

Cow's milk proteins/allergens

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Objective: The primary objective of this review is to provide updated data on the structure and function of the main cow's milk proteins (CMPs) identified as allergens and on the characterization of their epitopes.

Data Sources: The review represents a synthesis of basic literature and most relevant original recent publications on both topics of clinical and epidemiologic aspects of milk allergy and of milk protein's bio- and immunochemistry.

Study Selection: The expert opinion of the author was used to select the relevant data for the review.

Results: Most CMPs are potential allergens, even the proteins present at very low concentration. There are both conformational and linear epitopes, widely spread all along the protein molecules. They may be short fragments, located in hydrophobic parts of the molecule which comprise highly conserved sequences responsible for immunoglobulin E cross-reactivity with corresponding milk proteins of other mammals, including human beings. Those sequential epitopes have also been proposed as good markers of persistent allergy to CMPs.

Conclusions: No specific structure nor function is associated with allergenicity of CMPs. Variability and heterogeneity of the human immunoglobulin E response preclude anticipating the allergenic potential of any CMP or fragment thereof, as well as justify the need for being careful before using peptides for desensitization or proposing any milk protein hydrolysate in a diet for highly allergenic children.

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INTRODUCTION

Cow's milk allergy (CMA) is mainly an immunoglobulin (Ig)E-mediated hypersensitivity reaction, although other mechanisms and delayed manifestations have also been identified.¹ Reported incidence thus varies from 0.1 to 7.5%.^{2,3} CMA was diagnosed in 1.9 to 2.8% of the general population of infants <2 years old in various countries of northern Europe, but its incidence fell to approximately 0.3% in children older than 3 years.⁴ CMA usually develops in early infancy and is considered to be transient in most cases. However, CMA also occurs in adults; interestingly, in a retrospective study on 34 adults who were diagnosed in Switzerland between 1981 and 1991, women represented 91.2% of the study group.⁵

MILK PROTEIN COMPOSITION

Cow's milk contains approximately 30 to 35 g of proteins (CMPs) per liter. The action of chymosin (rennin), or the acidification of the milk to pH 4.6 enables two fractions to be obtained: lactoserum (whey) which contains approximately 20% of the CMPs, and coagulum (curd) which contains approximately 80% of the CMPs. Whey contains essentially globular proteins. The major ones, ie, β -lactoglobulin (b-Lg) and α -lactalbumin (a-Lac), are synthesized in the mammary gland, whereas others, such as bovine serum albumin (BSA), lactoferrin (Lf), or Igs, come from the blood. In the coagulum, the whole casein fraction (Cas) comprises four proteins coded by different genes carried on the same chromosome, ie, α S₁-, α S₂-, β -, and κ -casein. Each casein represents a well

defined chemical compound, but the different caseins associate with one another in solution. They cross-link to form ordered aggregates: micelles that are in suspension in the aqueous phase of lactoserum. The proportion by weight of the α S₁-, β -, α S₂-, and κ -casein in the micelles is relatively constant, approximately 37, 37, 13, and 13% of the protein content of the Cas.⁶ The main characteristics of the major CMPs appear in Table 1 adapted from Jost.⁷

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.

Changes in the quantitative pattern of proteins occur during lactation and in the composition of milk of other ruminant species (eg, buffalo, sheep, goat), but also of other mammals including man. Human milk does not contain b-Lg.

IgE REPERTOIRE AND PREVALENCE OF ALLERGENS IN MILK

Sensitivities to various CMPs have now been proven to be widely distributed and not limited to a single protein such as b-Lg.^{8,9}

Several constituents of milk have been identified as allergens, ie, recognized by specific IgE, or as antigens, ie, recognized by IgGs of nonatopic individuals, using various electrophoretic techniques associated with immunoblotting.

