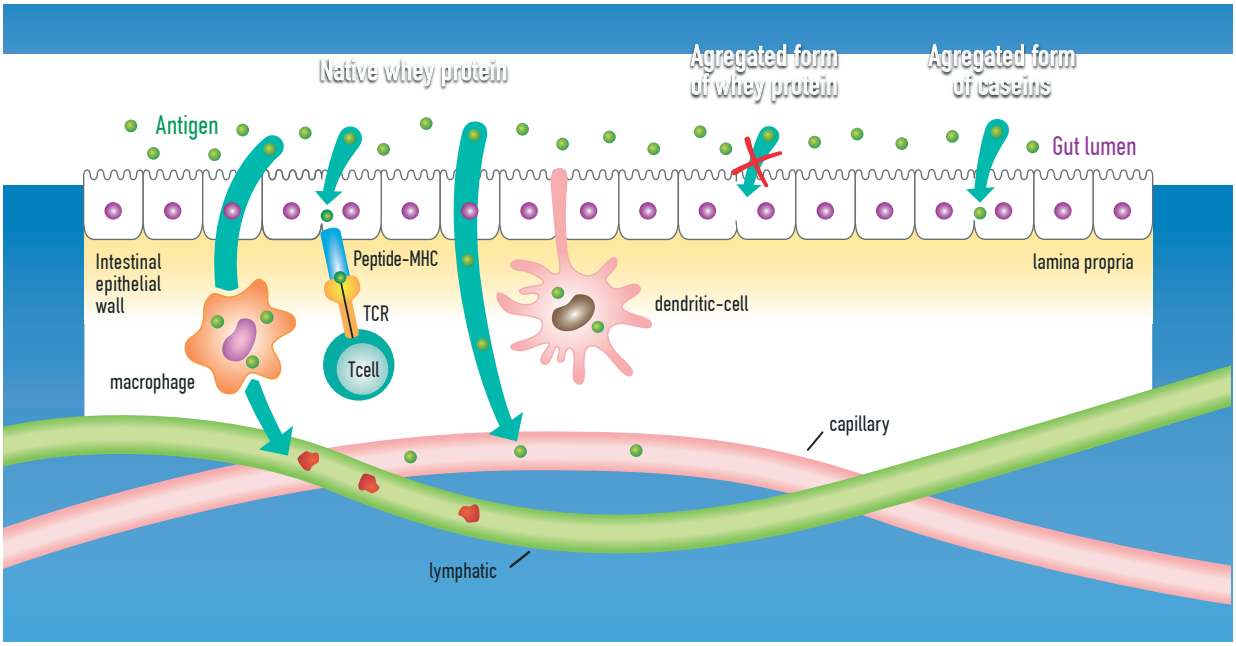

Fig. (2). Forms of intestinal epithelial uptake of cow's milk proteins induced by heat treatment.

however, cause clinical anaphylaxis. Since milk proteins are usually consumed in a pasteurized form in the westernised world, this may be of clinical relevance. The above findings support the hypothesis that food processing may contribute to the increasing prevalence of sensitization to food proteins in westernized countries [81].

High Pressure

High pressure is a relatively new processing technique in food industry. High pressure induces structural changes in milk proteins, such as denaturation and formation of aggregates which may affect the allergenic potential of cow's milk proteins [68]. In a recent study, the researchers used an *in vivo* mouse model and showed that b-Lg hydrolysates, obtained with chymotrypsin under atmospheric pressure or high-pressure conditions, lost their allergenicity [83]. Other studies have also suggested that the application of high pressure during enzymatic hydrolysis reduces the antigenicity and serum IgE binding properties of milk protein hydrolysates [84-87]. It was proposed that this reduction may be due to an enhanced accessibility of potentially immunogenic hydrophobic regions to the enzyme, that led in improved hydrolysis [88]. Therefore, the use of high pressure during hydrolysis of milk proteins may be an efficient method of producing hypoallergenic whey hydrolysates.

Furthermore, it was shown that the combination of enzymatic hydrolysis with heat treatment can increase the tryptic and peptic hydrolysis of a-Lac and b-Lg and reduce the allergenicity of milk [89, 90]. Recently, the effects of combined microwave and enzymatic hydrolysis on b-Lg and bovine whey proteins, on the human IgE-binding properties, were investigated [91]. It was found that microwave treatment increased hydrolysis of b-Lg and bovine whey proteins in comparison with conventional heating and the same proteolytic treatment. Microwave treatment at 200 W, in 3 min, enhanced the hydrolysis of b-Lg by pepsin and decreased its immunoreactivity significantly.

