

Immunological IgE Cross-Reactions of Bovine and Human α -Lactalbumins in Cow's Milk Allergic Patients

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Despite great homology with the equivalent human protein, bovine α -lactalbumin ($B \alpha$ -La), a major component of whey, has been identified as a major milk allergen. The aim of this study was to investigate the relationship between structure and IgE binding capacity in α -Las: (1) the importance of three-dimensional structure using native vs disulfide bridge-reduced $B \alpha$ -La; and (2) the incidence of amino acid sequence divergence on specific IgE cross-reactivity to human vs bovine α -La. Purified native, reduced and S-carboxymethylated $B \alpha$ -La and human α -La ($H \alpha$ -La) were prepared. Specific IgE of 20 sera from patients with clinically recognized cow's milk protein allergy and positive RAST® tests to $B \alpha$ -La were measured in original direct and competitive ELISA inhibition tests. All sera containing specific anti-native $B \alpha$ -La IgE also reacted with denatured protein, but the IgE levels were generally lower, showing that three-dimensional structure is an important feature in $B \alpha$ -La allergenicity but that sequential epitopes are also exposed after protein denaturation. Despite lower IgE levels, all sera also gave significant IgE responses to $H \alpha$ -La. Competitive ELISA inhibition confirmed results obtained by direct ELISA. The demonstrated IgE cross-reactivity between $B \alpha$ -La and $H \alpha$ -La could be related to the high degree of sequence homology between the two proteins but did not prove to have a clinical significance. However, it is of great interest for a study of the relationship between structure, IgE binding capacity and allergenicity in α -Las.

Keywords: Milk allergy, bovine α -lactalbumins, human α -lactalbumins, cross-reactions, structure-homologies, immuno-reactivity

INTRODUCTION

α -Lactalbumin (α -La) is a globular protein found in milk of several species. It corresponds to one of the two protein components of the lactose synthetase complex (Brodbeck *et al.*, 1967) and regulates lactose biosynthesis by modulating the specificity of galactosyltransferase (Brew *et al.*, 1968).

Bovine α -La (B α -La) is a major component of whey (22% of total whey proteins) with 14.4 kDa molecular weight. The complete primary sequence of α -La from several species has been established including the bovine (Brew *et al.*, 1970) and human (Findlay & Brew, 1972) protein. The monomeric polypeptide chain of B α -La contains 123 amino acid residues. The complete amino acid sequence of B α -La shows extensive homology with human α -La (H α -La) since 74% of residues are identical and another 6% chemically similar (Brew *et al.*, 1970; Findlay & Brew, 1972). The three-dimensional structure of globular α -La is stabilized by four disulfide bridges (Browne *et al.*, 1969; Warne *et al.*, 1974). Despite great homology with the human protein, B α -La has been identified as a major milk allergen (King, 1976) and 51% of cow's milk protein allergic patients were found to have anti-B α -La specific IgE (Wal *et al.* 1995a). It has been reported that anti-B α -La antisera react with α -La from other ruminants, but fail to do so with the parent proteins from non-ruminants (Johke *et al.*, 1964; Tanahasi *et al.*, 1968). Using rabbit anti- α -La antibodies, Prieels *et al.* (1975) stated the difficulty of correlating structural similarities of α -La from different species with antigenic behavior. Immunodiffusion techniques failed to relate in a simple manner cross-reactivities to amino acid sequences of different proteins.

The aim of this study was to investigate the relationship between structure and IgE binding capacity in α -Lacs. Twenty sera from patients with clinically recognized cow's milk protein allergy and positive RAST® tests to B α -La were used in direct and competitive ELISA inhibition tests. We first studied the importance of three-dimensional structure in IgE binding using native and disulfide bridge-reduced B α -La. Second, we also investigated the incidence of amino acid sequence divergence on cross-reactivity of specific IgE binding to B α -La vs H α -La.