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Table 1. Main Characteristics of the Major Milk Proteins

Proteins		Concentration (g/L)	kDa	No. amino acid residues per molecule	No. S-S bridges per molecule	Secondary structures (% of total chain)		pI
(Concentration % milk proteins)						α -helix	β -sheet	
Whey (20%) (~5 g/L)	b-Lg (10%)	3-4	18.3	162	2 +1 free SH	+++	15	5.3
	a-Lac (5%)	1-1.5	14.2	123	4	++	25	4.8
	Igs (3%)	0.6-1.0	150					
	BSA (1%)	0.1-0.4	66.3	582	17 +1 free SH	+++	50	4.9-5.1
	Lf (traces)	0.09	80	703	16			8.7
Whole casein (80%) (~30 g/L)	α S ₁ -Cas (32%)	12-15	23.6	199		+	4-15	4.9-5
	α S ₂ -Cas (10%)	3-4	25.2	207	1			5.2-5.4
	β -Cas (28%)	9-11	24.0	209		+	1-10	5.1-5.4
	κ -Cas (10%)	3-4	19.0	169	1	+++	14	5.4-5.6

S-S bridge, disulphide bridge; free SH, free cysteine.

Interestingly, most CMPs are both antigens and allergens, but the same proteins are not always recognized at the same time by IgG and IgE of the same individual.¹⁰

In vitro tests such as radioallergosorbent test or derived tests, and in vivo tests such as skin prick tests, sometimes confirmed by oral challenge, also permitted to outline the incidences of sensitization to the main CMPs.^{11,12} Quantitative enzyme-linked immunoadsorbent assay (ELISA) tests, specifically, enzyme allergosorbent test (EAST), for determination of specific IgE to highly purified isoforms of milk proteins, such as b-Lg (Bos d 5) variant B, Cas (Bos d 8), a-Lac (Bos d 4), BSA (Bos d 6), and Lf, have been used to study the variability of the affinity, specificity, and magnitude of the human IgE response.¹³ Figure 1 shows the concentration of the specific IgE in sera of 20 individuals allergic to milk. As can be seen, most proteins are involved at different extent, and some patients may even be only sensitized to

minor proteins, present at very low concentration in milk, such as BSA and especially Lf.

The study has been continued on a larger population of 92 allergic patients to milk, which confirmed the variability in the pattern of sensitizations.¹⁴ It showed that most of the patients were sensitized to several proteins. Only 26% were monosensitized; 17, 22, 20, and 15% of the patients were sensitized to two, three, four, and five allergens, respectively. Almost all possible combinations of allergens were observed. The main proteins by weight, Cas and b-Lg, but also a-Lac, appeared to be major allergens, as 65, 61, and 51% of patients were specifically sensitized to these proteins, respectively. However, proteins present in very low quantities, such as BSA, Igs, and Lf, also appeared to be of great importance, as 43, 36, and 35% of patients were sensitized to these proteins, respectively. Sensitivities to Cas, a-Lac, and b-Lg appeared to be closely related, whereas sensitivity to BSA was com-

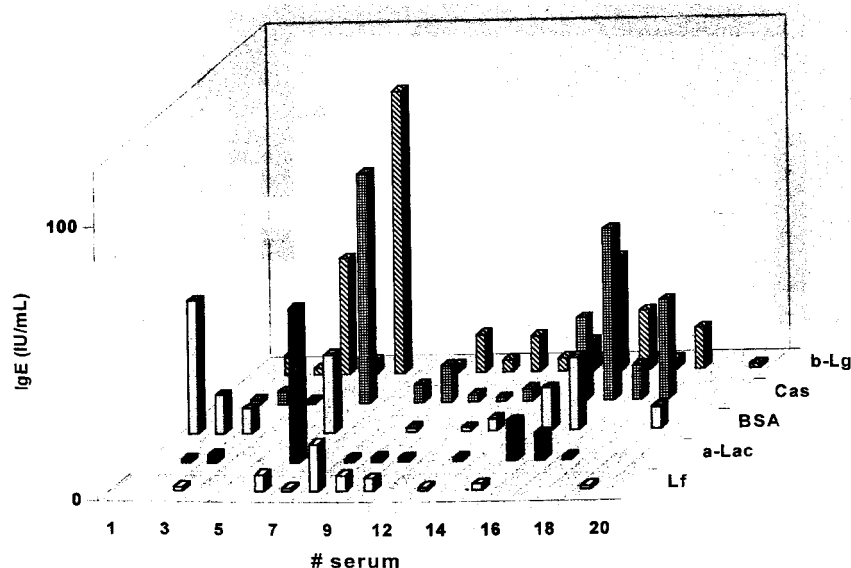


Figure 1. Enzyme immunoassay of specific IgE to five milk proteins (20 patients).

