

Allergenic epitopes of bovine α_{s1} -casein recognized by human IgE and IgG

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B-cell epitopes of bovine α_{s1} -casein, one of the major allergens of cow's milk, were identified by a screening method based on synthetic peptides. According to the known amino acid sequence of α_{s1} -casein, a set of 188 overlapping sequential decapeptides shifted by one amino acid was manually synthesized on polyethylene pins by the 9-fluorenylmethoxycarbonyl (Fmoc) method. Peptides were screened by an enzyme-linked immunosorbent assay (ELISA) specific for human IgE and IgG. Bound antibodies were detected by successive incubation with up to three polyclonal antibodies, the last one conjugated to horseradish peroxidase. Tested sera were from 15 patients with acute clinical reactions to cow's milk and IgE-specific reactions to bovine α -casein in the ELISA and immunoblot. Sera from 10 healthy subjects without remarkable reactions to cow's milk proteins were used as controls. All sera from allergic subjects showed reactions with three regions of α_{s1} -casein, corresponding to amino acids 19–30, 93–98, and 141–150. Furthermore, individual sera showed reactions with other parts of the protein. No essential differences in the epitope specificity of IgE and IgG were found. Inhibition of IgE binding to α_{s1} -casein with soluble synthetic peptides confirmed the results and revealed peptide CN-2 as the most inhibiting one.

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Cow's milk allergy (CMA) has a prevalence of about 0.3–7.5% in infants (1, 2). The major allergens in cow's milk are α_{s1} -casein and the whey protein β -lactoglobulin, both being major proteins of bovine milk, but virtually absent from human milk (3, 4).

Bovine α_{s1} -casein is a single-chain phosphoprotein of 199 amino acids, with 17 proline residues, uniformly distributed over the molecule, and a high content (45%) of hydrophobic residues (5). As a consequence of the high number of proline and hydrophobic residues, it exhibits not a defined tertiary structure but a so-called random coil conformation, stabilized by hydrophobic interactions (6, 7). Bovine α_{s1} -casein is an acidic protein, isoelectric point 4.1–4.5 (8), with up to eight phosphorylated serine residues and a highly negatively charged region including residues 41–77 (9). The lack of a defined tertiary structure suggests that α_{s1} -casein mainly contains sequential epitopes and only a few conformational epitopes. For this reason, predictions of antigenic regions, based on criteria such as hydrophilicity, surface probability, and chain flexibility are not very useful in this case.

Information about the epitopes of a protein can be obtained by many different methods. The classical way is to test the antigenic properties of peptides resulting from enzymatic or chemical fragmentation of the protein. This approach was applied to bovine α_{s1} -casein by Otani et al. (10–12) with rabbit and human sera. The method is restricted by the distribution of cleavage sites within the protein sequence. The size of the peptides is given by the chosen cleavage method, and the risk of destroying epitopes by cleavage is relatively high. Another means of epitope mapping is the immunologic testing of synthetic peptides according to the protein sequence. This procedure was also applied to bovine α_{s1} -casein (13–15) with mouse sera and cultures of lymphocytes. The validity of this method depends on the size of the peptides used.

A modified method for epitope mapping with synthetic peptides was developed by Geysen et al. (16). This approach is based on a set of overlapping sequential peptides synthesized onto a solid support, which can be directly used for ELISA. This epitope scanning method allows us to screen high numbers of small peptides and to identify epitopes with the

resolution of a single amino acid. Here we report the identification of IgE- and IgG-specific B-cell epitopes of bovine α_{s1} -casein by this scanning method. A set of overlapping decapeptides shifted by one amino acid according to the known amino acid sequence of bovine α_{s1} -casein was used to detect epitopes binding human IgE and IgG antibodies from sera of patients with CMA.

Material and methods

Purification of caseins

Commercially available bovine casein (C7891, Sigma) was further purified by DEAE anion exchange chromatography by means of the High-Load System (Pharmacia). An amount of 300 mg casein was dissolved in 4.0 ml 20 mmol Tris/HCl, pH 7.0; 4 mol urea; and 10 mmol 2-mercaptoethanol (buffer A) and applied onto a DEAE Sepharose FastFlow column (1.6 × 11.2 cm, Pharmacia) equilibrated with the same buffer. Caseins were eluted at a flow rate of 1.0 ml/min with a gradient of 0–350 mmol NaCl (in buffer A) in 100 min, followed by a 20-min wash with 350 mmol NaCl, a second gradient of 350–500 mmol NaCl in 10 min, and a final 20-min wash with 500 mmol NaCl. Eluted proteins were detected by absorption at 280 nm (Uvicord SII, Pharmacia). Pooled fractions were desalted (ultrafiltration cell, YM-10 membrane, Amicon) and lyophilized. The purity and identity of caseins were checked by SDS-PAGE (17) and by N-terminal amino acid sequencing with a gas-phase sequencer (Protein Sequencer 477A/120A, Applied Biosystems).