MATERIALS AND METHODS

Materials

H α -La was purchased from Sigma (St Louis, MO, USA). Human serum albumin was obtained from the Centre National de Transfusion Sanguine (Les Ulis, France). HPLC-grade water and acetonitrile (Licrosolv) were obtained from Merck (Darmstadt, Germany). Trifluoroacetic acid (TFA), phenyl isothiocyanate (PITC) and amino acids standard were from Pierce (Kontron Instruments, Lausanne, Switzerland). All other reagents were of analytical grade. The HPLC was performed with a HP1050 system (Hewlett Packard, Basel, Switzerland) consisting of an autosampler, a quaternary pump fitted to an on line degasser, a column heater and a diode array detector. The equipment used in ELISA consisted of an automatic microplate washer 120, an automatic dispenser (Autodrop) and a spectrophotometer (Multiskan MC) from Flow Laboratories (Helsinki, Finland). Microtiter plates (96F immunoplates I with certificate) were purchased from Nunc (Roskilde, Denmark).

Methods

Purification of α -lactalbumins. Native bovine α -La (NB α -La) was prepared and purified as described by Wal *et al.*, (1995b). Crude H α -La was purified by reverse-phase HPLC (RP-HPLC) on a C18 Nucleosil 300–5/ET 250 column (Macherey Nagel, Oensigen, Switzerland). Operating conditions were as follows: column temperature, 40°C; flow rate, 1 ml min⁻¹; solvent A, 0.1% (v/v) TFA in water; solvent B, 60% (v/v) acetonitrile in solvent

A. Elution was performed with a linear gradient established from 0 to 35% (v/v) solvent B in 15 min, 35 to 65% (v/v) B in 25 min and 65 to 100% (v/v) B in 10 min. Absorbance was recorded both at 214 and 280 nm.

Reduction and S-carboxymethylamidation of bovine α -lactalbumin. Reduction and S-carboxymethylamidation of B α -La disulfide bridges were performed according to a method derived from Swenson *et al.* (1982). B α -La was dissolved in 1.44 M-Tris HCl buffer, pH 8.6 in presence of a 3 M final concentration of guanidine HCl, 0.13 M-EDTA and 0.12 M-2-mercaptoethanol. After 4 h incubation in the dark at ambient temperature, 1.44 M-iodoacetamide was used for blocking free thiols. The reduced and S-carboxymethylamidated bovine α -La (RB α -La) was dialyzed against distilled water and lyophilized.

Amino acid analyses. The S-carboxymethyl amido L-cysteine (CMC) content in RB α -La was quantified as described by Cohen *et al.* (1989). After acid hydrolysis under vacuum in the presence of 6 M-HCl for 1 h at 150°C in a Pico-Tag station (Waters, Schlieren, Switzerland), amino acids were derivatized with PITC according to the method of Bidlingmeyer *et al.* (1984). The phenylthiocarbamyl amino acids were quantified by RP-HPLC on a C18 Pico-Tag column (Waters) using an HP 1050 HPLC system (Hewlett Packard).

Human sera. Twenty serum samples from allergic patients to cow's milk proteins were obtained in banks of sera from pediatrics and/or allergy departments of different hospitals in France. They were selected according to their positive RAST® tests to milk proteins, and particularly to B α -La.

The children/adult partition among these sera was as follows: five were obtained from 2–3-year-old children, ten from 5–12-year-old children and five from adults, i.e. more than 15 years. In this study, there was no sera from infants less than 2-years-old.

Specific IgE to the main milk proteins (whole casein fraction, α -La, β -lactoglobulin and bovine serum albumin (BSA) were determined according to Wal *et al.* (1995a). 8/20 sera had specific anti-B α -La IgE but also anti-whole casein fraction, anti- β lactoglobulin and anti-BSA IgE. 6/20 sera were IgE reactive to α -La plus two other milk proteins. 4/20 sera reacted to α -LA and one other milk protein, either whole casein fraction, β -lactoglobulin or BSA. 2/20 sera were only IgE reactive to α -La.

