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Specificity of the Human IgE Response to the Different Purified Caseins in Allergy to Cow's Milk Proteins

Abstract

Background: Cow's milk is one of the most frequent food allergens. Whole casein appears to be highly allergenic. It corresponds to an association of four different proteins, α_{s1} -, α_{s2} -, β - and κ -caseins in approximate proportions of 40, 10, 40, and 10%, respectively. Methods: These different components were thus purified and used as immobilized antigens in an original enzyme immunoassay to measure specific serum IgE response in a population of 58 children (median age 11 months) allergic to cow's milk who were sensitive to whole casein. Results: A great variability was observed in the affinity and specificity of specific IgE responses in milk-allergic patients' sera. 85% of the patients presented IgE against each of the four caseins. Statistically higher amounts of specific IgE were found to be directed against the most abundant fractions (α_{s1} - and β -casein). Co- and/or cross-sensitization to the different caseins were seen in most of the patients sensitive to whole casein. Conclusion: These results suggest that both distinct and common epitopes may occur on these different caseins. The major site of phosphorylation which is the most conserved domain in three caseins could be involved in the IgE response to casein and in immunocross-reactivity between these proteins.

Key Words

Cow's milk allergy
IgE antibodies specific
Enzyme immunoassay
Casein
 α_{s1} -Casein
 α_{s2} -Casein
 β -Casein
 κ -Casein

Introduction

Cow's milk is one of the most frequent food allergens. Incidences of 0.5–7.5% of newborn or young children suffering from milk allergy, i.e. with immediate-type reaction symptoms, have been reported [1, 2]. Most other studies have stated an average incidence of 2–3% in the whole population of infants under 1 year of age [3, 4]. β -Lactoglobulin, the main protein from bovine whey, has long been considered as the major allergen due to its absence in human milk [5, 6]. β -Lactoglobulin has been shown to be quite resistant to attack by digestive enzymes [7, 8] and among milk

proteins most easily crosses the intestinal mucosa [9]. However, it is now clearly established that other milk proteins can also sensitize genetically predisposed infants, and may elicit an IgE-mediated response [10–13]. Whole casein, which accounts for 80% of bovine milk proteins, is thus also considered as an important allergen, and specific IgE directed against whole casein have been detected in humans allergic to milk [12, 14–18]. We have recently used an enzyme immunoassay (EIA) to detect human specific IgE against purified cow's milk allergens to show that most of the sera from patients allergic to milk possess specific IgE against whole casein [19].

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Whole casein corresponds to an association of four different proteins, namely α_{s1} -casein, β -casein, α_{s2} -casein, κ -casein in approximate proportions of 40, 40, 10, and 10%, respectively. Their biosynthesis is controlled by four different genes and they have little primary structure homology. The four caseins are hydrophobic phosphorylated and/or glycosylated proteins, with a loose tertiary, highly hydrated structure. These caseins associate with each other in solution to form ordered structures, micelles. They are very susceptible to all proteinases and exopeptidases, but are not significantly affected by severe heat treatment. Casein (e.g. whole casein fraction) is often considered poorly immunogenic because of this flexible, noncompact structure. Moreover, after ingestion, most of this structure and the presentation of the potential antigens and/or allergens are assumed to be modified by acidic gastric conditions, action of digestive enzymes, and uptake through the intestinal mucosa. The study of the specific antibody response, and particularly the IgE response after oral sensitization, first to whole casein fraction, and then to each of its isolated purified constituents, may provide information valuable in elucidating the structure-allergenicity relationships of milk proteins. Whereas the characterization of certain epitopes has been reported for some caseins [20–22], little is known about their respective allergenicity. Some studies involving skin tests [13] or the determination of the specific IgE response to different caseins have been reported. However, these studies used only a limited number of sera or a mixture of α_{s1} - and α_{s2} -casein [11, 12]. We therefore investigated the specificity of IgE responses against each type of purified casein, using sera against the whole casein fraction from a large number of allergic patients. This study allowed us to evaluate the respective roles of each of these four allergens in the global IgE response to whole casein.

Materials and Methods

Reagents

Unless otherwise stated, all reagents were of analytical grade and from Sigma (St. Louis, Mo., USA). Human serum albumin (HSA) was from the Centre National de Transfusion Sanguine (Les Ulis, France). Free fatty acids were removed on activated charcoal at pH 3 [23]. After dialysis against water, purified albumin was freeze-dried.