IgE ELISA

Microtiter plates (medium capacity, Greiner, Germany) were coated with purified bovine α -casein (2 μ g/ml, 50 μ l/well) in 0.1 mol carbonate buffer, pH 9.6, at 4°C overnight. Subsequently, the plates were washed three times with phosphate-buffered saline (PBS), pH 7.2, 0.05% Tween-20 (PBS-Tween) containing 3% fish gelatin (blocking buffer). The plates were then incubated with 50 μ l/well human sera (diluted 1:25–1:1600 with blocking buffer) at 4°C overnight and washed three times with PBS-Tween. They were then successively incubated with rabbit antihuman IgE (1:200, 50 μ l/well, 4°C, 2 h), swine antirabbit Ig (1:200, 50 μ l/well, 4°C, 1.5 h), and rabbit anti-swine Ig conjugated with horseradish peroxidase (1:200, 50 μ l/well, 4°C, 1.5 h). Between incubations, the plates were washed three times with PBS-Tween. All antibodies were from Dako Diagnostika, Germany. Finally, the plates were incubated with 50 μ l/well of 10 mg 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS, Sigma) and 5 μ l 30% H₂O₂ in

20 ml 0.1 mol citrate-phosphate buffer, pH 5.0, for 20 min at room temperature. Absorption was measured at 405 nm with a microplate reader (Titertek Multiscan MCC/340, Flow).

Inhibition ELISA

Native α -casein and seven synthetic polypeptides (Biometra, Germany, and Abimed, Germany) corresponding to immunologic reactive and nonreactive regions of the protein were tested for inhibitory capacity. Microtiter plates were coated with bovine α -casein (2 μ g/ml, 50 μ l/well) in 0.1 mol carbonate buffer, pH 9.6, at 4°C overnight and then washed three times with blocking buffer. Synthetic peptides or bovine α -casein were dissolved in blocking buffer at a concentration of 40 μ g/ml. Subsequently, 60 μ l peptide solution and 60 μ l diluted patients' serum (1:50 in blocking buffer) were mixed and incubated 2 h at room temperature. Duplicates of 50 μ l/well were applied onto the plates and then incubated at 4°C overnight. Bound IgE antibodies were detected as described above. Finally, inhibition was calculated as follows:

$$\text{Inhibition (\%)} = \frac{(\text{control} - \text{test}) \times 100\%}{\text{control}}$$

Immunoblot

A mixture of bovine milk proteins (α -, β -, and κ -casein, β -lactoglobulin, α -lactalbumin, and serum albumin, 2.5 μ g each) was separated by vertical SDS-PAGE (80 × 70 × 0.7 mm, 15% T, 2.5% C) according to Laemmli & Favre (17). After electrophoresis, proteins were blotted onto PVDF membrane (Immobilon P, Millipore) by the Novablot system (Pharmacia). After blotting, membranes were incubated with blocking buffer overnight. Blocked membranes were mounted into a Miniprotean II Multiscreen (BioRad), and then 600 μ l human serum dilution (1:200 in blocking buffer) per slot was applied and incubated at 4°C overnight. For detection of bound IgG, blots were incubated with rabbit antihuman IgG labeled with horseradish peroxidase (1:200, 5.0 ml, 4°C, 2 h). Bound IgE was detected by successive incubation with rabbit antihuman IgE (1:200, 5.0 ml, 4°C, 2 h), swine antirabbit Ig (1:200, 5.0 ml, 4°C, 1.5 h), and rabbit anti-swine Ig conjugated with horseradish peroxidase (1:200, 5.0 ml, 4°C, 1.5 h). Between incubations, membranes were washed three times with 20 ml PBS-Tween. Bound antibodies were visualized by incubation with 10 mg 3,3'-diaminobenzidine and 10 μ l 30% H₂O₂ in 20 ml 50 mmol Tris/HCl, pH 7.3, for 5–10 min.

Epitope scanning

Preparation of pins. The Epitope Scanning Kit was obtained from Cambridge Research Biochemicals (Northwich, Cheshire, UK) and was used according to the manufacturer's instructions. The kit is based on the epitope-scanning method developed by Geysen et al. (16). The surface of inert polyethylene pins was activated by coupling spacer groups carrying a terminal amino group. 9-Fluorenylmethyloxycarbonyl (Fmoc) protected amino acid pentafluorophenylesters could be linked to the amino group of the spacer according to the Fmoc chemistry (18). Peptides were synthesized by sequential addition of the appropriate Fmoc amino acid at a time. The deprotected terminal amino group of the completely synthesized peptide was acetylated to remove the positive charge of the free amino group. Finally, the protecting groups of the side-chains were removed by treatment with trifluoroacetic acid. A set of 188 decapeptides shifted by one amino acid was synthesized on polyethylene pins according to the known amino acid sequence of bovine α_{s1} -casein (Swiss-Prot database, sw:cas1-bovin). The first peptide started with the second amino acid of the α_{s1} -casein sequence, and the last peptide ended with the last but one amino acid. The set of immobilized peptides was used to probe human sera from patients with CMA and from nonallergic subjects.