ELISA. Two-site immunometric enzyme immunoassays of specific human IgE was used as described by Wal *et al.* (1995b). Briefly, microtiter plates were coated with 100 μ l per well of a 5 μ g ml⁻¹ solution of each purified protein α -La in the coating buffer (0.05 M-sodium carbonate buffer, pH 9, 0.01% NaN₃). After incubation overnight at ambient temperature, plates were extensively washed with a 10 mM-potassium phosphate buffer, pH 7.4, 0.05% Tween-20. Plates were then saturated with 250 μ l ELISA buffer (0.1 M-potassium phosphate buffer, pH 7.4, 0.4 M-NaCl, 1 mM-EDTA, 0.1% human serum albumin and 0.01% sodium azide) per well, sealed and stored at 4°C until use. Plates were washed before use. For direct ELISA of human-specific IgE, 50 μ l of individual human serum at appropriate dilution were dispensed per well. After incubation overnight at room temperature, plates were washed. Then, 50 μ l of a solution of mouse monoclonal antibody (MAb) anti-human IgE (clone BS17) labelled with acetylcholinesterase (AChE) were added per well. Following incubation overnight with MAb-AChE, plates were washed and 200 μ l of Ellman's reagent prepared according to Ellman *et al.* (1961) and used as enzyme substrate were dispensed into each well. Absorbance was measured at 414 nm.

Six sera from individuals not allergic to cow's milk were used as negative controls. Specific human IgE quantification as IU (i.e. 2.4 ng ml⁻¹) was made by comparison with

dose-response curves obtained with a total IgE assay performed with known standards of human IgE (Pharmacia Prist Test Kit), as described by Grassi *et al.* (1986). The corresponding immunometric assays of total and specific IgE were performed under identical conditions: an anti-human IgE MAb (clone LE27), complementary to the BS17-AChE tracer, was used as capture antibody for the total IgE assay, instead of the immobilized, purified allergen preparation used for specific IgE determination. In such conditions, no interference with total nor with specific anti milk proteins IgG has been observed in specific IgE determination (Wal *et al.*, 1995b).

The background (i.e. non-specific binding) was defined as the mean value obtained on specific IgE determinations in six different control sera from non-allergic individuals. The inter-assay standard deviation (SD) was calculated on these six values and the detection limit of specific anti- α -La IgE was defined as the background plus $3 \times \text{SD}$.

To confirm direct determinations, human sera with specific IgE to α -La were also tested by competitive ELISA inhibition, when sufficient volumes of sera were available. Human sera dilutions were chosen according to the results of direct ELISA, in order to get a limited but significant signal (*ca.* 0.5 absorbance unit) in the absence of competitor. Concentrations of inhibitors ranged from 0.25 to 10 nmol mL⁻¹. The inhibitors at variable concentration (30 μ L in ELISA buffer) were pre-incubated for 18 h with human sera (30 μ L at appropriate dilution in ELISA buffer) at ambient temperature. Then 50 μ L of the mixture were transferred into the allergen/antigen-coated wells. The amount of tracer bound in absence of competitors (B_0) was determined by using an incubation mixture containing 30 μ L of ELISA buffer instead of sample and 30 μ L of different human sera. B_0 can also be considered as a control that corresponds to the binding in presence of an irrelevant, non- α -La-related, antigen, e.g. human serum albumin at a 15 nmol mL⁻¹ concentration. The inter-assay SD on B_0 was calculated on six values. All other steps of the competitive ELISA inhibition were performed as reported above. The results of competitive inhibition were expressed as % inhibition, calculated as $(1 - B/B_0) \times 100$ where B and B_0 represent the absorbance measured on the bound fraction in the presence or absence of α -lactalbumins competitors, respectively. Inhibitions were considered to be significant when the differences between B and B_0 were higher than $3 \times \text{SD}$ on B_0 , which corresponds to a *ca.* 15–20% inhibition.