Human sera (92 patients) were obtained from a bank of sera of hospital pediatricians. They originated from children (median age 11 months) who presented clinical symptoms of cow's milk allergy. These sera were tested for specific anti-whole casein IgE determination by using the original EIA developed in our laboratory [19]. Approximately 60% (58 sera) of the tested sera indeed had an IgE response to whole bovine casein and were selected for the study. Eight control sera were obtained from nonallergic human volunteers.

Specific rabbit polyclonal antisera for each of the κ -, α_{s1} - and α_{s2} -caseins and guinea pig antiserum against β -casein were obtained from Dr. L. M. Houdebine (INRA, Jouy en Josas, France). Affinity-purified polyclonal goat anti-guinea-pig antibodies were purchased from Jackson Laboratories (Baltimore, Md., USA). Buffers and reagents used for EIA were as follows: coating buffer: 0.025 M EDTA (disodium salt) pH 9.3 [24]; EIA buffer: 0.1 M potassium phosphate buffer pH 7.4, containing 0.1% HSA, 0.4 M NaCl, 0.001 M EDTA, and 0.01% sodium azide; washing buffer: 0.01 M potassium phosphate buffer pH 7.4, containing 0.05% Tween 20. Ellman medium contained 7.5×10^{-4} M acetylthiocholine and 5×10^{-4} M dithio-bis-nitrobenzoate in 0.1 M potassium phosphate buffer, pH 7 [25]. Solid-phase EIA was performed on 96-well microtiter plates (Nunc, Denmark) using automatic Titertek microtitration equipment (washer, dispenser, and reader) from Labsystems (Helsinki, Finland).

Preparation of the Whole Casein Fraction

Whole casein was isolated by isoelectric precipitation at pH 4.6 [26] of skimmed raw milk from a single cow homozygous at the four casein loci: α_{s1} -casein variant B, β -casein variant A1; α_{s2} -casein variant A, and κ -casein variant A. The precipitate was recovered by centrifugation, washed twice with water at pH 4.6, redissolved at pH 7 and precipitated again at pH 4.6. The precipitate was lyophilized and stored at -20°C .

Purification of the Different Caseins

Caseins were fractionated from the whole casein fraction by a set of successive and selective precipitations (fig. 1) [27–29]. Each recovered purified fraction was extensively dialyzed against water and lyophilized. Whole casein dissolved in water at pH 6.5 was precipitated by the addition of propanol (40%, v/v). After centrifugation, the pellet containing a highly enriched fraction in α_{s2} -casein was dissolved in 6 M urea before dialysis and lyophilization. The supernatant was dialyzed, freeze-dried and dissolved in 6.6 M urea. After dilution to 2.2 M urea, precipitation was achieved by lowering the pH to 1.3 with sulfuric acid. The supernatant mainly contained κ -casein which was concentrated by precipitation with ammonium sulfate (132 g/l), centrifuged, dialyzed and freeze-dried. The pellet containing a mixture of α_{s1} - and β -casein was dissolved in 6.6 M urea, diluted with an equal volume of water, and precipitation was achieved by lowering the pH to 4.7 with 1 M HCl. After centrifugation, the supernatant containing β -casein was collected, dialyzed and freeze-dried. The precipitate contained an enriched α_{s1} -casein fraction which was dissolved in 6.6 M urea, dialyzed and freeze-dried. The lyophilisate was redissolved in 50% ethanol and precipitated with 2 M ammonium acetate in order to eliminate traces of κ - and α_{s2} -caseins. The supernatant containing pure α_{s1} -casein was dialyzed and freeze-dried.

Final purification of each casein fraction was then achieved by anion exchange chromatography using a fast protein liquid chromatography (FPLC) system and a mono Q HR 10/10 column (Pharmacia, Sweden) equilibrated in 5 mM Tris buffer pH 8 containing 4.5 M urea. Samples were dissolved in the same buffer containing $6.4 \cdot 10^{-5}$ M dithiothreitol (DTT). The different caseins were eluted using a linear gradient from 0 to 0.31 M NaCl as previously described [30, 31]. The peaks corresponding to the purified fractions were collected, dialyzed against water and lyophilized.