pletely independent and is therefore not a good marker of milk allergy.¹⁴

A similar study, using the same methodology, has been performed by the same authors on approximately 80 sera collected from milk-allergic patients in the late 1990s, approximately 10 years after the previous one. The background and the conditions of the two studies were different. The patients were not the same, and no group was defined according to the intake and exposure to CMPs nor to the clinical manifestations of the patients which preclude any statistically significant comparison. However, the patterns observed may give interesting indications in the possible shift of sensitizations in the everyday conditions of life of allergic consumers. Indeed, a great variability in the number of sensitizing proteins was still noticed, but 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 has much decreased. Frequencies of sensitization to b-Lg, a-Lac, and Lf were 51, 19, and 21%, respectively, versus 61, 51, and 35% 10 years ago. Prevalence of sensitization to BSA has increased from 43% to 54%, but, as already noted, it is not linked to milk allergy. In parallel with the striking decrease of whey proteins is the increase of caseins in terms of importance as milk allergens (72% vs 65%),¹⁵ which has also been stressed in other studies.⁵

As noted no definite explanation can be brought for this evolution observed in the pattern of allergenicity of the different milk proteins, which may be attributable to modifications in technologic processes applied to milk and dairy products and/or changes in dietary habits of consumers.

STRUCTURE AND FUNCTION OF MILK PROTEINS

The primary structure and part of the secondary and tertiary structures of CMPs are known. However, caseins can not be crystallized, and their tertiary structure is approached by molecular modeling. Amino acid sequences of the main CMPs are given in Figure 2, A–E.

Whey Proteins

The major allergens of lactoserum are b-Lg and a-Lac.

b-Lg occurs naturally in the form of a 36-kDa dimer. Each subunit corresponds to a 162-residue polypeptide; the molecule possesses two disulfide bridges and one free cysteine (Fig 2A). This structure is responsible for the main physicochemical properties and also for interaction with casein during heat treatments.¹⁶ The relative resistance of b-Lg to acid hydrolysis as well as to proteases allows some of the protein to remain intact after digestion. This increases the probability that intact b-Lg will be absorbed through the gut mucosa. Indeed, b-Lg from ingested cow's milk, can be detected in human milk and may be responsible for colic in breast-fed infants.¹⁷

There are two main isoforms of b-Lg, ie, genetic variants A and B, which differ only by two point mutations on residues 64 and 118, aspartic acid and valine in variant A, and

glycine and alanine in variant B, respectively. The tertiary structure of b-Lg is known. It belongs to the lipocalin family and is considered to be a retinol-binding protein.¹⁸ Lipocalins bind and carry hydrophobic ligands. They share well conserved sequence homologies in their N-terminus portion, where tryptophan at position 19 is always present. Crystallography studies revealed very similar tertiary structure called β -barrel structure, with the same arrangements of 8 (or 10) antiparallel β -sheets.¹⁹ This kind of molecule, including α_2 -microglobulin, has a high allergenic potential and several allergens of animal origin belong to this family such as the major mouse urinary proteins, the major horse allergen Equ c 1, the major allergen from cockroach, Bla g 4.

a-Lac is a monomeric globular protein of 123 amino acid residues, with 14.4-kDa molecular weight, and four disulfide bridges (Fig 2B). It possesses a high-affinity binding site for calcium, and this bond stabilizes its secondary structure. It corresponds to one of the two protein components of the lactose synthetase complex and regulates lactose biosynthesis by modulating the specificity of galactosyltransferase.

The complete amino acid sequence of bovine a-Lac shows extensive homology with human a-Lac, as 74% of the residues are identical and another 6% chemically similar.²⁰

