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Allergy to Bovine β -Lactoglobulin: Specificity of Human IgE Using Cyanogen Bromide-Derived Peptides

Abstract

Background: Bovine β -Lactoglobulin (Blg) is a major allergen involved in allergy to cow's milk proteins. Hydrolyzing Blg did not totally suppress its allergenicity; moreover its immunoreactivity may be increased. The aim of this work was to evaluate the specificity of serum IgE to different fragments of Blg in a group of 19 individuals allergic to cow's milk. **Methods:** This study was performed using both direct and competitive inhibition ELISA involving immobilized native protein or peptides derived from Blg cyanogen bromide cleavage. **Results:** Analyses of responses to each peptide revealed a large number of epitopes recognized by specific IgE of human allergic sera. However, there were differences in the specific determinants recognized, depending on the serum. Generally, peptides (25–107) and (108–145) retained substantial proportions of the immunoreactivity of the whole protein. Two other peptides, i.e. (8–24) and (146–162), were less recognized but were not inert. **Conclusion:** The main conclusion is that many epitopes were identified all along the Blg sequence by specific anti-Blg IgE from allergic humans.

Introduction

The prevalence of adverse reactions to food, including essentially food allergy, has been estimated by several studies. Mean figures range from 1 to 2.5% of the adult population [1, 2] and 8% of children under 3 years of age [3]. Milk is a basic food component and plays a prominent role in the first years of life. However, milk allergy is a common adverse food reaction since it occurs in 2–3% of children under 2 years of age [4, 5] and even in 1% of the adults [6]. It is related to protein constituents. The most sensitizing proteins are: bovine β -lactoglobulin (Blg), caseins, α -lactalbumin and serum albumin which are recognized by serum IgE in 62–80, 60, 53 and 52% of patients allergic to cow's milk, respectively [7, 8].

Blg is a major whey protein in the milk of ruminants and some non ruminants; it has no homologous counterpart in

human milk. It is dimeric at physiological pH; the ruminant Blg monomer is a globular protein of 162 amino acids and a molecular weight of 18 kD; it contains 5 cysteine residues, 4 of which are involved in forming intrachain disulfide bridges [9]. There are two main isoforms of Blg, i.e. genetic variants A and B which only differ by two point mutations on residues 64 and 118 (D⁶⁴ and V¹¹⁸ vs. G⁶⁴ and A¹¹⁸ for variant A and B, respectively). Blg belongs to the lipocalin family and is considered as a retinol-binding protein [10]. The tertiary structure of these proteins is very similar and has a very high affinity for hydrophobic ligands. Crystallography has revealed very similar folding, called β -barrel structure, with the arrangements of 8 antiparallel β -sheets in spite of the mutations that have occurred during evolution [11]. This kind of molecule has a high allergenic potential and several allergens of animal origin belong to this family, including the major mouse urinary proteins

(mMUP), the major horse allergen Equ c 1 and a major allergen from cockroach, Bla g 4, which also has a very close structure homology [12].

Although the physicochemical properties of Blg are known, its biological function remains unclearly established. Its relative resistance to acid hydrolysis as well as to proteases allows some of the protein to remain intact after gastric digestion [13]. This increases the probability that intact Blg as well as digested fragments will be absorbed as antigens [14]. Indeed, Blg, from ingested cow's milk, can be detected in human milk [15]. Jackobsson et al. [16] demonstrated that milk from three mothers, whose children suffered from infantile colic, contained high amounts of Blg. The colic abated in all cases when the mothers ceased consuming cow's milk.

Haddad et al. [17] detected serum IgE from allergic patients using RAST tests with the total tryptic hydrolysate of Blg even when no serum IgE could be detected with native Blg. Few studies have been performed with human sera from patients with cow's milk allergy. These studies only determined the specificity of either one serum against various peptides [18] or of a limited number of sera against a single epitope [19, 20]. In this study, we investigated the immunoreactivity of the protein using peptides resulting from the cyanogen bromide (CNBr) hydrolysis of Blg. The IgE specificity and relative affinity for each purified peptide were tested by direct and competitive inhibition ELISA using 19 sera from patients allergic to cow's milk and particularly to Blg.

Materials and Methods

Materials

Human serum albumin (HSA) was from the Centre National de Transfusion Sanguine (Les Ulis, France). Unless otherwise stated, all chemicals were of analytical grade and from Sigma (St. Louis, Mo., USA).