Pin ELISA. Specific binding of IgG or IgE to the immobilized peptides on the pins was measured by the ELISA described above, which was modified for this purpose. All incubations of the pins with antibodies or reagents were performed in flat-bottom microtiter plates (Greiner, Germany). Pins were blocked with 250 μ l/well 10% fish gelatin in PBS-Tween for 6 h with agitation at room temperature, and then washed three times with 200 ml PBS-Tween for 5 min each time. The blocked pins were incubated with human serum (diluted 1:200 in blocking buffer, 200 μ l/well, 4°C, overnight). In the IgE pin ELISA, bound IgE was detected by successive incubation with rabbit antihuman IgE (1:500, 200 μ l/well, 4°C, 2 h), swine antirabbit Ig (1:500, 200 μ l/well, 4°C, 2 h), and rabbit antiswine Ig conjugated with horseradish peroxidase (1:500, 200 μ l/well, 4°C, 2 h). In the IgG pin ELISA, blocked pins were incubated with horseradish peroxidase-labeled rabbit antihuman IgG (1:500, 200 μ l/well, 4°C, 2 h) to detect bound IgG. Between all incubations, pins were washed three times with 250 ml PBS-Tween. Finally, the pins were incubated with 200 μ l/well of 20 mg ABTS and 10 μ l 30% H₂O₂ in 40 ml 0.1 mol citrate-phosphate buffer, pH 5.0, for 30 min at room temperature. Absorption

was measured at 405 nm with a microplate reader (Titertek Multiscan MCC/340, Flow). The pins were reused after stripping off the bound antibodies, according to the manual of the kit.

Patients

In this study, sera from 15 patients with CMA, 12 male and three female, aged between 4 months and 30 years (median 21 months), were tested. All patients had a history of anaphylactic or immediate reactions to cow's milk, such as angioedema, bronchospasm, and urticaria. They all had a positive oral provocation test with cow's milk and a positive skin prick test with purified bovine casein, 10/15 had high levels of specific IgE to casein (f78, class >2) determined by the CAP RAST system (Pharmacia), and all showed IgE-specific reactions to α -casein in the ELISA and the immunoblot. Control sera were obtained from seven male and three female healthy subjects, aged 10 months to 13 years (median 16.5 months), who had no history of atopy or allergy to cow's milk and immeasurably low levels of specific IgE to bovine casein (RAST class 0). They showed negligible reactions to α -casein in the IgE ELISA and the IgE immunoblot. Both groups of subjects were not age-matched.

Hydrophilicity plot

A hydrophilicity plot of bovine α_{s1} -casein was calculated by the program PEPTIDSTRUCTURE of the HUSAR program package at the German Cancer Research Center, Heidelberg. The hydrophilicity was calculated according to the algorithm of Hopp & Woods (19) with a window of seven residues.

Results

Sera from patients with CMA and from nonallergic subjects were tested in the IgE ELISA and the immunoblot for their reactions with bovine α -casein. Fifteen patients' sera with positive results in the IgE ELISA (>0.2 OD) and distinct reactions to α -casein in the IgE immunoblot (Fig. 1) were used for epitope scanning. Most sera from patients with CMA showed reactions to several milk proteins in the immunoblot specific for IgG and IgE, respectively. Sera from nonallergic subjects showed no remarkable reactions to α -casein, in either the IgG or the IgE immunoblot, and no or only weak reactions to other milk proteins.

In all pin ELISA scans, the background of about 0.3–0.5 absorption units was very high, rendering the identification of the epitopes difficult. An absorption value higher than the mean absorption of the scan plus one standard deviation was taken

