

Allergy to bovine β -lactoglobulin: specificity of immunoglobulin E generated in the Brown Norway rat to tryptic and synthetic peptides

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Summary

Background Animal models which reflect the induction and development of food-allergic reactions are important in the identification of allergenic potential of food proteins and peptides. A number of rat strains, including PVG, Hooded Lister and Brown Norway have been shown to produce immunoglobulin (Ig) E antibodies to food proteins as well as to inhaled allergens. Previous work in our laboratory using the Brown Norway (BN) rat has shown that specific IgE is produced following administration of ovalbumin and milk products via both enteral and parenteral route; this has allowed us to rank ovalbumin, lactoferrin and bovine serum albumin in terms of their inherent allergenic potential and has enabled us to demonstrate that milk protein allergens recognized by the systemically-sensitized animal are consistent with those recognized by sera from cow's milk-allergic patients (the most common allergens recognized were β -lactoglobulin and the α , β and κ -caseins).

Objective To demonstrate that the BN rat model can be used to identify the major IgE-binding peptides from β -lactoglobulin, a known human food allergen, and that those IgE-binding peptides are similar to those recently identified using sera from cow's milk-allergic patients.

Methods BN rats were exposed to β -lactoglobulin or to semiskimmed milk via the intraperitoneal route in the presence of the adjuvant carrageenan. Specific IgE raised against β -lactoglobulin was determined by a direct enzyme immunoassay using acetylcholinesterase substrate; specific IgG responses were also monitored. Overlapping synthetic peptides and tryptic peptides were used within the ELISA to identify the major and minor IgE-binding immunoreactive sequences.

Results In terms of comparative immunogenicity, there appeared to be sequences that were predominantly IgE- or IgG-reactive. IgE-dominant regions were amino acid sequences 21–40, 41–60, 107–117 and 148–168 whereas sequences 1–24, 67–77, 82–92, 85–95 and 117–127 appeared more selective for IgG antibody recognition. An increased capacity to induce specific IgE was observed when the allergen was present in the context of whole food.

Conclusions These studies provide evidence that the immune system of the BN rat and humans — at least in the case of milk allergens — is recognizing similar protein allergens and indeed, at the molecular level, similar peptide epitopes.

Keywords: bovine β -lactoglobulin, Brown Norway rat, cow's milk allergy, ELISA, peptides

Clinical and Experimental Allergy, Vol. 29, pp. 1696–1704. Submitted 22 October 1998; revised 23 January 1999; accepted 20 May 1999.

Introduction

Allergic reactions to food and dietary components are increasing throughout the industrial world and perceived as a serious problem in adults as well as children. The number of incriminated foods are also increasing even if the number of well-documented cases reported are still relatively low compared with those reported for inhaled allergens [1,2]. The introduction and availability of imported foods into the diet and the high level of industrial food production are no doubt important contributory factors in the development of allergenicity and the severity of symptoms reported [3,4]. Furthermore the protein structure and the protein composition of foods may be modified by new technological processes, by breeding and, in particular, by genetic engineering. Thus, there is an increasing need for both the agro-food industry and the health authorities to develop assay systems to test for the potential allergenicity of food proteins and peptides and to identify the immunoglobulin (Ig) E-immunoreactive sequences (epitopes) giving rise to food hypersensitivity reactions.

However, whereas information from animal experiments have clarified the extent to which gut immune responses depend on age, dose and frequency of antigen administration, and the conditions needed to induce vigorous immune reactions [5–7] there is as yet no established animal model for screening potentially reactive sequences in protein allergens. Several studies have demonstrated oral sensitization to milk proteins using a guinea pig model [8,9]. However, there are significant differences in the isotype of reaginic antibody produced by guinea pigs compared with other animal species and limited tools for studying its immune system. Strains of both mice and rats on the other hand developed specific IgE antibodies to food proteins and other allergens. The Brown Norway (BN) rat strain in particular has been shown to be a high IgE responder strain [10] and thus, to a certain degree, can be said to resemble atopic humans.

Several laboratories have demonstrated that the BN rat produces IgE after oral administration of ovalbumin [7,11,12] an antigen perceived as highly allergenic by the rat immune system [13]. However, in general, IgE responses are not consistently evoked using oral administration [14], and the majority of experimental animal studies of IgE-mediated hypersensitivity have been conducted using parenteral administration in association with a specific adjuvant to increase the magnitude of IgE production.