The ELISA equipment (Labsystem) consisted of an automatic microplate washer, an automatic dispenser autodrop and a multiscan spectrophotometer. Microtiter plates were 96F maxisorp immunoplates I certificate from NUNC (Denmark).

The HPLC experiments were performed using a Waters Chromatograph (Milford, Mich., USA) equipped with two M510 pumps and a Wisp 712 automatic injector. Absorbances were measured using an SPD-10A UV-VIS detector (Shimadzu, Tokyo, Japan).

The protein liquid chromatography system (Pharmacia, Uppsala, Sweden) comprised a GP-250 gradient programmer equipped with two P500 pumps, and a detector. Amino acid composition was determined using an amino acid analyzer (Biotronik LC 3000, Munich, Germany).

Methods

Preparation and Purification of Blgs and Peptides Derived from Native or Reduced and S-Carboxymethylated Blg

Highly purified bovine Blg variant B (Blg) was prepared as described by Wal et al. [21]. Reduced and S-carboxymethylated Blg (RCM-Blg) was prepared according to a method partially derived from Swenson et al. [22]. Lyophilized Blg was dissolved in 1 M Tris-HCl pH 8 reducing buffer in the presence of 3 M guanidine hydrochloride, 5 mM EDTA and 27 mM DL-dithiothreitol (DTT) (corresponding to a 10-fold molar excess of DTT over the thiol concentrations). 1.47 M iodoacetic acid (1 ml in 1 M NaOH) was added for blocking the free thiols. After 4 h incubation in the dark, the reaction was stopped by addition of β -mercaptoethanol (50 μ l) and the RCM-Blg was dialyzed against water containing 0.1 M NaOH and lyophilized.

Absence of residual native Blg was checked using HPLC under the following conditions: column Vydac, C4, 300 Å, (250 $\times$ 10 mm); elution at a 2 ml/min flow rate: 0–2 min 35% buffer B (0.04% trifluoroacetic acid (TFA)-acetonitrile) in buffer A (0.1% TFA-water), then a linear gradient from 35 to 45% buffer B from 2 to 30 min. Retention times of RCM and native Blg were then 15 and 18.5 min, respectively. Under these conditions, no peak corresponding to native Blg can be detected with a detection limit corresponding to approximately 1% of the protein content.

Peptides Derived from Native or RCM-Blg. Native Blg or RCM-Blg (100 mg) was dissolved in 10 ml of 70% formic acid containing 45 mg CNBr and hydrolysis was carried out for 24 h at 28°C. The CNBr digest was dried using a Speedvac apparatus (Savant, Farmingdale, N.Y., USA). The peptides derived from the protein CNBr cleavage were separated by HPLC on a semipreparative 300 Å C18 Vydac column (250 $\times$ 10 mm, Shandon, Eragny, France) maintained at 40°C and equilibrated with a mixture of solvent A (H₂O-0.05% TFA) and solvent B (acetonitrile, 2-propanol, water v/v/v 2:1:2 and 0.05% TFA) at a 3 ml/min flow rate. Elution was achieved in two gradients: an 11-min linear gradient from 50 to 54% of solvent B, followed by an 18-min linear gradient from 58 to 75% of solvent B.

Peptide Identification and Purity. Peptide purity was checked by HPLC on a C18 analytical column (300 Å, 5 μ m, 10 nm, 4.6 $\times$ 250 mm, Delta-pak). Elution was achieved with a linear gradient from 20 to 60% of the same solvent B (acetonitrile, 2-propanol, water v/v/v 2:1:2 and 0.05% TFA) over 40 min at a 0.8 ml/min flow rate. Peptides were identified by analysis of their amino acid composition using a Picotag station (Waters).

Human Sera

Nineteen serum samples from milk-allergic patients were obtained from banks of sera from pediatric and/or allergy hospital departments.

Specific IgE to the main milk proteins (whole casein fraction, α -lactalbumin, lactoferrin, Blg and bovine serum albumin) were determined according to Wal et al. [21]. Two sera were monosensitive to Blg (S2, S5), 2 sera were sensitive to three allergens: i.e. whole casein fraction, α -lactalbumin and Blg (S4, S7), 8 sera were sensitive to four allergens i.e. whole casein fraction, α -lactalbumin, Blg, and either bovine serum albumin (S19), or lactoferrin (S1, S3, S8, S13, S17) or to α -lactalbumin, Blg, bovine serum albumin and lactoferrin (S9, S15) and 7 were sensitive to the five purified allergens tested (S6, S10, S11, S12, S14, S16, S18). Three sera were obtained from 2- to 3-year-old children, 10 from 5- to 12-year-old children and 6 from adults (more than 15 years old). There was no serum from infants less than 2 years old.