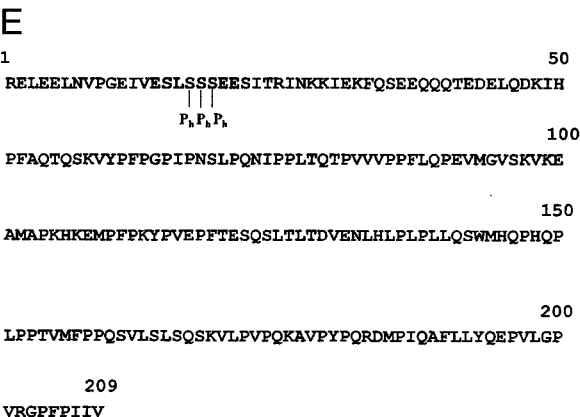
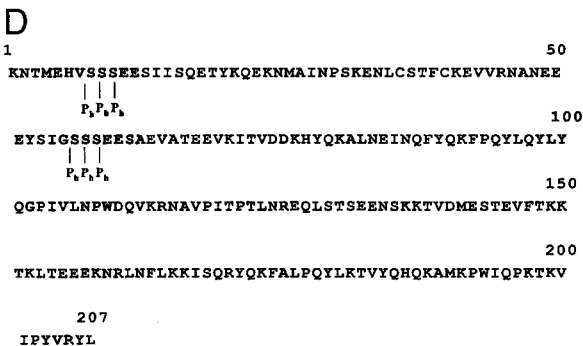
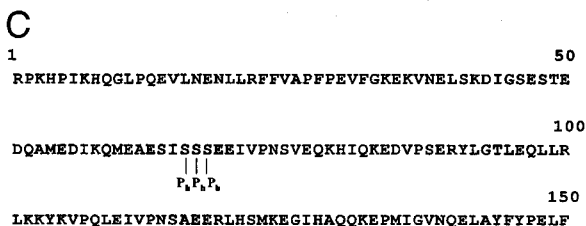
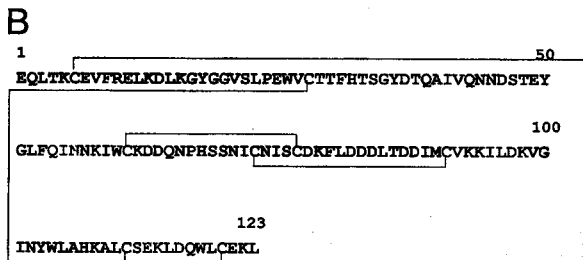
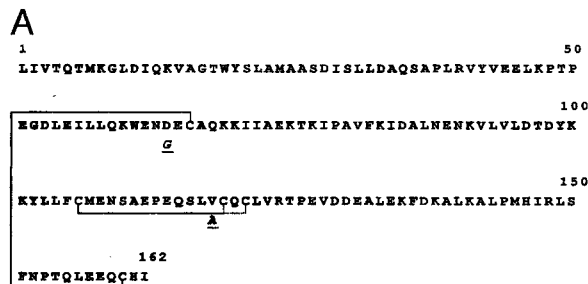
Caseins

As already mentioned, Cas comprises four different proteins, ie, α_S -, α_2 -, β -, and κ -casein, which have little primary structural homology (Fig 2, C–E).^{21–23} However, the caseins display similar characteristics that differ greatly from those of whey proteins.

α - and β -caseins are the major constituents of Cas. These are calcium-sensitive caseins, whereas κ -casein is not. They share common structural features. They have no rigid tertiary structure but a "random coil" conformation that is stabilized by hydrophobic interactions. Caseins are often considered poorly immunogenic because of this flexible, noncompact structure. Caseins are not significantly affected by severe heat treatments, but are very susceptible to all proteinases and exopeptidases and are rapidly and extensively degraded during digestion in the gut. α - and β -caseins have a dipolar structure, comprising a globular hydrophobic domain and a highly solvated and charged domain, with amphipathic properties. A striking common characteristic is the homology in the acidic peptide sequence containing the cluster of phosphoserine residues: f(13–21) in β -casein, f(62–70) in α_S -casein, f(7–12) and f(55–60) in α_2 -casein variant A (Fig 2, C–E).

Finally, the heterogeneity of milk proteins is complicated by their genetic polymorphism, resulting in several variants which are characterized by point substitutions of amino acids or by deletions of peptide fragments of varying size. They may also differ from posttranslational modifications such as phosphorylation and glycosylation.²⁴

The same or closely homologous proteins, sharing the same structural, functional, and biologic properties are present in milk of other ruminant species. As a consequence, a high IgE cross-reactivity between ewe's, goat's, and cow's



milk casein occurs in most patients with CMA, and therefore, adverse reactions can occur in patients allergic to cow's milk who consume milk from other ruminants.²⁵ However, the IgE response may also be quite specific with manifestations occurring after ingestion of sheep's or goat's cheese but not of cow's milk or other dairy products.²⁶