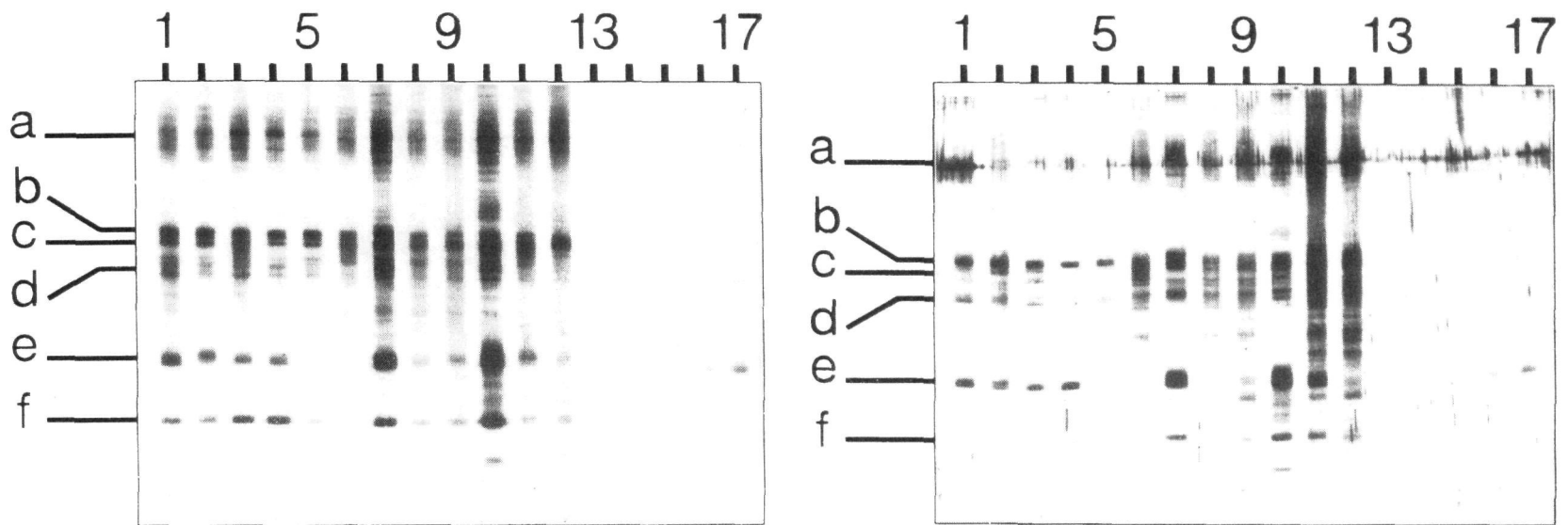


Fig. 1. IgG (left) and IgE immunoblot (right) of various cow's milk proteins; a) serum albumin, b) α -caseins, c) β -casein, d) κ -casein, e) β -lactoglobulin, f) α -lactalbumin. Lanes 1–12: sera from patients with CMA; lanes 13–17: sera from controls without CMA. Diffuse band below a) in IgE blot is artifact caused by impurity of reagents.

as a criterion for positive rating. A comparison of all scans (Fig. 2) revealed three groups of pins showing high reactivity with all sera from patients with CMA. These groups correspond to amino acids 19–30, 93–98, and 141–150 of the bovine α_{S1} -casein sequence (Fig. 3), thus comprising many unpolar and aromatic amino acids. Furthermore, individual sera showed positive reactions with other regions of the α_{S1} -casein; e.g., the adjacent regions of the sequences mentioned above or the amino acids 50–70, 125–134, 172–183, and 189–198. In contrast to the commonly recognized sequences, these regions contain a high number of polar and charged amino acids. The results were compared with scans of 10 sera from nonallergic subjects, which had shown no remarkable reactions in the ELISA and the immunoblot. The scans of this group gave essentially the same results as the scans of the patients, but the reactions were less intense.

Six sera from the patients with CMA and six from the healthy controls were tested in the IgG pin ELISA. A comparison of the IgE and IgG pin

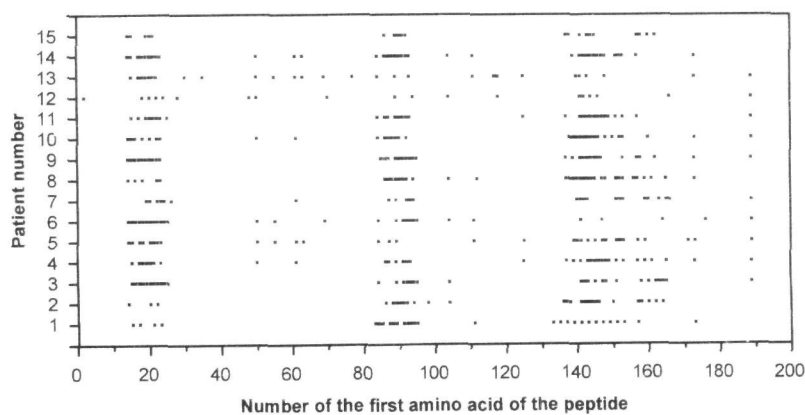


Fig. 2. Comparison of results of IgE pin ELISA with sera from patients with CMA. Dots mark peptide pins which gave absorption greater than mean absorption of ELISA plus one standard deviation. Pins are labeled by number of first amino acid of decapeptide according to amino acid sequence.