Enzyme Immuno-assays

Blg or peptides were passively adsorbed on microtiter plates (100 µl/well at 5 µg/ml in 50 mM carbonate buffer, pH 8.5) for 24 h at room temperature. After washing, each well was saturated with 300 µl of EIA buffer (0.1 M phosphate buffer, pH 7.4, 0.4 M NaCl, 1 mM EDTA, 0.01% sodium azide and 0.1% human serum albumin; HSA). Plates were stored overnight at 4°C before use. Specific human serum anti-Blg IgE were measured using the ELISA test previously described [21]. Under the conditions used, the detection limit was 0.1 IU/ml and no interference with serum IgG was observed [21].

As far as peptides were concerned, no absolute quantification of specific IgE was done, but absorbance was measured under similar conditions and the results were expressed as percent of the response (e.g. absorbance) measured with native Blg.

To confirm and better characterize the specificity of the sera, the same ELISA tests were performed after 4 h preincubation at room temperature of human sera and dilutions of different inhibitors, i.e. 0.03–20 nmol/ml of each peptide and 0.003–2 nmol/ml of native Blg.

The results of competitive inhibition were expressed as percent inhibition, calculated as $(1 - B/B_0) \times 100$, where B and B₀ represent the absorbance measured on the bound fraction in the presence or absence of Blg-derived competitors, respectively. Inhibitions were considered as significant when the signal measured in the presence of the CNBr peptide (B) was higher than the mean of the background signal measured with a Blg-unrelated control peptide $+3 \times$ standard deviation ($n = 6$). This corresponds to approximately 15% inhibition. In all these tests, non specific binding was evaluated using a Blg-unrelated peptide as negative control.

Results

Peptides Derived from CNBr Cleavage

CNBr cleaves the carboxyl side of the methionine residues. Figure 1 shows the elution pattern of the different peptides obtained after purification of the fragments derived from CNBr hydrolysis of either native (e.g. A₁, A₂), or RCM-Blg (e.g. B₁, B₂). These peptides have been numbered according to their retention time. Each peptide appeared as two peaks: the major peak corresponded to the native peptide resulting from the chemical treatment and the second one corresponded to a terminal homoserine lactone form instead of the methionine residue. As native Blg contains two disulfide bridges involving cysteine residues 66 and 160 on the one hand and 106 and 119 or 121 on the other, its cleavage generates a fragment comprising two peptide fragments (25–107) and (148–162) linked by a disulfide bridge. Consequently, to obtain every peptide devoid of interchain disulfide bridges and covering the entire amino acid sequence of Blg, it was necessary to hydrolyze both native Blg and RCM-Blg.

The peptides were identified as follows from the analyses of their amino acid composition (data not shown): A₁ corresponded to sequence 8–24 of Blg, A₂ corresponded to

sequence 108–145, B₁ corresponded to sequence 146–162 and B₂ corresponded to sequence 25–107 of the Blg molecule.

Detection of Specific Anti-Peptide IgE in Sera from Allergic Patients

Results Obtained Using Direct EIA. In the 19 sera from patients allergic to cow's milk protein, the specific anti-Blg IgE titers ranged from 1 to 320 IU/ml (table 1). Specific anti-Blg and anti-peptide IgE remained undetectable in the 4 control sera from subjects without known allergy (data not shown). In 37% of the sera, specific IgE were significantly detectable for each peptide, whilst the sera of 3 patients failed to respond to either peptide. One serum displayed a reactivity against only 2 peptides, and at least 4 sera had specific IgE against 1 peptide and 4 against 3 peptides (21% each).

For 37% of the sera, the sum of the IgE responses to the different CNBr-peptides was in the same order as the anti-Blg specific IgE response. Peptide recognition was lower than Blg recognition in 53% of the sera, and higher in only 2 cases. However, the sum of the mean intensity of the responses calculated for the 4 peptides in the 19 sera represented approximately 95% of the Blg response (fig. 2).