RESULTS

After purification, each allergen NB α -La, RB α -La and H α -La was eluted as a single homogenous peak in RP-HPLC (result not shown). The CMC content of RB α -La quantified by amino acid analysis was 7.58 residues CMC mol⁻¹ which indicated a reduction rate of the disulfide bonds of 95%, confirming a complete reduction of the four disulfide bridges.

Sera were tested for their content of specific IgE to NB α -La, RB α -La and H α -La using direct ELISA. No signal (e.g. specific IgE against any of the allergens) was detected in control sera from non-milk allergic individuals. The results of this screening on 20 allergic patients are reported in Table 1. In each case, specific IgE response was quantified as IU mL⁻¹ as described in methods. The detection limit for specific IgE directed against NB, RB or H α -La slightly varied depending on immobilized antigen. It was determined from the non-specific binding measured with negative control sera (see Methods) and could be estimated to 0.1 IU mL⁻¹. A great variability was observed in specific IgE titers among these sera. No relationship had been evidenced between mono- or multi-sensitization of the allergic patient sera to B α -La only or to B α -La and other milk proteins, and the specificity and intensity of IgE response towards NB, RB and H α -La. Indeed, some of them exhibited a low (i.e. < 1 IU mL⁻¹) although significant anti-NB α -La IgE levels, e.g. sera 10, 15 and 18. Two sera (6 and 13) were particularly distinguishable from this panel according to their high specific IgE contents to NB α -La (32 and 33 IU mL⁻¹, respectively). This confirmed that B α -La is a very strong allergen for some individuals.

TABLE 1. Specific IgE response (IU ml⁻¹) to the native bovine, reduced bovine and human α -lactalbumins in direct ELISA assay. Detection limit: 0.1 IU ml⁻¹

Serum number	Specific IgE (IU ml ⁻¹)		
	Native bovine α -La	Reduced bovine α -La	Human α -La
1	13.0	1.3	0.9
2	2.7	3.4	0.9
3	11.0	0.4	1.2
4	1.5	2.4	0.4
5	3.0	1.0	0.4
6	32.0	31.0	3.5
7	1.3	0.8	0.9
8	2.6	0.25	0.35
9	1.3	0.5	0.5
10	0.75	0.4	0.45
11	9.0	0.8	2.8
12	9.0	4.5	3.2
13	33.0	2.2	3.1
14	3.0	0.3	0.2
15	0.5	0.2	0.2
16	8.0	3.5	0.75
17	2.0	0.25	0.45
18	0.75	0.3	0.5
19	6.0	1.2	3.7
20	12.0	0.25	1.7

Using reduced B α -La as immobilized allergen, all sera gave a significant IgE response although sometimes with much lower IgE levels than with the native protein. Since the majority of human sera contained higher specific IgE titers to the native α -La, it is a clear evidence that the three-dimensional structure of B α -La is an important feature in its allergenicity. However, nine out of 20 sera had significant anti-RB α -La IgE titers higher than 1 IU ml⁻¹. Similar or higher IgE binding capacity to reduced α -La than to native protein was observed in sera 2, 4 and 6. These data suggest the existence of highly reactive sequential epitopes besides conformational epitopes in B α -La.

The reactivity of these sera to H α -La was thus also investigated and all gave a significant IgE response. However, the IgE titers to H α -La were always much lower than those to NB α -La and ranged between 6 to 71% of the IgE response reached with NB α -La. The comparison of IgE responses to H α -La and RB α -La showed that 10 sera had a better recognition of H α -La than of RB α -La and gave an IgE response one to three times higher to H α -La than RB α -La. On the other hand, half of the sera recognized the denatured B α -La better than the native human homologous counterpart.

To confirm results from direct determinations, sera containing IgE to the different α -Lacs were also analyzed by competitive ELISA inhibition when sufficient volumes of sera were available. Therefore, only six sera (1, 4, 5, 6, 13, 20) were used to perform indirect ELISA using NB α -La as immobilized allergen. A total (e.g. 100%) IgE binding inhibition to immobilized NB α -La was obtained with all sera tested when using a NB α -La solution at 1 nmol ml⁻¹ concentration as inhibitor. This showed a clear competition between soluble and immobilized allergen, meaning that the IgE antibodies were really specific for NB α -La. This confirmed the direct ELISA results. Once again all controls, e.g. with non-allergic sera or with a non- α -La related, irrelevant antigen used as inhibitor, gave negative results.