Lactic Acid Fermentation

Proteolytic enzymes can be produced during fermentation by lactic acid bacteria (LAB) [92]. Hydrolysis of milk proteins by Lactobacillus fermentation may affect milk digestibility and production of bioactive peptides. Proteolysis can cause the breakage of some epitopes and may decrease milk allergenicity [93, 94]. Several studies revealed that the antigenic properties of milk proteins can be decreased by various LAB strains. Lactobacillus delbrueckii subsp. Bulgaricus CRL 656, fragmented b-Lg and its epitopes, and reduced their recognition by the IgE of allergic children [95]. Similarly, IgE binding on αs1-casein and β-casein reduces significantly after proteolytic activity of Lactobacillus fermentum IFO3956 and Lactobacillus helveticus A75 [96, 97].

Alterations of antigenicity and allergenicity vary with the species of LAB and conditions of fermentation. It was shown that lactic acid fermentation reduces b-Lg antigenicity in skim milk and whey more than 90% and 70%, respectively [98]. Another study also suggested that the fermentation with lactic acid bacteria can significantly reduce the antigenicity of α-Lac and b-Lg in skim milk [99] Combined strains of L. helveticus and S. thermophilus most effectively reduced the antigenicity of α-Lac and b-Lg (inhibition rate 87%, and 95%, respectively).

CROSS REACTIVITY AND ASSOCIATED CLINICAL ASPECTS

The heterogeneity of milk proteins is further complicated by their genetic polymorphism, and the resultant several variants. The latter differ in amino acids point substitutions or deletions of peptide fragments [100]. They may also differ

in posttranslational changes such as phosphorylation and glycosylation [100]. The same or closely homologous proteins, with the same structural, functional, and biologic attributes are also present in milk from other ruminant species (Table **3**). As it has already been mentioned, there is high IgE cross-reactivity between ewe's, goat's, and cow's milk casein in most patients with CMA. Therefore, allergic reactions can occur in patients allergic to cow's milk if they consume milk from other ruminants [44]. On the other hand, the IgE response may also be quite specific with allergic symptoms occurring only after ingestion of a specific ruminant's dairy products [101]. Selective allergy to goat's or sheep's milk has been reported in children who are tolerant to bovine milk and its products [102]. IgE antibodies recognized as1-caseins, as2- caseins and b-caseins of goat's and sheep's milk, but not cow's milk [103].

Horse (mare) and donkey milks differ widely in protein distributions from cow's milk, having lower proportion of caseins and higher numbers of electrophoretic bands of whey proteins [5]. Recently, allergy to artiodactyls (even-toed ungulates, *i.e*. 'pawed animals') and ruminants, such as cow, sheep and goat, was attributed to the 'kosher epitope' [104]. All 24 patients allergic to CMA had possitive skin prick test (SPT) to deer, ibex (wild mountain goat) and buffalo milk, while on the contrary, only 20% and 25% were found positive to pig's and camel's milk, respectively. Interestingly, only partial cross-reactivity of human antibovine IgE with reindeer b-Lg exists, despite the fact that reindeer is a ruminant [105]. A randomized, crossover study demonstated that donkey's milk was better tolerated and more effective than goat's milk in ameliorating atopic dermatitis in children with CMA. In food challenges of 26 children, 23 exhibited a positive reaction with goat's milk and 1 with donkey's milk [106]. A prospective study [107] of 46 children with CMA clearly demonstrated that the majority of children (80%) are able to tolerated donkey's milk. IgE cross-reactivity with donkey's milk proteins was very weak. However, the study used heated donkey's milk and fresh and skimmed cow's milk, which may have affected tolerability significantly. Furthermore, 80% of children with a history of severe allergic reaction to cow's milk reacted to donkey's milk as well [108].