The highest IgE-binding capacity was measured with peptide (25–107) both in terms of frequency (since 79% of the serum IgE recognized this peptide), and signal intensity (42% of the Blg signal, fig. 2). Three of the 4 monospecific sera (i.e. S5, S11, S15) displayed specific and exclusive reactivity towards this peptide. The specific IgE response of the serum S5 against native Blg was exactly equivalent to that measured against the immobilized peptide (25–107); the Blg immunoreactivity was exclusively contained in this (25–107) sequence. Similar figures were obtained with fragment (108–145) although the frequency of recognition was lower (68 vs. 79%). The lowest IgE-binding capacities were revealed with peptides (8–24) and (146–162) (fig. 2). IgE binding to peptide (8–24) was the lowest in terms of mean intensity of the response since the signal measured with peptide (8–24) was 10% of the signal obtained with whole native Blg while in terms of frequency only 37% of sera bound peptide (146–162). In addition, the fourth monospecific serum (S10) and the dispecific serum (S17) both recognized peptide (108–145).

Results Obtained Using Competitive Inhibition ELISA of IgE Binding to Immobilized Blg. Competitive inhibition was performed using native Blg or RCM-Blg, unpurified total hydrolysates and finally purified peptides as competitors. Binding inhibition of specific IgE to Blg by increasing concentrations of various competitors was tested and results