Specific IgE against celeriac and roasted peanuts has been successfully induced in parenterally immunized BALB/c mice [15] and studies using the BN rat strain have shown that the range of milk allergens recognized by systemically-sensitized BN animals are consistent with the allergens identified in sera obtained from cow's

milk-allergic patients [13,16]. More recent studies on sera obtained from allergic patients have shown specific IgE towards several peptides derived from β -lactoglobulin by tryptic cleavage [17]. Further studies by us using BN rats have therefore focused on the effect of one specific milk allergen protein, namely, β -lactoglobulin. Interestingly, studies where animals were exposed to semiskimmed or whole milk before exposure to β -lactoglobulin demonstrated that a more sustained IgE production and a higher level of antibody was induced as compared with when the BN rats were exposed only to β -lactoglobulin [18]. These experiments provide some support for the hypothesis that an enhanced entry of β -lactoglobulin into antigen-presenting cells would occur when the allergen is presented in the content of an emulsion [19].

We now demonstrate that a good correlation can be shown to exist between the major immunoreactive peptide sequences of β -lactoglobulin identified in sera obtained from cow's milk-allergic patients [17] and the peptide sequences identified as immunoreactive with sera obtained from β -lactoglobulin-sensitized rats. These findings lend further support to the suggestion that the high IgE-responder Brown Norway rat is a valuable predictive model for screening allergen-inducing food constituents.

Materials and methods

Animals

Brown Norway 6–7-week-old rats were obtained from Harlan UK (Bicester, UK) and supplied from dams that had been maintained on milk and ovalbumin free 'BeeKay' (BK) diet. The rats were reared and maintained on the BK diet under standard housing conditions with both food and mains tap water freely available.

Allergens

Semiskimmed milk was obtained from a commercial dairy company (Express Dairy, Morden, UK) and used on the day of purchase. β -lactoglobulin (β -LG) was obtained from Sigma Ltd (Poole, UK) and β -LG variant B was prepared and purified by INRA (Gif-sur-Yvette, France) as previously described [16].

Treatment

Groups of BN rats were injected intraperitoneally (i.p.) with either isolated β -lactoglobulin, semiskimmed or whole milk (co-administered with carrageenan as IgE-selective adjuvant). Animals were sensitized to 100 μ g β -lactoglobulin alone injected on days 0 and 7 or days 0 and 14 or

exposed i.p. to 500 µg semiskimmed or 500 µg whole milk on days 0 and 7 followed by 100 µg β -lactoglobulin on days 14 and 28. Six separate groups of five rats each were used to determine the effects for each induction regimen. Animals were bled at weekly intervals from the lateral tail vein. Sera were assessed for the presence of specific IgE and IgG by ELISA. A total of 280 sera samples were assayed for specific IgE to β -lactoglobulin.

Peptides

β -lactoglobulin (variant B) was digested by trypsin using an enzyme : substrate ratio of 2.5% molar overnight at 37°C. The tryptic hydrolysate was first purified by anion exchange chromatography. Each anion exchange fraction was further purified by HPLC on a semipreparative C18 column eluted with a three-step gradient using solvent A (0.0115% TFA in water) and solvent B (60% acetonitrile, 0.1% TFA in water), comprising (a) a linear gradient from 0 to 22.5% of solvent B, (b) a linear gradient from 22.5 to 50% of solvent B, and (c) a linear gradient from 50 to 60% of solvent B. Peaks were collected and characterized by amino acid composition analysis [19]. INRA also determined the IgE capacity of some synthetic peptides corresponding with the tryptic fragments.

Linear synthetic peptides designed from the templates of β -LG A and B were supplied by the Institute of Food Research (Norwich, UK). Peptides LL7, GB32, LL1, LL2, LL9, GB39, LL3, LL10, LL4, LL8 and LL6 were synthesized on a Novasyn Crystal Automated synthesiser (Calbiochem-Novabiochem, Nottingham, UK). Following peptide cleavage from the resin and removal of protective groups on reactive side residues, the purity of each peptide was checked by reverse-phase HPLC using a C18 column and FAB mass spectrometry. Synthetic peptides, β -LG5, β -LG1, β -LG3, β -LG4, β -LG6, β -LG7, β -LG8, Pep1 and β -LG9 were supplied by Unilever Research (Colworth, UK). These linear synthetic peptides were used at the BIBRA laboratories.

Enzyme immunoassays

Determination of specific IgE was performed as previously described [20]. Monoclonal mouse-antirat IgE (MARE-1) and biotinylated MARE-1 were supplied by Serotec (Oxford, UK), production of F(ab¹)₂ fragments of MARE-1 antibodies and conjugation of the fragments to the tetrameric (G₄) form of acetylcholinesterase (AChE) were performed according to Grassi *et al.* [21]. Antigens (β -LG or β -LG peptides) were used at a concentration of 5 µg/mL in EDTA buffer pH 9.3, control or test serum diluted to 1/20 or 1/100, Ellman's reagent [22] added as the substrate and the absorbance of the wells was measured at 410 nm

or 414 nm after 1 h of enzymatic reaction. The results were expressed in absorbance (OD) units.