IgE-BINDING EPITOPES ON MILK PROTEINS

Whey Proteins

b-Lg. Using EAST and EAST inhibition tests on sera of 19 and 46 allergic patients to milk, Selo et al²⁷ have studied the IgE-binding capacity of isolated and purified fragments of b-Lg, obtained either by chemical cleavage using cyanogen bromide (CNBr) or by tryptic proteolysis. Most sera recognized several peptides, and the specificity of the human IgE response appeared to be quite variable and heterogeneous. The peptides most easily recognized by >90% of the patients were fragments f(41–60), f(102–124), and f(149–162), each of them accounting for 10 to 15% of the whole b-Lg immunoreactivity. The C-terminus (149–162) peptide forms a turn, adopting a short structure of an α helix, and appears to be quite mobile according to the crystal structure.¹⁸ Peptides (41–60) and (102–124) form loops stabilized by either hydrogen or disulfide bonds which are presented at the surface of the molecule, and are therefore accessible to antibodies. Less familiar epitopes could be classified into two groups. The first one comprises peptides (1–8), (25–40), and (92–100), recognized by 52 to 65% of sera; the second consists of peptides (9–14) and (84–91), recognized by 40% of sera and of fragments (78–83) and (125–135).

Heinzmann et al²⁸ have used overlapping decapeptides in the Pin-ELISA method to investigate B-cell epitopes in 14 individuals with CMA, 8 of them with a history of acute systemic reactions and 6 with delayed skin reactions after contact with cow's milk. The main linear epitopes identified were among those previously described, and no difference was found between the two groups of patients that led the authors to the conclusion that the recognition pattern of b-Lg IgE-binding epitopes was not predictive of the clinical type of reaction.

These results concerning the inhibition of IgE binding to b-Lg by isolated fragments were mostly obtained in the presence of large quantities of peptides; it is generally accepted that the hydrolysis of milk proteins considerably reduces their allergenicity.^{7,29} For this reason, it is interesting to recall the work of Haddad et al,³⁰ who have shown that specific IgE from 10 patients with CMA recognized enzymatic digestion products of b-Lg by pepsin or trypsin + trypsin (10 of 10 patients), and that the recognition of pep-

Figure 2. A, Amino acid sequence and location of disulfide bridges of b-Lg: variant A and variant B. B, Amino acid sequence and location of disulfide bridges of a-Lac. C, Amino acid sequence of α S₁-casein (P_h: phosphate group). D, Amino acid sequence of α S₂-casein (P_h: phosphate group). E, Amino acid sequence of β -casein (P_h: phosphate group).

tides was even better than that of the intact molecule in 4 of 10 patients. They concluded that the digestive processes unmask new allergenic epitopes. Selo et al²⁷ confirmed that CNBr and/or tryptic cleavage may allow the presentation of determinants which were not accessible to the antibodies on the whole native protein and thus enhance protein immunoreactivity in 10% of patients. Sensitization thus involved many epitopes, sometimes composed of short linear sequences that are widely spread all along the b-Lg molecule.

a-Lac. a-Lac is a major component of whey. Unlike b-Lg, which is absent from human milk, a-Lac is found in the milk of several species including human.

Maynard et al³¹ showed an IgE cross-reactivity between bovine and human a-Lac in patients with CMA, which could be related to the high degree of sequence homology between those two molecules. The same authors have also investigated the human IgE-binding capacity of tryptic digest fragments of native and denatured a-Lac in 19 allergic human beings.³² Of the 19, 11 sera recognized only the whole protein, whereas 8 had IgE able to bind isolated tryptic fragments. The peptide-comprising loop (60–80), which has a disulfide bridge and is itself attached by another disulfide bridge to peptide (91–96), considered to be an antigenic region on animal model,³³ was not identified as a major IgE-binding epitope for humans. Further, IgE-binding sequences were located in hydrophobic regions, such as fragment (99–108), of the a-Lac molecule, where antigenicity is very unlikely to be predicted. Evidence of binding to small peptides, such as f(6–10):S:S:f(115–123) and f(109–123), was produced. In some sera, reduced peptides such as f(59–94) exhibited a similar or a higher IgE-binding capacity than the native corresponding fragment, suggesting the existence of sequential epitopes exposed through protein denaturation. Further, a high IgE-binding capacity was shown to be associated with sequence (17–58) and with the C-terminal end (109–123), which have 81% and 87% homology, respectively, with the corresponding human sequences, showing that a high degree of homology does not exclude allergenic reactivity.