Purity of the four isolated caseins was assessed by HPLC using a Waters 600E system, a Waters 486 detector (Milford, Mass., USA) and a 300-Å C4-Vydac column (250×4.6 mm/Shandon France). The samples (about 1 mg of whole or purified caseins) were dissolved

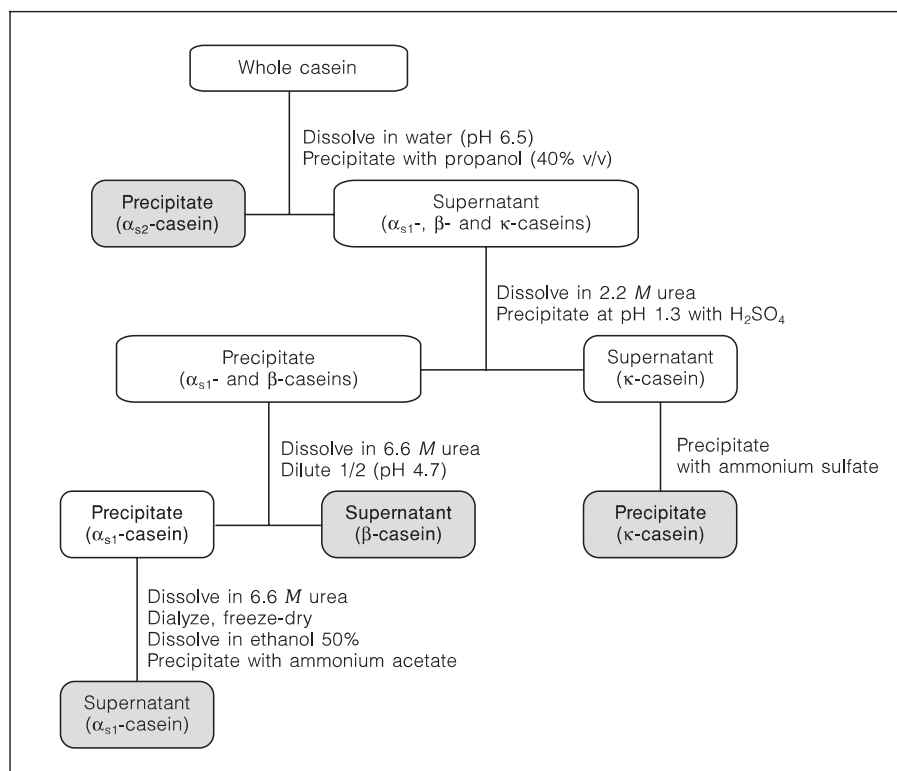


Fig. 1. Outline of the preparation of enriched fractions of the different caseins from the milk of one single cow homozygous for the variants B, A1, A, A of the respective α_{s1} -, β -, α_{s2} -, and κ -caseins.

(1 mg/ml) in 8 M urea containing 0.1% DTT. Each sample was diluted 4-fold in solvent A and was injected (200 μ l, corresponding to 50 μ g). Elution was performed using a 40 min linear gradient from 25 to 40% of solvent B (B: acetonitrile/TFA 0.04%) in solvent A (A: H₂O/TFA 0.1%) at a flow rate of 1 ml/min and was monitored at 220 nm.

Preparation of Enzyme Conjugate

Acetylcholinesterase (AChE, EC 3.1.1.7) was purified from the electric organs of electric eel *Electrophorus electricus* by affinity chromatography [32]. The tetrameric form of AChE (G₄ form) was used to label monoclonal antibodies (mAbs). The characteristics of this preparation have been described elsewhere [33, 34]. AChE activity was measured by the colorimetric method of Ellman [25].

Purified anti-rabbit IgG (mAb 173) [35] and antihuman IgE (mAb BS17) [36] mAbs, as well as polyclonal goat anti-guinea-pig antibodies were treated with pepsin in acidic medium to obtain F(ab')₂ fragments. Reduction of these fragments by β -mercaptoethylamine yielded Fab' fragments which were subsequently covalently coupled to AChE pretreated with N-succinimidyl-4-(N-maleimidomethyl)-cyclohexane-1-carboxylate as previously described [37].

Enzyme Immunoassays

Microtiter plates were coated with each of the purified caseins at a concentration of 5 μ g/ml in 25 mM EDTA solution pH 9.3 [24]. After 24 h incubation, plates were washed and saturated with EIA buffer. These immunoplates coated with purified caseins were then used to perform two different kinds of immunoassays.