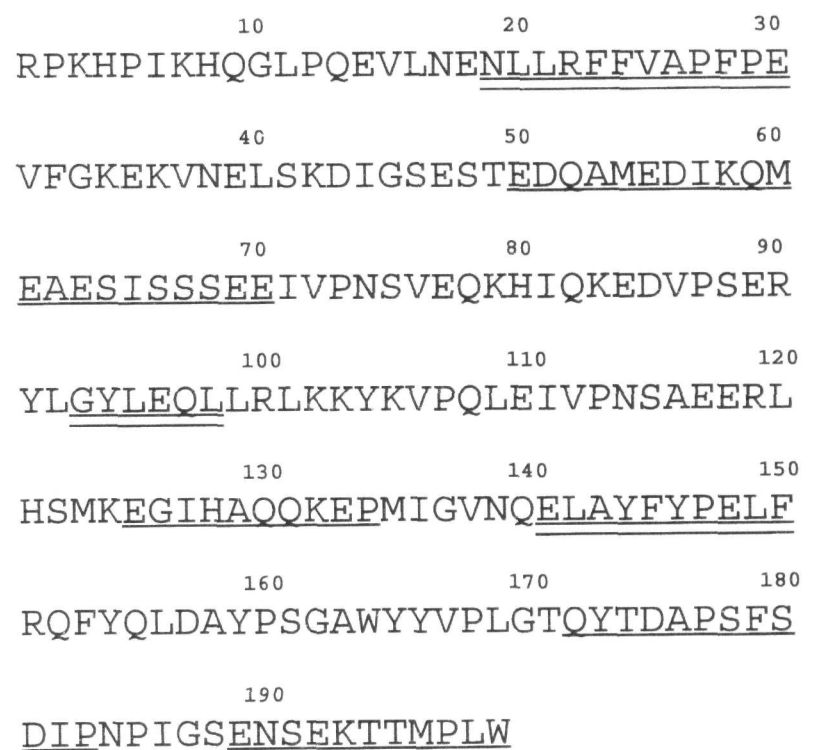


Fig. 3. Amino acid sequence of bovine α_{S1} -casein (Swiss-Prot database, sw:cas1_bovin). Detected major epitopes are double underlined; minor epitopes are single underlined. Signal peptide was omitted.

ELISA scans (Fig. 4) showed that the epitopes recognized by IgG were essentially the same as the IgE-specific epitopes. No significant differences in the antibody binding to the major epitopes could be observed. In a few cases, the binding of IgG to some minor epitopes, e.g., to the carboxy-terminal peptide or amino acids 50–70, was reduced compared to the IgE binding.

From one child with CMA, a first serum was available at the age of 10 months and a second at 2 years. The scans with these sera (Fig. 5) showed a remarkable increase of the reactivity to the identical epitopes with age, in agreement with an increase of the values in the IgE ELISA and the CAP RAST.

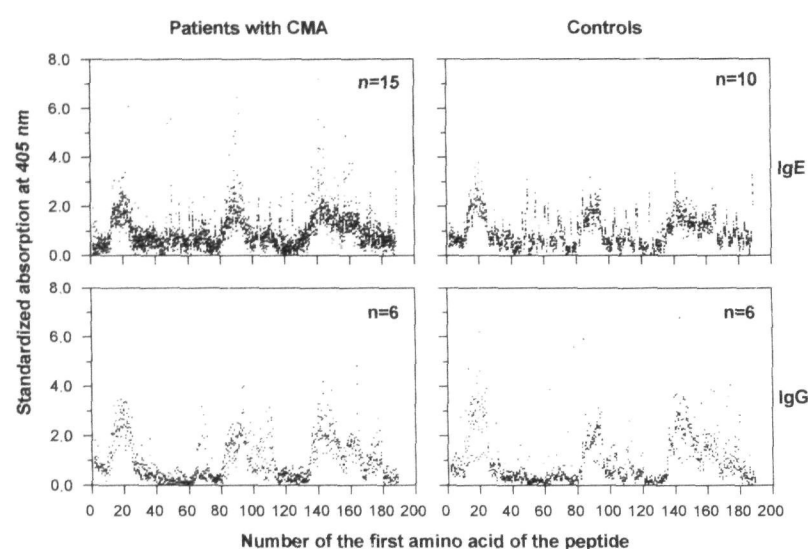


Fig. 4. Overlays of IgE and IgG pin ELISA scans of patients with CMA and of controls. Top: IgE scans of patients (left) and controls (right); bottom: IgG scans of patients (left) and controls (right). For comparison, measured absorption values of each scan were standardized by subtracting background and dividing by mean absorption.