Jarvinen et al,³⁴ using overlapping decapeptides, have identified several IgE- and IgG-binding epitopes of b-Lg and a-Lac. They confirmed previous results, including the variability of the antibody responses to various regions of the molecules. Interestingly, they correlated the presence of IgE to multiple linear epitopes with persistent (vs transient) CMA and suggested it could be a good marker to identify the patients that would have a lifelong CMA.

Sharma et al³⁵ have compared the three-dimensional structures of b-Lg and a-Lac on which they have located allergenic epitopes. In both proteins the loops are well defined and tightly held at the two ends by antiparallel strands. Based on these structural data, the authors have searched for similar sites in other milk proteins and they identified two such sites widely separated in Lf, one each in the N- and C-lobes: sequences (82–89) and (572–579), respectively. They form loops that can be precisely superposed with allergenic loops

of b-Lg and a-Lac, suggesting that these fragments are allergenic epitopes of Lf.

Caseins

Bernard et al³⁶ showed that polysensitizations to the different casein fractions which is currently observed in allergic patients to whole casein were very likely attributable to both co-sensitization and cross-sensitization through common or closely related epitopes.

As already noted, there is poor sequence homology between the different caseins. However, a striking but common characteristic is the homology in the acidic peptide sequence containing the cluster of phosphoserine residues: f(13–21) in β -casein, f(62–70) in α S₁-casein, and f(7–12) + f(55–60) in α S₂-casein variant A. This sequence, which is the only conserved region, may play an important part in allergenicity and cross-reactivity of the three calcium-sensitive caseins, especially as it has already been described as being immunoreactive³⁷ and resistant to digestive degradation.³⁸

α -Caseins. The numerous works of Otani et al³⁹ on CNBr and tryptic peptides of α S₁-casein have highlighted the role of the phosphorylated regions in antigenicity and have described six allergenic and/or antigenic fragments, of which the main one was the large peptide (61–123). Spuerger et al,⁴⁰ using overlapping synthetic decapeptides in immunoblot and direct and competitive ELISA techniques have noticed no major difference between IgE- and IgG-binding regions. Three sequences, (19–30), (86–103), and (141–150), were recognized by sera of all 15 patients with CMA, the most immunoreactive one being peptide (86–103). It must be emphasized that these three major epitopes are located in hydrophobic regions of the molecule, where they are not accessible to antibodies unless the casein is denatured or degraded (eg, during digestive processes).

Chatchatee et al,⁴¹ using overlapping peptides, have identified 6 major and 3 minor IgE-binding as well as 5 major and 1 minor IgG-binding regions on α S₁-casein, using sera from 24 children with milk allergy. The highest intensity of IgE binding was in regions (17–36), (69–78), (109–120), and (173–194) recognized by 75, 46, 53, and 63% of the patients, respectively. Interestingly, differences were observed in two groups of patients with persistent or transient CMA. Sequences (69–78) and (173–194) were recognized by 67% and 100% of the patients >9 years of age with persistent allergy, respectively, but by none of the children <3 years old, who are likely to outgrow CMA. f(15–36) was the most frequently recognized region by IgG, but f(69–78) was not found to be an IgG-binding epitope. In contrast to IgE binding, no difference in IgG binding between the two groups was observed.

Screening for IgE antibodies specific to these epitopes that are differently recognized by patients with different histories of CMA, and particularly to linear epitopes, may thus be useful for identifying children who will have persistent milk hypersensitivity.^{41,42}

Using two naturally occurring genetic variants of α S₂-casein differing by the number of clusters of phosphoserine

Table 2. Immunoreactivity of β -Casein-Derived Peptides

β -Casein derived peptide	Rabbit IgG	Human IgG	Human IgE
1-25	+	-	+
1-60	+	-	+
26-60	+	-	-
61-93	+	-	-
29-93	+	-	+
94-102	+	-	-
103-109	+	-	-
110-144	+	-	+
132-144	-	-	+
157-185	+	+	+
186-209	-	+	+

residues and the native and chemically dephosphorylated tryptic fragment (1-25) from β -casein, Bernard et al⁴³ have shown that in most patient sera, the IgE response to caseins is affected (ie, much decreased) by modifying or eliminating the major phosphorylation sites. Posttranslational modifications such as phosphorylation are thus events that may greatly affect the IgE-binding capacity of caseins.