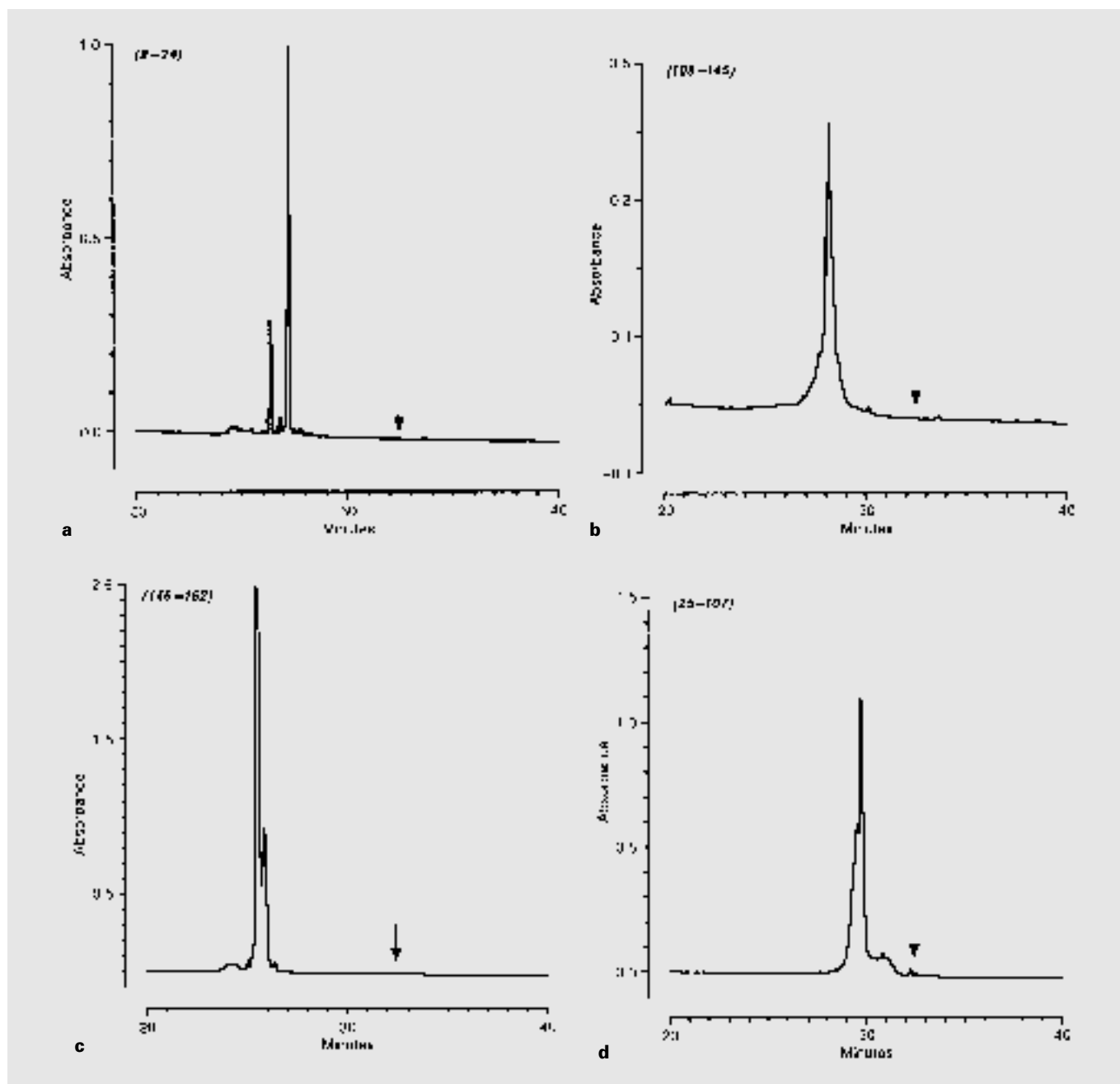


Fig. 1. HPLC profiles of purified CNBr-derived peptides from native Blg, i.e. peptides (8–24) (**a**) and (108–145) (**b**) and RCM Blg, i.e. peptides (25–107) (**c**) and (146–162) (**d**). The arrow indicates the retention time of native Blg.

were expressed as percent inhibition at an inhibitory concentration of 1 μ M.

The inhibition curves obtained with sera S2 and S13 when using proteins and total CNBr digests as competitors illustrate the two tendencies noted in the pattern of responses (fig. 3). In all cases, RCM-Blg was recognized by the sera, indicating that chemical treatment did not abolish the

immunoreactivity. In contrast, CNBr digests inhibited the binding of the specific IgE to the native Blg to various extents. Inhibition of IgE binding capacity from serum S13 was efficient whatever the competitor, whereas the CNBr digests were not as efficient competitors as Blgs in inhibiting binding of specific anti-Blg IgE from serum S2.

Table 1. Determinations of the specific IgE from allergic patients to Blg and CNBr peptides

Peptide	Serum																		
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19
(8–24)	15	25	10	6	0	7	0	37	0	0	0	0	12	0	0	1	0	1	3
(108–145)	34	50	27	31	0	10	67	70	0	8	0	0	30	0	0	9	20	10	25
(146–162)	19	0	16	29	0	0	0	10	0	0	0	0	7	0	0	6	2	8	0
(25–107)	33	50	6	15	100	6	150	46	0	0	9	0	23	0	14	12	13	17	50
Native Blg	320	4	173	150	1	40	3	10	18	19	17	6	69	8	14	97	15	60	32

Binding of IgE from allergic patients to Blg and CNBr-peptides was measured by direct ELISA. Data represent the amount of anti-Blg IgE present in the sera expressed as IU/ml. For each peptide, results are expressed as percent of the response to native Blg. No signal was detectable using 4 control sera from non allergic patients and/or a control peptide (data not shown). Zero means < detection limit (i.e. 0.1 IU IgE/ml)

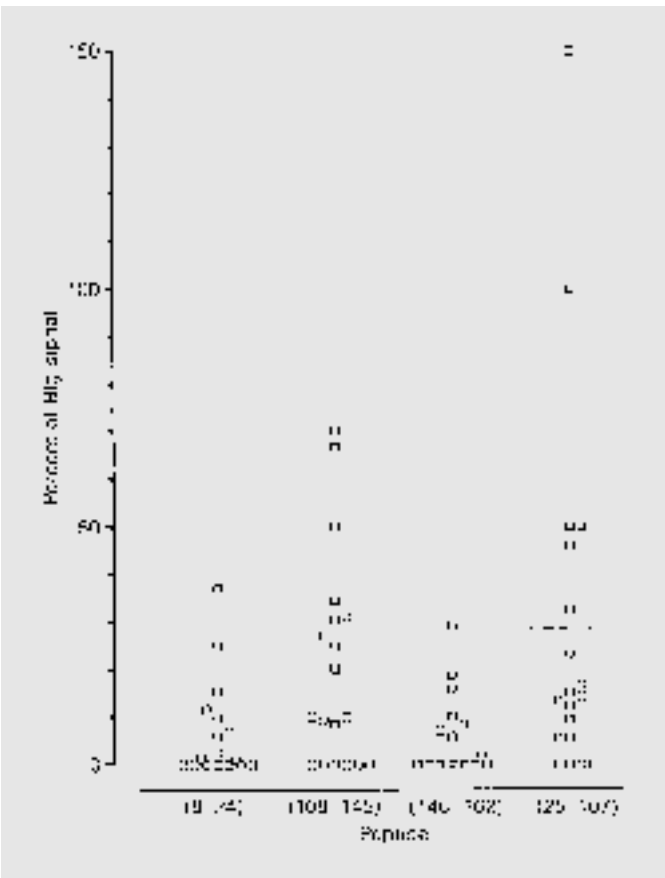


Fig. 2. Blg and CNBr peptide binding capacity to IgE from allergic patients measured by direct ELISA. Data represent the specific IgE titer present in the sera expressed as a percentage of the anti-Blg IgE response. The mean intensities of the responses are represented as horizontal bars.