In a first series of experiments, we checked the presence of any contamination in the different purified proteins. These tests involved

either rabbit or guinea pig polyclonal antibodies specific for each casein. Dilution curves were plotted to establish optimized concentrations of these antisera. Competitive inhibition assays were performed to characterize the four caseins. A portion of the optimal working dilution of each specific antiserum (i.e. dilution which gave less than 50% of maximal tracer binding) was preincubated for 4 h at 20°C with serial dilutions of each of the four caseins before addition (50 μ l) to the wells of the corresponding coated plates. After 18 h incubation at 20°C, plates were extensively washed with 0.01 M phosphate buffer pH 7.4 containing 0.05% Tween 20. Rabbit or guinea pig IgG bound to the solid phase were then revealed by addition of 50 μ l mAb 173-AChE conjugate or AChE-labeled (2.3 Ellman units/ml) polyclonal anti-guinea-pig antibodies for 4 h at 20°C. After washings, Ellman's reagent (200 μ l) was distributed into each well and the absorbance was measured at 414 nm. Classical competitive curves were plotted and the sensitivity of the assays was characterized by the concentration of the competitor inducing a 50% reduction in the binding observed in the absence of competitor (B/B₀ 50%). Protein contaminants were screened by comparing the sensitivities determined using as a competitor the same casein as that used for the coating and the three other caseins.

The second series of EIA experiments was carried out to screen the IgE response of the allergic patient sera to the different coated caseins. Procedure and quantitative determination of specific IgE were performed as previously described [19]. Briefly, after incubation of diluted sera (50 μ l/well) with coated casein, IgE bound to immunoplates were revealed by the addition of 50 μ l of BS17-AChE conjugate (3 Ellman units/ml). After a further incubation for 18 h at 20°C, plates were washed and Ellman's reagent (200 μ l) was added to reveal AChE

activity. Specific IgE were quantified as previously described [19, 36]. This immunometric assay proved to be totally specific for human IgE, presenting no cross-reactivity with other classes of immunoglobulin (especially IgG) and made possible the detection of specific IgE lower than 0.1 IU.

Competitive EIA of the different human sera was used to characterize the recognition of the different caseins by specific IgE. These IgE inhibition EIA were carried out by incubation of increasing concentrations of the different caseins (25 μ l in EIA buffer) with appropriately diluted immune sera (25 μ l) for 6 h at room temperature followed by incubation of these mixtures with immobilized whole bovine casein. Subsequent steps were as previously described for direct EIA.

Statistics

Due to the non-Gaussian distribution of the specific IgE levels, analysis was performed using nonparametric statistical methods (Friedman test and Spearman rank correlation test).

Results

Purification and Characterization of the Four Different Caseins

Each of the four different fractions recovered after the set of successive precipitations contained more than 80% of the desired casein, as observed during the subsequent FPLC purification (data not shown). The HPLC chromatograms of the purified κ -, α_{s2} -, α_{s1} - and β -caseins are shown in figures 2B–E, respectively. The HPLC profile of whole casein (fig. 2A) indicated that α_{s1} - and β -caseins are the most abundant. Each of the four caseins was well separated from the others under our chromatographic conditions and no significant contamination was observed. The chromatographic behavior of each of the four caseins appeared quite different: α_{s1} -casein corresponded to a single peak; α_{s2} - and β -caseins presented a second minor peak; κ -casein appeared as the most heterogeneous compound, corresponding to at least three different entities.

Competitive inhibition EIA tests performed with specific rabbit or guinea pig polyclonal antisera were used as an alternative approach to check the absence of residual cross-contamination of these different purified molecules. In each case, the binding of the specific antibodies to the corresponding immobilized antigen was totally inhibited by the homologous protein. On the other hand, no reduction in binding was observed with the three other proteins used as competitors, except for α_{s2} -casein which slightly inhibited the binding of specific antibodies to immobilized α_{s1} - and β -caseins. This could be due either to slight contamination or to minor cross-reactivity of the antisera with the other caseins. Maximum contamination of α_{s2} -casein by 4% β -casein and α_{s1} -casein was estimated.

Specific IgE Determination in Allergic Patient Sera

Of the selected 58 sera, IgE to whole casein fraction ranged from 0.3 to 1,030 IU/ml (mean 43.2 IU/ml and median 8.1 IU/ml) as shown in table 1. Each of the four caseins was recognized by almost all the sera, since anti- α_{s1} , anti- α_{s2} , anti- β and anti- κ -casein IgE antibodies were detected in 57, 55, 53 and 53 of the 58 sera, respectively. The presence of specific IgE against each of the four caseins was detected in 49 of the 58 sera. Interestingly, only a single case of monospecificity (recognizing only κ -casein) was observed. In all cases, detected IgE were specific to the coated purified casein since competitive experiments with the corresponding casein totally abolished the binding.