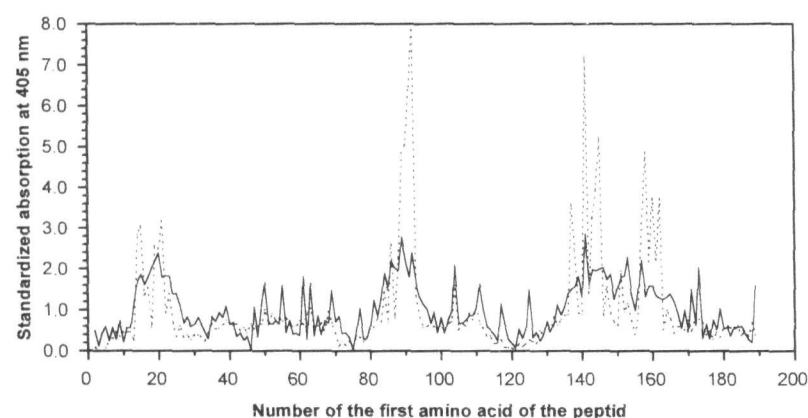


Fig. 5. IgE pin ELISA scan of boy with CMA at two different ages. Solid line indicates the scan of 10-month-old child; dashed line scan at age of 2 years. Measured absorption values of both scans were standardized by subtracting background and dividing by mean absorption.

Inhibition of IgE binding to α -casein by synthetic peptides (Table 1 and Fig. 6) was tested with 12 sera from patients with CMA. Peptide CN-2 inhibited IgE binding of all patients' sera, and peptide CN-3 of all but one sera. All other peptides showed inhibition of IgE binding of only some sera.

Table 1. ELISA inhibition of IgE binding to α -casein by synthetic peptides and their amino acid sequences

Peptide	Amino acids	Sequence	% Inhibition ^a
CN-1	20–31	LLRFFVAPFPEV	6.7 (0.0/39.6)
CN-2	86–103	VPSERYLGYLEQLRLKK	19.0 (2.8/42.2)
CN-3	141–150	ELAYFYPELF	8.2 (0.0/19.7)
CN-4	1–10	RPKHPIKHQG	4.9 (0.0/14.4)
CN-5	34–45	KEKVNELSKDIG	4.8 (0.0/18.2)
CN-6	58–73	KQMEAESISSSEEIVP	2.9 (0.0/15.0)
CN-7	188–199	SENSEKTTMPLW	6.8 (0.0/27.7)
α _{S1} -casein	1–199	—	96.7 (90.9/99.2)

^a Mean inhibition; in parentheses, minimum and maximum values.

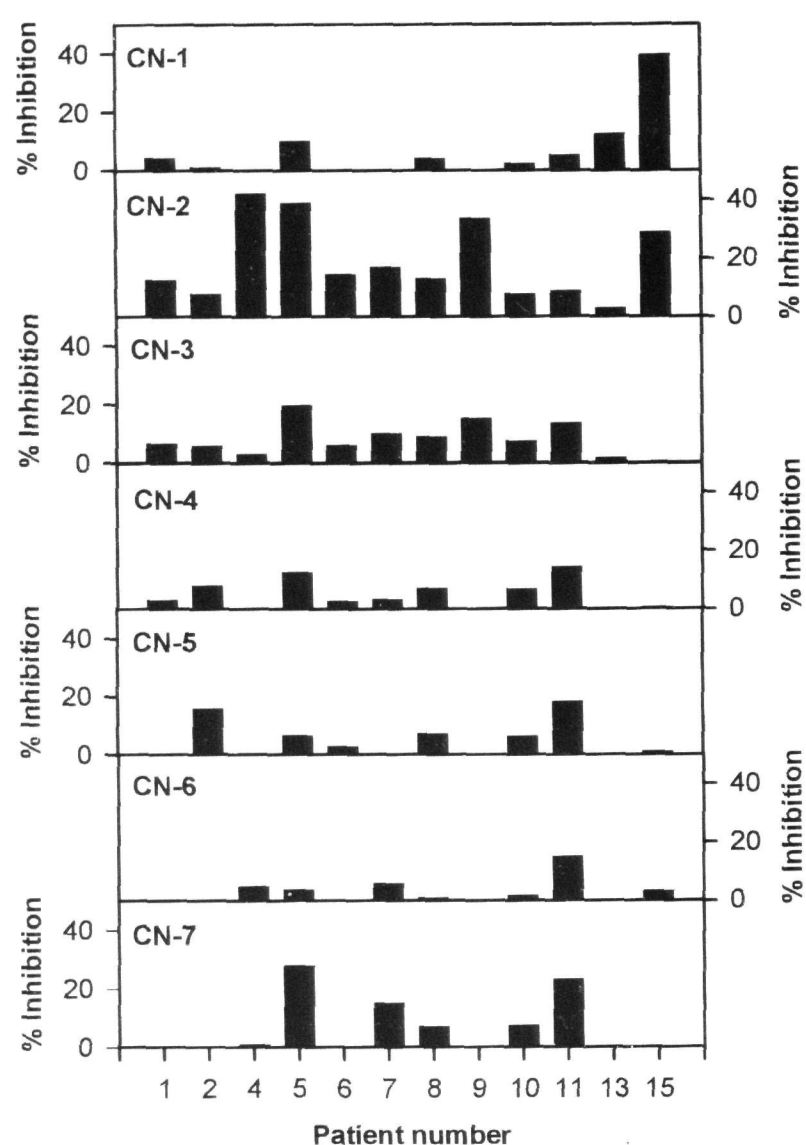


Fig. 6. ELISA inhibition of IgE binding of α _{S1}-casein by synthetic peptides tested with 12 sera from patients with CMA. Numbers of patients are identical with numbers of Fig. 2.