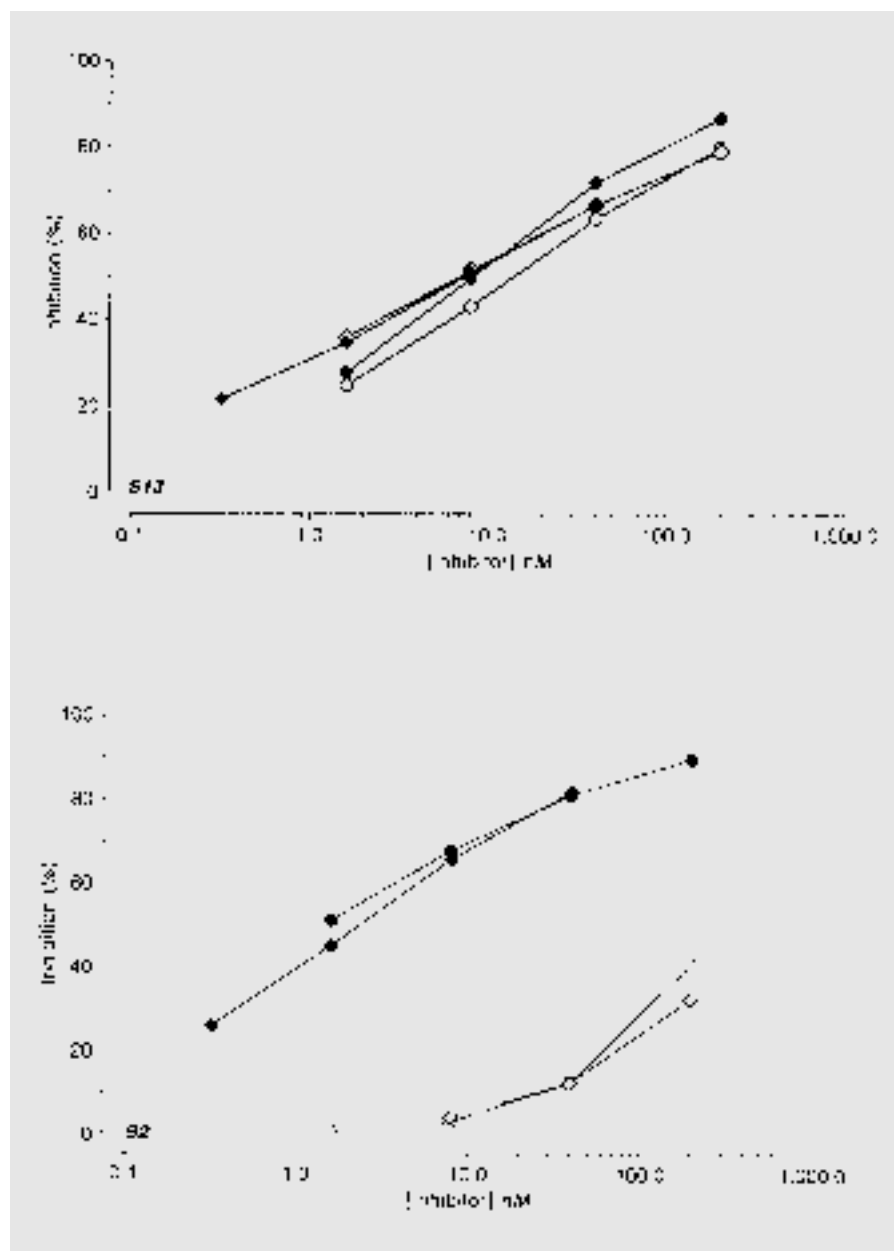
Table 2. Binding inhibition of specific anti-Blg IgE to immobilized Blg by 1 μ M Blg and CNBr peptides

Peptide	Serum								
	S1	S2	S3	S4	S6	S8	S13	S18	S19
(8–24)	0	0	34	0	6	26	28	6	0
(108–145)	12	8	28	30		57	16	32	6
(146–162)	0	0	0	0	0	28	20	0	0
(25–107)	2	3	36	38	10	22	37	0	10
Native Blg	76	72	82	79	50	62	57	92	80

By reference to the inhibition curves obtained by competitive inhibition ELISA tests, the capacity of the competitor to inhibit the binding between the specific IgE present in the human sera and the coated protein was calculated for an inhibitor concentration of 1 nmol/ml. Values represent the percentage calculated as $(1 - B/B_0) \times 100$ where B is the signal measured in the presence of 1 μ M of inhibitor and B0 corresponds to the signal measured without any inhibitor. No value is given when an assay was not performed. No inhibition was measured using a control peptide (data not shown). Zero indicates no significant binding inhibition, i.e. mean of the signal (B) < mean of the background signal measured with a Blg-unrelated control peptide +3 $\times$ standard deviation (n = 6).