Using RB α -La as competitor, no significant inhibition could be observed at concentrations up to 10 nmol ml^{-1} in sera 13 (result not shown) and (Figure 1(c)). This agreed with direct ELISA where low amounts of specific anti-RB α -La IgE were found in these two sera (Table 1). In serum 6, the IgE binding inhibitions were identical using either NB α -La or RB α -La as competitors at 1 nmol ml^{-1} . This was in correlation with the similar IgE titers in this serum to the two forms of B α -La (Table 1). High inhibitions of IgE binding to NB α -La by RB α -La were also observed with serum 4 (Figure 1(a)) and with serum 5 (Figure 1(b)). Same observations were made with H α -La as competitor. Indeed, the IgE binding to NB α -La was either not (serum 4) or highly inhibited (serum 5 and 20) by soluble H α -La at 1 nmol ml^{-1} as shown in Figures 1(a), (b) and (c), respectively. However, when using either RB α -La or H α -La as competitors, the % inhibitions reached were always lower than those obtained with NB α -La. In both cases a maximum 60% inhibition was completed at a 1 nmol ml^{-1} RB α -La or H α -La concentration and remained constant even when the competitor concentration was increased up to 10 nmol ml^{-1} . This suggests that among the population of anti α -La(s) polyclonal IgE antibodies, reacting with B α -La and/or H α -La, the binding to H α -La involves IgE of lower affinity than the binding to B α -La. Moreover, as shown in Figure 1(c), the IgE binding to immobilized BN α -La was more inhibited by H α -La than by RB α -La. These observations from competitive inhibition experiments entirely agreed with direct ELISA results (Table 1).

DISCUSSION

The relationship between structure and IgE binding capacity on α -La was investigated using 20 sera from cow's milk protein allergic patients. All of these sera came from patients who presented clinical symptoms of allergy to cow's milk proteins and contained significant amounts of specific anti-NB α -La IgE. Indeed B α -La has already been shown to be a major milk allergen and the cut-off for a positive result in specific IgE determination is in good accordance with what was originally described (Wal *et al.*, 1995a). As already noticed, using acetyl cholinesterase as a label increases the sensitivity of the determination compared to other classical enzyme immunoassays, which also allows working on minute amounts of serum. An IgE titer of 0.1 IU ml^{-1} thus corresponds to a very strict criterion of positivity and is highly significant. Three different molecular forms of α -La were used as models in order to look at the importance of three-dimensional structure, as well as the incidence of amino acid sequence divergence, on IgE binding capacity. The influence of spatial conformation was evidenced by comparison between the reference native B α -La and the denatured form obtained by disulfide bridge reduction (RB α -La). Because of low degree of divergence with NB α -La, H α -La was also tested for IgE binding capacity.

All selected sera also reacted with both denatured protein (RB α -La) and H α -La but the IgE levels reached were generally lower than with NB α -La. The results on IgE binding in direct ELISA showed the presence of sequential epitopes exposed after protein denaturation besides the pre-eminent conformational epitopes in NB α -La. For three sera (2, 4, 6), the IgE responses towards these sequential epitopes were of the same order of magnitude or higher than those registered with NB α -La. It means that in this last case, sequential epitopes were particularly reactive. Indeed we have already shown that IgE binding to NB α -La and to large peptides such as sequence (17G-K58) confirmed the importance of conformational epitope(s) but a clear evidence for IgE binding to smaller peptides has also been reported. Moreover, in some sera, reduced and S-alkylated peptide (59I-K94) was shown to exhibit a similar or higher IgE binding capacity than the native corresponding fragment, suggesting the existence of sequential epitope(s) (Maynard *et al.*, 1997). Such a high specificity towards a denatured protein or towards denatured parts of the protein probably reflects a specific mechanism of food allergy. Stimulation of the immune system and IgE response induction take place after gastric and intestinal digestion of NB α -La then absorption and processing of the fragments. The immuno-stimulating