False-positive IgE determination was never observed, neither on control plates coated only with HSA using allergic patient sera nor on plates coated with specific antigens using nonallergic volunteer control sera.

Marked heterogeneity was observed in the intensity of the IgE response. The highest specific IgE response among the different sera appeared to be directed to α_{s1} -casein and the lowest to κ -casein, mean values of IgE titers against α_{s1} -, β -, and α_{s2} - and κ -casein being 20, 14.6, 13.7 and 7.6 IU/ml, respectively. As shown in figure 3, the distribution of the IgE titers against the four caseins in the different sera appeared significantly different. Indeed a large proportion of the sera (35–40%) exhibited low titers (ranging from 0.1 to 1 IU/ml) against α_{s2} (fig. 3B) and κ -casein (fig. 3A), but 50% of them contained more than 1.2 IU/ml of specific IgE to both allergens. By contrast, the IgE response to α_{s1} - and β -casein was more homogeneous (fig. 3C, D), i.e. similar percentages of sera presented low (<2.5 IU/ml), intermediate (2.5–10 IU/ml) or high (>20 IU/ml) IgE titers. Therefore, it may be calculated that half of the sera contained more than 2.7 IU/ml of anti- β -casein or more than 4.2 IU/ml of anti- α_{s1} -casein IgE. Accordingly, the statistical analysis indicates the following rank order of potency in inducing an allergic response: $\alpha_{s1} > \beta \gg \alpha_{s2} = \kappa$.

In the case of sera responding to at least three caseins (54 sera), correlation values between the responses to two different caseins were estimated by the Spearman rank correlation test. The highest value (0.72) was observed for the responses to both α_{s2} - and β -casein, while the IgE concentrations with the different caseins were always correlated. A similar statistical analysis also revealed a high correlation between the responses against the whole casein and the different purified fractions (the highest value being 0.87 for α_{s1} -casein).

We compared for the same serum the amplitude of the IgE responses to individual caseins with the response to the whole casein fraction. Additivity of the responses was ob-