Table 2 gives the values of the inhibition of the binding of specific IgE to immobilized Blg, measured for a 1 μ M concentration of inhibitor, in 9 out of the 19 sera for which enough volume was available. No inhibition was observed with the control peptide. Marked inhibition (50–92% inhibition) was observed using native Blg, even if it was not always total. By itself, no peptide inhibited the binding of the IgE to immobilized Blg as efficiently as the native protein. With some sera (S1, S2, S6, S18, S19), the sum of inhibitions due to the different fragments was lower than the inhibition observed with the native Blg, indicating the existence

Fig. 3. Competitive inhibition curves obtained using various antigens (native Blg, RCM-Blg or peptides) as competitor of the binding between the human IgE from two allergic patients (S2 and S13) and the coated Blg. Data represent an inhibition percentage calculated as $100 - (100 \times B/B_0)$ where B and B₀ correspond to the measured absorbance in the presence or absence of inhibitor, respectively. Using a control peptide, no inhibition was measured in the two cases (data not shown). ♦ = Native Blg is used as competitor; ● = RCM-Blg issued as competitor; ◇ = total CNBr hydrolysate of native Blg is used as competitor; ○ = total CNBr hydrolysate of RCM-Blg used as competitor.



of conformational epitope(s) involving most of the whole native protein. On the opposite, with other sera the inhibition observed with the whole Blg can be obtained by addition of the inhibitory action of each peptide. In some sera (i.e. S8, S13), this sum even largely exceeded the maximum inhibition observed with whole Blg, probably because of the presence in these sera of IgE antibodies exhibiting a higher affinity for the different fragments than for the whole protein. This may be due to a better availability of the epitopes for IgE when presented on peptides and/or to the fact

that structural features on CNBr fragments better mimic the sensitizing Blg-derived peptides formed after digestive processes.

In keeping with the results noted with direct EIA, the best competitors were peptide (108–145) and (25–107) which sometimes inhibited more than 30% of IgE binding to Blg (S3, S4, S8, S13, S18). Except for S3, S8, S13, the peptide (8–24) provoked no or weak inhibition of IgE binding to Blg. Peptide (146–162) appeared to be the less efficient competitor. Finally, we noted that the serum IgE of

each patient presented a specific pattern. For example, whilst peptide (146–162) was generally a poor inhibitor of IgE binding to Blg, it efficiently inhibited this binding in the case of sera S8 and S13.

Discussion

The direct ELISA tests used proved to be well adapted for the quantitative determination of anti-Blg IgE, as already described [21]. As far as peptides were concerned, we have chosen to express their IgE-binding capacity as a percentage of the immunoreactivity of the whole intact protein in order to permit valuable comparisons between the different peptides as already described [23]. Moreover, competitive inhibition ELISA tests confirmed all the results and tendencies obtained with the direct tests. During the coating by passive adsorption, interactions between the allergen molecule (protein or peptide) and the plastic solid phase of the microtiter plate may occur which may induce some slight structural modifications on the immobilized allergen, such as partial unfolding and denaturation. This phenomenon has already been observed and discussed [21] and can explain the difference in IgE affinity for the immobilized allergen versus the same allergen in solution. It should be noticed that in food allergy, IgE seem to better recognize coated ('modified') than soluble ('native') allergen since inhibition of the binding of IgE to the immobilized allergen cannot always be totally inhibited by increasing concentrations of the same allergen in solution. This may be due to the fact that sensitization occurs after processing of the protein particularly during the digestion and that the immune system is stimulated by modified or partially destructured proteins or fragments. This phenomenon thus does not appear as an artifact due to interactions with a hydrophobic plastic adsorbent. Moreover very similar results have been obtained in anti-Blg IgE determination using hydrophilic adsorbent in classical RAST® system.