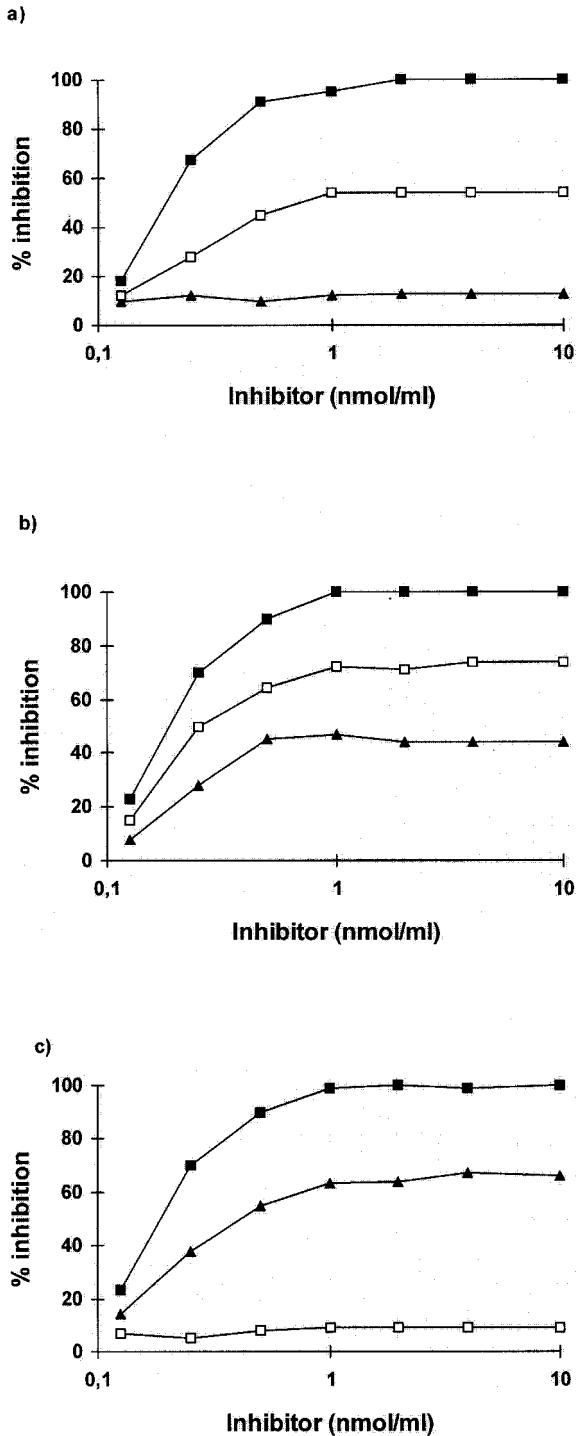


FIG. 1. Competitive inhibition ELISA tests: Increasing concentrations of native bovine α -La (■), reduced bovine α -La (□) and native human α -La (▲) were pre-incubated with a) serum number 4, b) serum number 5 and c) serum number 20 in order to inhibit the IgE binding to immobilized native bovine α -lactalbumin.