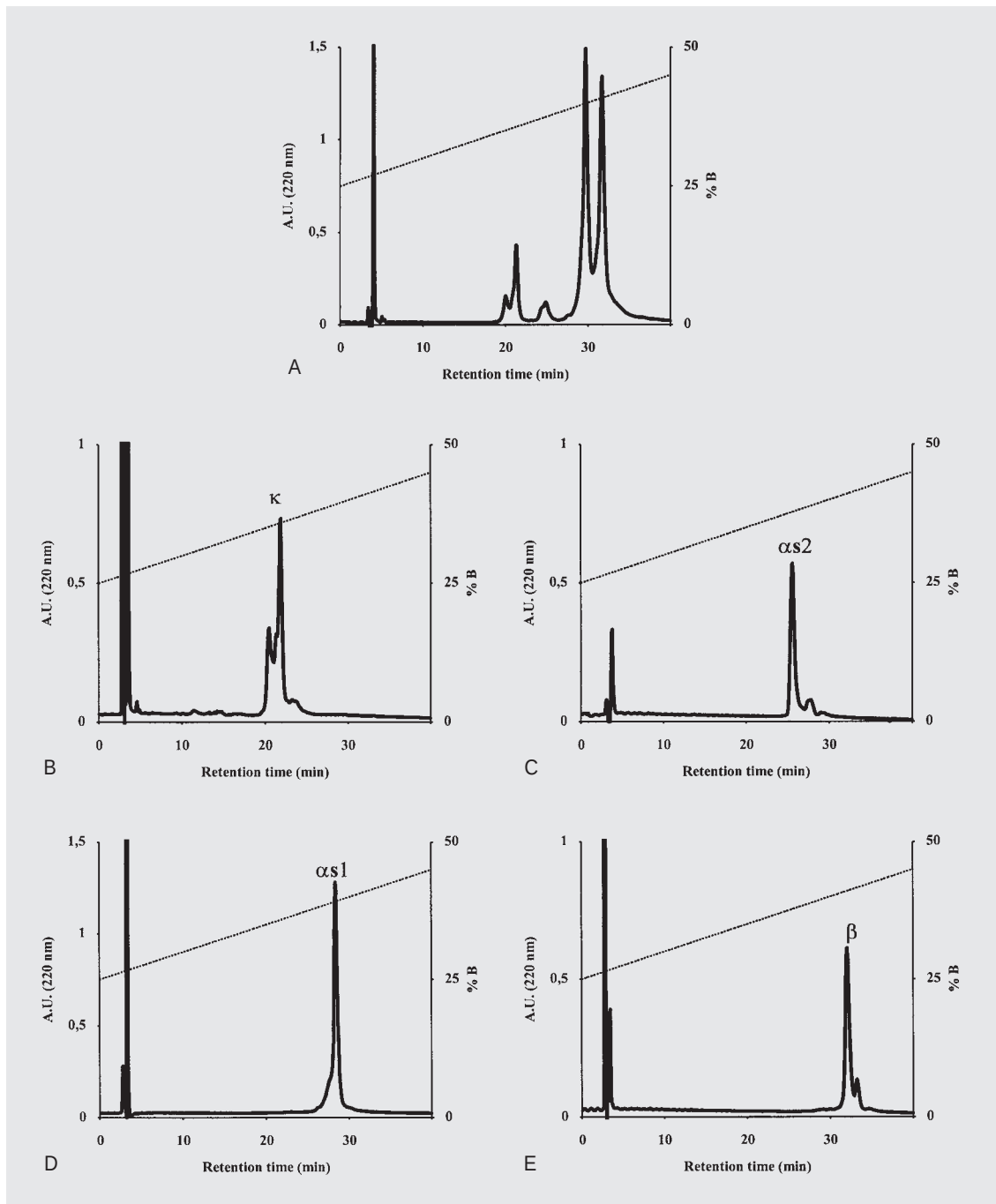


Fig. 2. RP-HPLC chromatograms of the different purified caseins: bovine whole casein (A), κ -casein (B), α_{s2} -casein (C), α_{s1} -casein (D) and β -casein (E).

Table 1. Specific anti-whole casein IgE in 58 sera of cow's milk-allergic patients

Serum No.	Specific anti-whole casein IgE, IU/ml	Serum No.	Specific anti-whole casein IgE, IU/ml
S2	2.7	S59	1,030.0
S3	5.2	S60	21.4
S6	107.0	S61	78.2
S8	8.2	S62	180.0
S9	13.2	S63	24.0
S13	5.8	S64	2.0
S15	49.0	S65	4.3
S16	141.0	S66	23.0
S17	14.4	S67	2.9
S18	35.0	S68	10.0
S21	14.5	S69	2.5
S22	17.6	S70	2.8
S24	2.1	S71	4.4
S25	22.4	S72	5.6
S30	2.0	S73	1.8
S31	25.7	S74	7.7
S32	155.1	S75	0.4
S34	23.1	S77	2.6
S35	10.7	S79	5.0
S36	1.0	S81	137.0
S39	8.1	S83	77.2
S48	16.5	S84	5.1
S52	2.3	S85	3.4
S53	40.7	S86	0.3
S54	54.5	S87	40.0
S55	21.4	S89	1.3
S56	7.0	S90	0.5
S57	2.7	S91	1.6
S58	2.0	S92	18.3

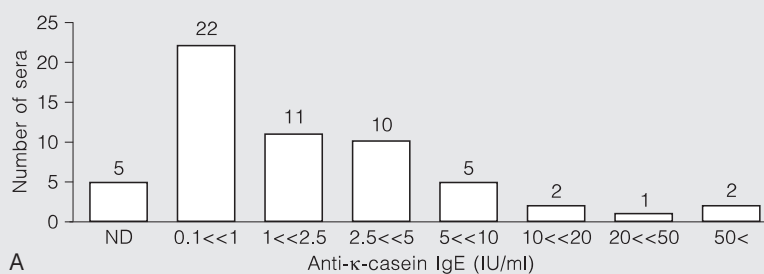
Table 2. Analysis of the specific IgE binding of some characteristic sera to the different immobilized caseins (expressed as % binding/immobilized whole casein)

Immobilized casein	Serum No.				
	S18	S16	S6	S9	S8
Whole casein	100	100	100	100	100
κ -Casein	1	4	31	61	12
α_{s2} -Casein	16	92	6	42	55
α_{s1} -Casein	99	20	59	78	98
β -Casein	5	16	62	51	74
Sum of the binding to the different caseins	121	132	158	232	239