Our results show unambiguously that the RCM protein was recognized by anti-Blg human IgE antibodies present in the sera of allergic patients. Cleavage of the intrachain disulfide bounds within the Blg and consequently the likely loss of the conformation of the molecule had little effect on its immunoreactivity. These findings are in agreement with those of Huang et al. [24] concerning rat IgE, but contrast with the responses measured by these authors using rabbit IgG.

The immunoreactivity of the CNBr peptides in terms of binding capacity to specific human IgE was evaluated by direct and competitive tests which appeared to be well cor-

related. Total CNBr digests of native Blg and RCM-Blg were equally efficient in inhibiting human IgE binding to coated Blg. However, chemical cleavage of Blg directly affected its immunoreactivity towards specific human IgE in different proportions depending on the patients. With some sera, the total digests (either from native Blg or RCM-Blg) were as efficient as the whole intact protein in inhibiting specific IgE binding to coated Blg. With other sera, the binding inhibitions obtained with the two total hydrolysates were lower than those obtained using intact Blg. We therefore compared the sum of the IgE responses to CNBr peptides with IgE responses to native Blg, and found that (1) CNBr-chemical cleavage of Blg completely suppressed recognition by specific anti-Blg IgE in 3 human sera (S9, S12, S14); (2) for some sera (37%), the cleavage had no effect, suggesting that the integrity of the epitopes was included in the fragments; (3) In another group of sera (52%), cleaving the Blg lowered its immunoreactivity perhaps due to the involvement of conformational epitopes in the binding to antibodies, and (4) in the last group of sera (10%), chemical cleavage enhanced protein immunoreactivity, suggesting that cleavage allows the presentation of determinants which were not necessarily accessible to the antibodies on the whole native protein. This might correspond to patients expressing antibodies to Blg fragments derived from natural digestion processes. These findings confirm the results of Haddad et al. [7] who obtained positive RAST tests towards Blg fragments whilst the same sera gave negative results with the native protein. This again highlights the difficulty of using immunized animals to mimic human sera.

These experiments revealed similarities and differences with studies on mice by Takahashi et al. [25], rats by Huang et al. [24], and rabbits by Otani et al. [18]. Takahashi et al. [25] described 4 fragments, 3 essentially within peptide (25–107) and the 4th (3–4 times lower in intensity) within peptide (146–162). This fits with the results of Huang et al. [24] who demonstrated that the immunoreactivity was correlated with peptide length. This observation is in agreement with our results but not with the results of Otani et al. [26] who found no immunoreactivity within peptide (8–24). These differences in antigen/allergen specificity between antibodies produced in various hyperimmunized/sensitized animals might be due to species specificity, as pointed out by Takahashi et al. [25]. In their study of whale sperm myoglobin in 3 strains of mice, Berzofsky et al. [27] concluded that the antibody specificity depended on structural characteristics of the antigen, but was also controlled by host factors (e.g. immune response genes). So the use of an immunized animal antiserum may be critical in the study of

protein immunoreactivity, when performed in order to predict the diversity and specificity of the human IgE responses of an allergic population. The difference in immunoreactive determinants of α -lactalbumin between sera from immunized animals and sera from allergic patients has also been suggested by Maynard et al. [23].

In the present study, 10% of the sera recognized the cleavage fragments better than the native protein and our results clearly show that there are numerous epitopes on the Blg molecule and that antibodies from a single patient can recognize more than one epitope (37% of the sera had specific IgE towards the four fragments). Accordingly, a study using smaller Blg fragments covering the entire protein sequence would be particularly interesting.

Direct and competitive EIA indicate that the big (25–107) peptide retained a substantial proportion of the IgE immunoreactivity of the whole protein. Serum S5 had IgE exclusively directed against this peptide. Indeed, this sequence which comprises half of the molecule includes the epitope defined by Ball et al. [20] using human sera, two antigenic determinants described by Takahashi et al. [25] with mice sera and one described by Otani et al. [18] using a single human serum. Peptide (108–145) also includes a

sequence described by Adams et al. [19]. This peptide, like peptide (25–107), is sometimes better recognized than the native protein itself. This peptide is also the only peptide recognized by serum S10. The responses to peptide (8–24) were as frequent as the responses to peptide (108–145), but less intense. Otani et al. [28] first found this peptide to be non immunoreactive [28], but more recently they have described it as immunoreactive, without providing data on the relative importance of the response [18]. Studies of smaller peptides will be required to precisely define Blg epitope binding and the relative importance of these epitopes. Such an approach would contribute to the better characterization of allergic epitopes and of the structure-immunoreactivity relationship in food allergy mechanisms.

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