fragments could therefore be different to those present and exposed in the native structure. Despite lower IgE levels, all sera gave also significant responses to H α -La. This cross-reactivity could be related to the high degree of sequence homology between B α -La and H α -La (Figure 2) (Findlay & Brew, 1972). The 20 allergic patients to B α -La thus presented a polyclonal IgE antibody response with a high variability both in specificity and affinity as evidenced by direct and inhibition ELISA. A contact with B α -La or with fragments derived from B α -La after digestive processes may have induced production of specific IgE to parts of this protein presenting high homology to corresponding human ones. Then, a great part of the polyclonal IgE populations could cross-react with H α -La. Moreover we have recently demonstrated that a high IgE binding capacity was associated with sequence (17G-K58) and to the C-terminal end (109A-L123) of B α -La which were recognized with a high frequency and accounted for a significant part of the whole molecule allergenicity. Indeed these two peptides (17G-K58) and (109A-L123) have a very high homology (i.e. 81 and 87%, respectively) with the corresponding human sequences (Maynard *et al.*, 1997). This means that a high degree of homology with the human corresponding sequence does not seem to *a priori* prevent a protein or derived fragments from having an allergenic potential or at least from an immunological cross-reactivity with IgE. However, competitive inhibition ELISA tests demonstrated that at most, 60% of the IgE binding to immobilized NB α -La could be inhibited by soluble H α -La, while a 100% inhibition was observed using soluble NB α -La, which suggests that IgE affinity to H α -La was very likely to be smaller than to NB α -La. The presence of such low affinity specific IgE may be not related to clinical symptoms and thus cannot lead to relevant conclusions on the allergenic risk in groups at risk such as nursing women and their infants. Moreover, even if the requisite amino acid side chains are present, slight alterations in their environment may be sufficient to reduce IgE binding capacity and thus allergic potential. The X-ray crystal structures (at a 1.7 Å resolution) of B α -La and H α -La and α -La are available from the Protein Data Bank (Brookhaven National Laboratory). Alignment of primary structures according to Huang & Miller (1991) and comparative protein modeling using Swiss-PdbViewer (Guex & Peitsch, 1997) showed the following main features. Both structures are very similar and cannot be distinguished easily. There is 76% homology between the two proteins. Of the amino acid residues 30% are accessible to solvents but this proportion increases to 50% in regions where residues differ indicating that divergences are not randomly located but rather concentrated at the surface of the molecule. Concerning the alignment of the α -carbon (C α) atoms of the peptide chain, four regions appear to present a divergence (i.e. a distance significantly higher than 1.7 Å) between the two α -La(s). These are sequences (11–15), 46, (63–67) and 114. The most divergent one is sequence (11–15) in which the distance between the C α of aspartyl residues 14 of the two α -La(s) is 3.14 Å and 4.7 Å between the two lateral chains. Actually, the amino acid sequence between B α -La and H α -La is different only in region (8–11) (Figure 2) but as a consequence, this divergence also affects sequence (11–16) where amino acid residues are the same but where lateral chains are thus significantly shifted. This also occurs for regions 46 and (63–67). Although there are some substitutions in the amino acid sequence (Figure 2), three-dimensional conformations are very close in both B α -La and H α -La. However, small divergences due to amino acid substitution and shifts in C α position add up and may accentuate their effects on lateral chain location of subsequent amino acids. Therefore, they can affect interactions between these two different domains, i.e. 46 and (63–67), which are spaced on the primary sequence but are brought close together by the folding of the molecule. Indeed, in H α -La, the distance between C α residue 46 and 65 is 7.7 Å but it is 11.3 Å in B α -La. So, no major nor even any visible difference occurs in the overall tertiary structure of the whole H α -La molecule when compared to the B α -La. No major difference even occurs when considering individually and separately the conformation of each region of the molecule where amino acid sequence diverges. However, divergences can be expressed and concentrated in other parts