β -casein. The antigenic and allergenic structures have been extensively researched by Otani et al.³⁹ The results are quite variable depending on the experimental conditions. They are assembled in Table 2. At least six antigenic sites comprising linear epitopes have thus been evidenced on the β -casein molecule. The authors noticed the role of sequence -K-X-K-E-, found in peptides (94-102) and (103-109). Similar sequences containing one acidic and two basic amino acid residues are also present in antigenic regions of lysozyme. They also stressed that sequence (14-21), which comprises four phosphorylated serine residues, was involved in the antigenicity of f(1-25).

Surprisingly, β -casein, which is abundant in human milk (approximately 5 g/L) and exhibits substantial (eg, 50%) sequence homology with the human counterpart, induces a high IgE response. Actually, Bernard et al⁴⁴ have detected IgE against human β -casein in sera of patients with CMA. This tallies with previous results of Cantisani et al,⁴⁵ who had observed IgE cross-reactivity between bovine and human milk proteins. The 50% identity between human and bovine β -caseins correspond to conserved regions widely spread all along the molecules. However, two of them have been shown to be responsible for cross-reactivity. One contains the major sites of phosphorylation, f(5-14) and f(14-23) in human and bovine β -casein, respectively. The other one corresponds to sequences (118-132) and (127-141) of bovine and human β -casein, respectively, and comprises a predicted α -helical segment, -N-L-H-L-P-, at positions (123-127) and (132-136) in human and bovine β -casein, respectively.⁴⁴ As for human and bovine a-Lacs, those cross-reactions may involve specific IgE of low affinity. They have not been related to clinical

symptoms and can not lead to conclusions of clinical or epidemiologic significance.

CONCLUSION

The main conclusion is the multiplicity and diversity of allergens in milk and, for each allergen, of molecular immunoreactive structures (epitopes) that are involved in CMA. Because of the great variability of human IgE response, no single protein nor particular structure accounts for a major part of milk allergenicity.

Polysensitization to several proteins most often occurs; it is observed in approximately 75% of patients with CMA, with a great variability of the IgE response both in specificity and intensity. Even if the proteins most frequently and most intensively recognized by IgE seem also to be the most abundant in milk (casein and b-Lg), all milk proteins appear to be potential allergens—even those that are present in milk in trace amounts (Lf).

A great diversity is also notable where immunoreactive molecular structures are concerned. In each allergen, IgE-binding regions appear to be numerous and widely spread along the protein molecule.

No definite relationship can be established between structure and allergenicity. In addition to characteristics of genetic origin such as amino acid sequence, secondary structure and three-dimensional configuration, posttranslational modifications may play an important part in allergenicity. In addition to the conformational epitopes, IgE-binding studies also showed the presence of linear epitopes, which might be of clinical value as good markers to predict persistent versus transient allergy to CMPs. Those linear epitopes are often located in hydrophobic parts of the molecule and are inaccessible to antibodies in the native conformation of the protein and that become available after denaturation/degradation of the protein during the digestive processes. Peptides as short as 12- to 14-amino acid residues have thus been demonstrated to account for a significant part of the allergenicity of the whole molecule in some patients. These peptides do not seem to share specific sequences that could be considered responsible for allergenicity. However, at least for caseins, the well conserved region corresponding to the major site of phosphorylation is one of the regions most frequently recognized by IgE, and with the highest intensity. Indeed, as shown on β -casein and a-Lac, IgE-binding domains of CMPs are not located in regions in which the structure is the most divergent from that of the corresponding human protein. A high degree of homology with the corresponding human sequence does not seem to prevent peptidic fragments from allergenic reactivity. Finally, it must be emphasized that most of these data on structure allergenicity relationship are based on studies of IgE-binding capacity or IgE immunocross-reactivity. Their clinical significance and their incidence or relevance in terms of choice of hydrolyzed formula or management of desensitization therapy for highly allergic patients need to be further investigated.

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