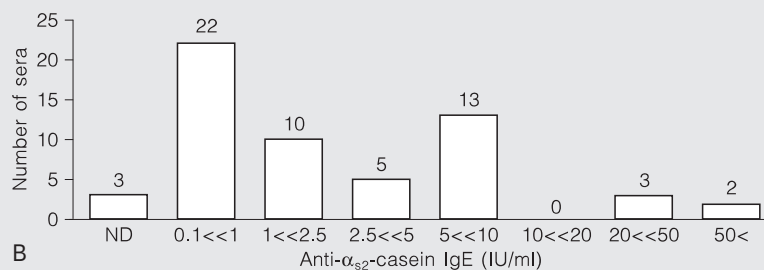
served for 34 of the 58 sera since the sum of IgE titers to the individual caseins roughly corresponded to the IgE titer measured against whole casein (S16 and S18 being representative of these sera; table 2). The IgE response was mainly directed against one type of casein (i.e. α_{s1} -casein for S18 and α_{s2} -casein for S16).

Unexpectedly, for the 24 other sera the sum of the individual IgE responses largely exceeded (sometimes by 2-fold or more) the IgE response to the whole casein (S6, S9 and S8 in table 2). There was a more homogeneous distribution of the different detected anti-casein IgE.

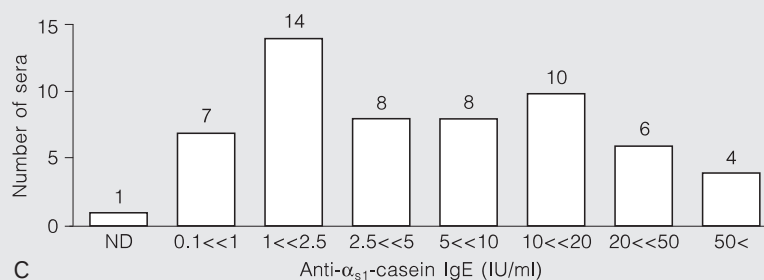
Competitive EIA experiments on whole casein-coated plates performed with these human sera preincubated with the different purified caseins corroborated this phenomenon. Inhibition was amplified in many sera since the total inhibition corresponding to the added effect of each of the different competitors (e.g. α_{s1} -, α_{s2} -, β - and κ -caseins) is then more than 2-fold higher than the inhibition due to the same amount of whole casein. A good correlation was ob-



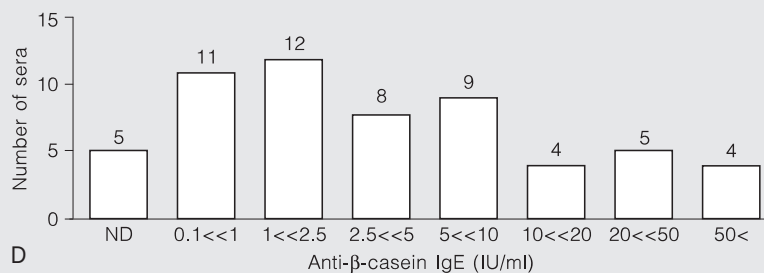
A



B



C



D

Fig. 3. Analysis of the quantitative distribution of the specific IgE responses to the four caseins using EIA: κ-casein (A), α_{s2}-casein (B), α_{s1}-casein (C), and β-casein (D). ND = <0.1 IU IgE/ml.

served between the two sets of experiments since sera exhibiting enhancement when tested with the different coated caseins generally displayed a greater inhibition when the four purified caseins were assayed against the coated whole casein.

Discussion

The analysis of the specificity of the IgE response to each type of casein requires their prior purification from the whole casein. After precipitation and anion exchange chromatography, the different types of casein were recovered in 'pure' form, as checked both by HPLC and immunoanalytical experiments. The chromatographic behavior of κ-casein, which appeared as multiple peaks, may be explained

by the various degrees of glycosylation and/or phosphorylation, or by a possible copurification of para- κ -casein, a protein resulting from the partial hydrolysis of κ -casein. The minor peak observed for β -casein probably also corresponds to a hydrolytic product of this compound, while in the case of α_{s2} -casein the minor peak likely resulted from aggregation (since after collection of the narrow peak, the same profile was exactly reproduced upon a repeat run under the same conditions). These results are in agreement with findings in the literature [38].

An immunological study using rabbit or guinea pig polyclonal antisera specific for each type of casein proved the absence of contamination, except for β - and α_{s1} -casein which may have been present in trace amounts (at most 4%) in the α_{s2} -casein fraction. These results demonstrated the recovery of highly purified individual caseins, allowing efficient analysis of the allergic immune response of human patients.