	1	5	10	15
H α -La	H ₂ N- Lys -Gln- Phe -Thr-Lys-Cys-Glu- Leu -Ser-Gln- Leu -Leu-Lys-Asp- <u>Ile</u> -			
B α -La	H ₂ N- Glu -Gln- Leu -Thr-Lys-Cys-Glu- Val - Phe -Arg- Glu -Leu-Lys-Asp- <u>Leu</u> -			
	20	25	30	
H α -La	Asp -Gly-Tyr-Gly-Gly- Ile -Ala-Leu-Pro-Glu - Leu - Ile -Cys-Thr- Met -			
B α -La	Lys -Gly-Tyr-Gly-Gly- Val -Ser-Leu-Pro-Glu-Trp- Val -Cys-Thr- Thr -			
	35	40	45	
H α -La	Phe-His-Thr-Ser-Gly-Tyr-Asp-Thr-Gln-Ala-Ile-Val-Glu-Asn- Asp -			
B α -La	Phe-His-Thr-Ser-Gly-Tyr-Asp-Thr-Gln-Ala-Ile-Val-Glu-Asn- Asn -			
	50	55	60	
H α -La	Gln -Ser-Thr-Glu-Tyr-Gly-Leu-Phe-Gln-Ile- Ser -Asn-Lys- <u>Leu</u> -Trp-			
B α -La	Asp -Ser-Thr-Glu-Tyr-Gly-Leu-Phe-Gln-Ile- Asn -Asn-Lys- <u>Ile</u> - Trp-			
	65	70	75	
H α -La	Cys-Lys- Ser - Ser -Gln- Val -Pro-Gln-Ser-Arg-Asn-Ile-Cys- Asp -Ile-			
B α -La	Cys-Lys- Asp - Asp -Gln- Asn -Pro- His -Ser- Ser -Asn-Ile-Cys- Asn -Ile-			
	80	85	90	
H α -La	Ser-Cys-Asp-Lys-Phe-Leu- Asn -Asp- Asn - <u>Ile</u> -Thr- Asn - Asn -Ile-Met-			
B α -La	Ser-Cys-Asp-Lys-Phe-Leu- Asp -Asp- Asp - <u>Leu</u> -Thr- Asp - Asp -Ile-Met-			
	95	100	105	
H α -La	Cys- Ala -Lys-Lys-Ile-Leu-Asp- Ile -Lys-Gly-Ile-Asn-Tyr-Trp-Leu-			
B α -La	Cys- Val -Lys-Lys-Ile-Leu-Asp- Lys - Val -Gly-Ile-Asn-Tyr-Trp-Leu-			
	110	115	120	
H α -La	Ala-His-Lys-Ala-Leu-Cys- Thr -Glu-Lys-Leu- <u>Glu</u> -Gln-Trp-Leu-Cys-			
B α -La	Ala-His-Lys-Ala-Leu- Cys- Ser -Glu-Lys-Leu- <u>Asp</u> -Gln-Trp-Leu-Cys-			
	123			
H α -La	Glu-Lys-Leu-COOH			
B α -La	Glu-Lys-Leu-COOH			

FIG. 2. Comparison of the amino acid sequences of human and bovine α -lactalbumins. Differences in amino acid residues are indicated in bold. Chemically similar residues are underlined.

of the molecule and particularly affect interaction between domains which can slightly but significantly alter the presentation of preferential sites of binding of IgE.

On the other hand, an eventual specific genetic regulation and a major histocompatibility complex restricted response may prevent the induction of an IgE response to homologous (human) α -La in atopic patients as demonstrated in the mouse by Horiuchi *et al.* (1990). To our knowledge, no case of anaphylactic shock triggered by H α -La in B α -La hyperreactive patients has been reported. The response levels and the likely low IgE affinity to H α -La observed in our study only reflects immunological cross-reactivity and could not be related to an eventual induction of specific anti-H α -La IgE synthesis. Immunological cross-reactions with IgE binding are often observed between more or less related proteins. They may involve low affinity specific IgE and cannot always be related to clinical symptoms and thus cannot lead to relevant conclusions on the allergenic risk in groups at risk such as nursing women and their infants. The clinical or pathological significance of such *in vitro* immunological cross-reactions should be confirmed by *in vivo* studies such as skin tests and provocation tests. However, available antibodies exhibiting a restricted specificity make it possible to locate particular antigenic/allergenic sites on the molecule. These properties of IgE cross-reactivity are therefore of great interest for a study of the relationship between structure, IgE binding capacity and allergenicity in α -La (s) but also as characteristics of other food allergens.

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