The protein expression of human milk is quite different from cow's and is more similar to horse's and donkey's milk, which are relatively poor in casein [109]. Another important difference of human milk from that of mammalian milks is that it lacks b-Lg. Despite this, a significant sequence homology between proteins of human milk and that of other bovines is present and this has led to the suggestion that cross-reactivity between conserved regions of bovine and human casein may exist [44, 110]. IgE antibodies to human milk were detected in over 80% of cow's milk allergic patients [111]. Both cross-reactive IgE-reactive human antigens such as a-Lac and non cross-reactive human milk antigens were identified, and skin prick tests were elicited with human milk. Both bovine and the corresponding human milk peptides existed in infants with CMA who had symptoms even when their mothers were no longer ingesting cow's milk products. They only show resolution of symptoms when breastfeeding was discontinued. However, low-level IgE binding to human milk peptides was also infrequently seen in infants who responded to maternal milk elimination. [112]. Surprisingly, it has rarely been observed IgE binding to a human peptide in the absence of binding to the corresponding bovine peptide, suggesting sensitization primarily to endogenous human milk.

Except for being an essential allergen in milk, serum albumin is also found in meat and is involved in the co-sensititization to milk and beef, reported in 13–20% of cow's milk-allergic children [113]. As far it concerns bovine meat

Table 3. Comparisons of amino acid sequences of major proteins from different mammalian species.

	Sequence Homology (%)							
	Cow Versus Water Buffalo	**Cow Versus Ewe**	**Cow Versus Goat**	**Cow Versus Pig**	**Cow Versus Donkey**	**Cow Versus Horse**	**Cow Versus Camel**	**Cow Versus Human**
Whey								
α-lactalbumin	99.3	97.2	95.1	74.6	71.5	72.4	69.7	53.2
β-lactoglobulin	96.7	93.9	94.4	63.9	56.9	59.4	Absent	absent
Serum Albumin	NA	92.4	71.2	79.9	74.1	74.5	NA	76.6
Caseins								
a s_1	95.3	88.3	87.9	47.2	NA	43.3	44.2	31.9
a s_2	95.0	89.2	88.3	62.8	60.0	NA	58.3	NA
B	97.8	92	91.1	67.0	-	60.5	69.2	56.5
K	92.6	84.9	84.9	54.3	-	57.4	58.4	53.2
Average	**96.1**	**91.1**	**87.6**	**64.2**	**62.8**	**62.4**	**60.0**	**58.4**

Modified from 4
NA= not available

allergy, monoclonal antibodies specific for BSA cross-reacted only with sheep serum albumin, whereas the reaction with serum albumins from other mammals was related with the proximity of the phylogenetic relationship between species [103]. Because of the potential for cross-reactivity between serum albumin present in milk, meat and epithelia of different mammals, patients with persistent CMA were examined for sensitization to BSA, meats and animal dander [114]. Seven of eight patients were sensitized to milk, BSA and animal dander recognized serum albumin in different meats (beef, lamb, deer and pork), epithelia (dog, cat and cow) and cow's milk, despite being tolerant to heated meats and their sera having shown no recognition to heated meat extracts. The authors deduced that patients with allergy to both BSA and cow's milk should avoid raw meats and furry pets. Adverse reactions in cow's milk-allergic individuals consuming soy milk have been attributed to a 30-kDa, glycinin like protein from soybean that cross-reacts with cow's milk casein [115]. However, cross sensitization in children who tolerate well soy milk was also observed in 17% [104]. Furthermore, a 10% incidence of soy allergy, mainly non-IgE mediated, was reported, in infants suffering from CMA and having taken soy formula [116].

Hence, although goat's milk should be avoided because of risk of cross-reactivity, mare's and donkey's milk are promising alternative protein sources especially if they are appropriately modified. Camel's milk would be another interesting alternative in case one could cope with the problems of availability.

CONCLUDING REMARKS

The diversity that characterizes the many allergens in milk and, the many epitopes within each allergen are of great interest. Because of the great variability of human IgE response, neither a single protein nor a particular structure accounts for a major part of milk allergenicity. Polysensitization to several proteins is the norm and occurs with great variability in specificity and in intensity of the IgE response. Proteins that are more abundant in milk are those most intensively intensively recognized by IgE, however, all proteins, even those that are present in tiny amounts may be potential allergens.

There is no definite relationship between structure and allergenicity. The significant homology between milk from cow, sheep and goat results in clinical cross-reactivity. On the other hand, mare's or donkey's milk may be tolerated by some individuals. Industrial processing technologies (glycation, enzymatic hydrolysis and lactic acid fermentation) can be used to effectively reduce the allergenicity of milk proteins and a combination of different technologies may further improve the resultant product.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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