Determination of specific IgG was performed using sheep antirat IgG-horseradish peroxidase (SARGP) as the tracer [20]. Antigens (β -LG or synthetic peptides) were used at a concentration of 10 µg/mL in bicarbonate buffer pH 9.6. Following washing the plate was blocked before use with MeggablockTM 1/500 for 2 h. The serum samples were diluted 1/8000 in PBS and 100 µL added to the wells. Samples were incubated for 18 h at 20°C, followed by further incubation with MeggablockTM with SARGP for 1 h at 37°C. A positive sample was then detected (after washing) with K-BlueTM substrate incubated for 15 min, stopped with 2 mol/L H₂SO₄ and measured at 450 nm. Results were expressed as OD units and all measurements performed using a DIAS MR 7000 plate reader and BioLinx software (Dynatch Laboratories, Billingshurst, UK).

Statistical analysis

Where relevant, data was analysed using Student's unpaired *t*-tests.

Results

To investigate which binding regions of β -lactoglobulin the specific IgE antibody induced in Brown Norway rat recognized, two different induction regimens were used in these studies. Furthermore, mapping of the allergenic peptide sequences was carried out independently in our two separate laboratories and under somewhat differing conditions of identification and screening.

As stated in Materials and methods the BN rats were sensitized either by β -lactoglobulin alone on days 0, 7 and/or 14, or by semiskimmed or whole milk on days 0 and 7 followed by exposure to β -lactoglobulin alone (boosted response). Specific IgE levels obtained from all regimes ranged from 40 to 2000 mOD units, levels of IgE greater than 200 mOD units were regularly found on days 13 and 42 using the 'boosted' regimen (Fig. 1). All samples were positive for IgG at a 1/8000 dilution.

The question of whether differing induction regimens might induce targeting of different immunoreactive regions of the β -LG molecule as well as the effect of time after induction of initial sensitization was investigated at the BIBRA laboratories during the course of performing recognition profile assays on the sera samples. Identification of reactive sequences was carried out using 20 synthetic peptides (covering the whole bovine lactoglobulin molecule as well as some overlapping sequences) on sera samples obtained 14, 21, 27 and 42 days after exposure. Thirty-one samples were selected for separate determinations of the IgE-binding capacity of tryptic peptides

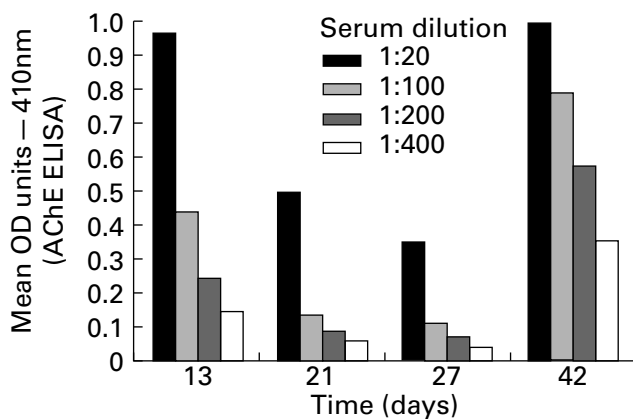


Fig. 1. Example of an experiment demonstrating a semiskimmed milk-based IgE-boosting regimen. Animals were sensitized using 500 μ g of semiskimmed milk on days 0 and 7 followed by 100 μ g of β -lactoglobulin on days 14 and 28. Serum samples were taken on days 13, 21, 27 and 42 and specific IgE was determined by ELISA as detailed in the Materials and methods section. The experiment was performed on six occasions using groups of five male rats (* $P < 0.05$).

prepared by INRA (covering the whole bovine lactoglobulin molecule) and INRA-selected synthetic peptides. The peptides had been prepared for studies of IgE epitope mapping of human sera, and although it was desirable to use the same peptides for all the rat sera studies to compare the capacity

of the BN rat to recognize similar peptide fragments, the amount of tryptic peptides produced was very low (because of the need for extensive purification) so that only 31 samples of rat sera could be analysed by INRA.

Rat sera samples were defined as positive if they contained IgE antibody at an optical density (OD) greater than control sera plus three standard deviations [23], but only sera containing β -LG-specific IgE at levels higher than 200 mOD (0.200 OD) were used in the peptide assays. Sixty-three samples out of the 180 sera samples analysed had OD levels higher than 200 mOD. This precaution was taken as linear synthetic peptides are generally less immuno-reactive than the native protein molecule.