Discussion

All patients with CMA selected for epitope scanning showed positive results in the IgE ELISA to α -casein and a distinct IgE-specific reaction to α _{S1}-casein in the immunoblot (Fig. 1). The immunoblot is only a qualitative criterion, but it allows us to distinguish between reactions to α _{S1}-casein and those to other caseins and milk proteins. The control sera were from subjects with no clinical history of food allergy and atopy, low levels of total IgE (RAST class 0), and no IgE-specific reactions to cow's milk proteins in the immunoblot or the ELISA (<0.1 OD).

The immunoblots (Fig. 1) of cow's milk proteins revealed that sera from nonallergic controls (lanes 13–17) contained no remarkable amounts of specific antibodies compared with sera from patients with CMA. Surprisingly, no considerable differences between the IgE and IgG immunoblot were found; only individual small differences were observed. For example, the sera of lanes 7 and 10 showed distinctly higher reactivity in the IgG blot, especially to κ -casein and α -lactalbumin. In contrast, the sera in lanes 11 and 12 gave stronger reactions in the IgE

blot than in the IgG blot. These differences in the reactions do not correlate with the severity of CMA. Most patients with CMA showed in the IgE blot strong reactions to α -casein and β -lactoglobulin and only weak reactions to α -lactalbumin and to serum albumin.

The background absorption of all IgE and IgG pin ELISA scans was very high compared to the background in the IgE or IgG ELISA (0.03–0.04 OD). However, the polyclonal antibodies used for detection were the same in ELISA and pin ELISA. Moreover, unspecific binding of human IgG and IgE to the fish gelatin used as blocking agent is unlikely since sera were diluted with blocking buffer for both tests. Therefore, the reason for the high background might be the extremely high peptide loading of about 50 μ g peptide/pin (20). Because of this high antigen loading, inhibition of IgE binding by competing IgG antibodies recognizing the same epitopes can be excluded. Compared with the concentration of total IgG in the serum dilution (approx. 100 pmol/well), the peptide loading of about 50 nmol/pin represents a 500-fold molar excess of peptide. The excess over α_{s1} -casein-specific IgG is considerably higher; thus, IgE and IgG antibodies do not compete for binding sites.

The comparison of all scans with patient sera (Fig. 2) turned out two groups of peptides: peptides reacting with all sera of patients with CMA and peptides interacting with only a few of them. Due to the binding prevalence to the first group of peptides, these regions of the protein represent the so-called major epitopes. They are characterized by a high content of nonpolar and aromatic amino acids (Fig. 3) and are located in hydrophobic regions of the protein, shown by the hydrophilicity plot of α_{s1} -casein (Fig. 7). In the random-coiled α_{s1} -casein or the micelles formed by caseins in milk, the major epitopes are probably buried in the

hydrophobic interior of the molecule or micelle and thus are not directly accessible to antibodies. Therefore, it seems likely that these epitopes are only accessible to the immune system after the disruption of the casein micelles or denaturation and digestion of the casein molecules.

In contrast, the peptides of the second group corresponding to the minor epitopes are characterized by a high content of polar and charged amino acids (Fig. 3) and are located in hydrophilic regions of the protein (Fig. 7). One of these epitopes (amino acids 50–70) is part of the highly negatively charged region of α_{s1} -casein and contains four phosphorylation sites, which were not phosphorylated in the synthetic peptides on the pins. The minor epitopes might be exposed on the surface of α_{s1} -casein molecules or casein micelles because of their hydrophilic character. A more detailed discussion of the accessibility of the epitopes to antibodies would be highly speculative, since reliable information about the folding of the α_{s1} -casein polypeptide chain is still lacking.

In the IgE pin ELISA, the sera of the healthy controls showed the same reaction pattern as the sera of patients with CMA but with slightly lower intensities (Fig. 4). In contrast, their reactivity in the IgE ELISA was significantly lower compared with patient sera, but a direct comparison of both ELISA methods is critical since the IgE pin ELISA is a more qualitative method and a quantitative comparison of sera is difficult. In the pin ELISA, the competition between IgE and IgG is strongly reduced due to the high antigen loading of the pins (approx. 50 nmol/pin). On the other hand, in the IgE ELISA, the antibodies compete for the limited amounts of antigen (approx. 4.2 pmol/well).