IgE screening of the allergic sera demonstrated the involvement of each of the four caseins, as previously reported [11] for a very limited number of sera. This indicates that each casein, even if poorly represented like α_{s2} - or κ -casein, could trigger an IgE response since most sera (>90%) contained specific anti- α_{s2} or anti- κ -casein IgE. Moreover, the frequency of positive responses was essentially the same for all types of caseins, which contrasts with a previous report that $\alpha_{s(1 \text{ and } 2)}$ - and κ -caseins might induce the major frequency of the allergenic response [12]. Instead, our results show that each type of casein could induce a marked IgE response. Interestingly, β -casein which is abundant in human milk and exhibits substantial homology with human counterpart can induce a high IgE response.

Statistical analysis of the specific IgE quantifications indicated the prominent role played by α_{s1} - or β -casein compared to α_{s2} - and κ -casein, as the targets of the antibodies present in the sera of allergic patients. Half of the 58 sera contained more than 4.2 IU/ml of anti- α_{s1} -casein antibodies, corresponding values for anti- β , anti- κ - and anti- α_{s2} antibodies being 2.7, 1.2 and 1.2 IU/ml, respectively. Interestingly, the relative proportions of these median values appear closely related to the concentrations of these four caseins in bovine milk (12, 9, 3 and 3 g/l, respectively). The intensity of the IgE immune response induced by individual casein seems to be directly dependent on its concentration in bovine milk.

Polysensitization was observed since 99% of the 58 allergic sera exhibited positive responses to more than one casein, while 88% contained IgE against each of the four types of caseins. Statistical analysis revealed in addition the existence of important correlations between the IgE re-

sponses to the different caseins. However, in all cases of polysensitization, our results do not discriminate between two possibilities: either the sera contain different specific antibodies, each reacting with one casein species, or they contain antibodies cross-reacting with different casein species (cross-reactive allergenicity), the two possibilities not being mutually exclusive.

The comparison of the IgE responses to the whole casein and to its different fractions strengthened the notion of interference between these two mechanisms. For some sera, the sum of the IgE titers against the different types of caseins corresponded to the IgE titer against the whole casein. In these sera, the different caseins were recognized by different and specific IgE populations, suggesting that IgE responses to individual caseins were independent. By contrast, for other sera, the sum of the IgE titers to the different caseins largely exceeded the IgE titer to whole casein, probably because of the presence in these sera of IgE antibodies cross-reacting with different caseins through common or closely related epitopes. Competitive EIA experiments strengthened this last hypothesis.

Interestingly, although three of them have a common ancestral origin [39, 40], bovine caseins have undergone marked evolution and present few sequence homologies. The most remarkable sequence is in the region containing the major site of phosphorylation. It is conserved in bovine α_{s1} - (sequence 66–70), α_{s2} - (sequences 8–12 and 56–60) and β -caseins (sequence 17–21), as well as in the caseins of other species. Hence, this sequence may play an important part in cross-reactivity, in particular it has already been described as immunoreactive [41], and resistant to digestive degradation [42–44]. Further studies will be necessary to analyze the allergenic role of this sequence and to define the major epitopes present on each casein.

However, whole casein represents a complex of four different components. The ultrastructural features displayed by whole casein involved several interactions leading to the formation of exposed regions for the different caseins. Nevertheless, during digestion this multicomponent structure undergoes physicochemical modifications induced by the different digestive media (bile salts, acidic gastric conditions) and by enzymatic cleavages. Such modifications could decrease immunogenicity and allergenicity of casein as discussed in the ESPACI document [45]. However these different processes might result in reorganization and disruption of whole casein. This would lead to exposure to the immune system of some zones initially inaccessible in whole casein. IgE subpopulations can then be produced against these sequences which are unable to find the corresponding epitopes in the whole casein. Purification of each

of the different caseins can lead at least to partial reorganization and modification of molecular folding, resulting in the exposure of regions hidden in whole casein. These newly exposed parts of the purified molecule could account for the recognition by some IgE which are unable to reach them in whole casein. A comparative study of IgE immunoreactivity against whole casein of different species is also currently undertaken to get a clearer insight into the species-specific differences recently described by Wüthrich and Johansson [46].

This information improves the understanding of the humoral response to milk proteins during allergy. It must also be taken into account when developing a casein-derived hypoallergenic formula since it now clearly appears that (1)

specific IgE are widely spread even to poorly represented casein or to casein having a high structural homology with the human counterpart, (2) cross-sensitization between the different caseins largely occurs and (3) hydrolysis of casein could lead to a hidden highly reactive epitope becoming available for IgE recognition.

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