The inhibition experiments revealed peptides CN-1, CN-2, and CN-3 as the most inhibiting peptides (Table 1), but only peptide CN-2, which gave the highest inhibition (mean 19.0%, maximum 42.2%), showed inhibition with all sera (Fig. 6). Peptides CN-1 and CN-3 inhibited the IgE binding of 7/12 and of 11/12 sera, respectively. These three peptides correspond to the detected major epitopes showing reactions with all patients' sera in the IgE pin ELISA. Peptides CN-4, CN-5 and CN-6 inhibited IgE binding of only a few sera and gave lower inhibition values, a finding which is in fairly good agreement with the results of the IgE pin ELISA. These peptides represent minor epitopes or non-reactive regions of the α_{s1} -casein. Peptide CN-7, which is identical with the carboxy-terminus of the protein, revealed high inhibition capacities with some sera and no inhibition with others. This behavior correlates very well with the reactivity of the corresponding pins in the IgE pin ELISA.

Previous research on the epitopes of bovine α_{s1} -casein used relatively large peptides of the protein.

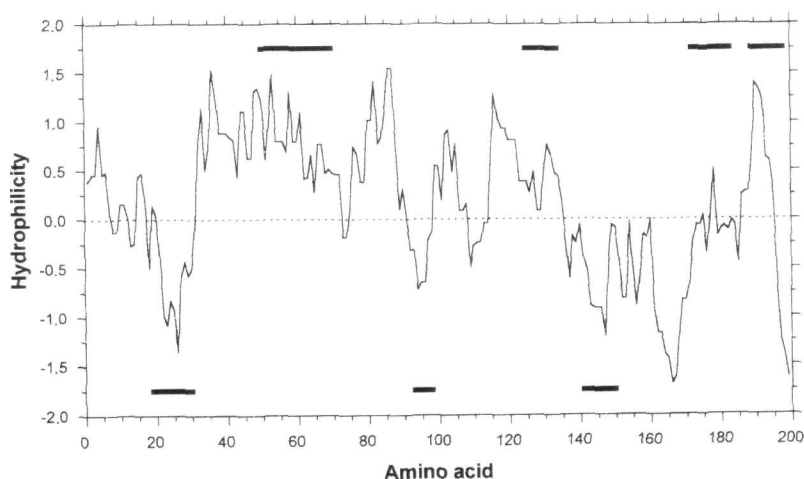


Fig. 7. Hydrophilicity plot of bovine α_{s1} -casein calculated according to algorithm of Hopp & Woods (19). Window was set to seven amino acid residues. Positive values indicate hydrophilic regions. Bars at bottom of diagram mark major epitopes, and bars at top minor epitopes.

Ametani et al. (13) and Otani et al. (10–12) worked with enzymatically or chemically cleaved α_{s1} -casein. The resulting peptides were often very long; therefore, localization of the epitopes was difficult. In some cases, the epitopes were destroyed during cleavage, especially by cleaving with chymotrypsin, which is specific for hydrophobic residues. The results of Enomoto et al. (14) and Kim et al. (15) are partially in good agreement with our results, but differ remarkably in some points. For example, they found high antibody titers and T-cell stimulation with peptide 91–110 which correlates to the high reactive major epitope of amino acids 93–98. On the other hand, they found no antibody reactivity to peptide 16–30, which includes the major epitope 19–30 of our studies. The reasons for these discrepancies might be due to the fact that we used sera from patients with CMA and not sera from immunized mice.

Comparison of the scans with serum from a boy at two different ages (Fig. 5) showed a striking increase in reactivity to the major epitopes with age, correlating with an increase of the values in the IgE ELISA (from 0.47 to 0.74 OD). Also the reaction was more specific at the age of 2 years than at the age of 10 months. Only a limited number of pins, corresponding to the major epitopes, showed an increase in IgE binding, while the reactivity of the other pins was not remarkably altered. The immune response changed from a relatively unspecific to a highly selective reaction to α_{s1} -casein-derived peptides, demonstrating clearly the maturation of the B-cell immune response to antigens with age. This phenomenon was a single observation which had to be tested on other patients.

The immunoblots showed clearly that the amount of α_{s1} -casein-specific antibodies differs remarkably between the group of patients with CMA and the group of healthy controls. However, the IgE and IgG pin ELISA scans revealed no differences between both groups in the pattern of epitopes recognized by IgE and IgG. All the B-cell specific reactions discussed in this paper cannot explain the different clinical reactions of subjects with and without CMA. Therefore, the reactivity of T-cells to peptides derived from α_{s1} -casein has to be analyzed and compared with the reported findings to elucidate allergy to food antigens such as cow's milk.

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