Sera samples were analysed in batches of 12 in the BIBRA laboratories, and a minimum of 24 assays were performed for each specific linear peptide when determining the overall recognition profiles. A typical series of results is illustrated in Fig. 2. Major regions recognized, i.e. with a mean percentage binding greater than 1% of the specific IgE response to native β -LG were the peptides Ser²¹-Arg⁴⁰ (21–40, GB32), Val⁴¹-Lys⁶⁰ (41–60, LL9) and Arg¹⁴⁸-Ile¹⁶² (148–162, LL6); Leu⁵⁵-Ala⁶⁷ (LL3, 54–67) and Lys⁶⁰-Pro⁷⁹ (60–79, LL10) were also recognized by rat IgE.

No differences were evident in the recognition of the peptide sequences recognized at different time points after immunization (days 21, 27 and 42) when either of the regimes, i.e. β -lactoglobulin alone or milk-based regimens

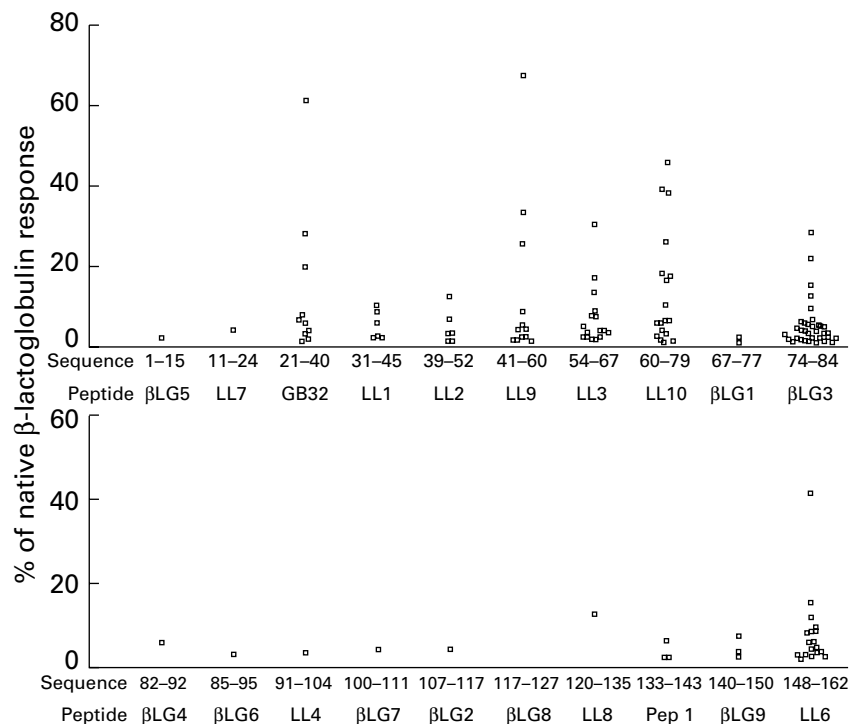


Fig. 2. Recognition of synthetic peptide or tryptic peptide sequences by sera from Brown Norway rats sensitized to β -lactoglobulin. Serum samples were analysed in batches of 12 in the BIBRA laboratories and a minimum of 24 assays were performed for each specific linear peptide. Measured by direct enzyme immunoassay, the results are expressed as a percentage of the IgE responses to the whole β -lactoglobulin protein.

	Peptide sequences				
	GB32 21–40	LL9 41–60	LL3 54–67	LL10 60–79	LL6 148–162
<i>β-LG alone</i>					
Nr-samples analysed	60	61	68	32	65
Mean percentage (all samples)	0.1	0.1	0.5	0.4	0.7
Standard deviation (all samples)	0.5	0.3	2.4	0.9	2.7
Maximum response	2.5	1.7	8.2	4.2	15.8
Frequency of response (%)	16.7	9.8	22.1	37.5	27.7
<i>Milk-based regimens</i>					
Nr. samples analysed	24	65	68	33	67
Mean percentage (all samples)	4.7*	2.6*	0.6	2.7**	1.4
Standard deviation (all samples)	13.5	9.8	1.4	3.9	5.4
Maximum response	61.6	68.2	8.2	7.2	42.3
Frequency of response (%)	41.7	50.8	47.1	66.7	38.8

Statistical analysis of β -lactoglobulin vs milk-based regimens. * $P < 0.01$; ** $P < 0.001$.

(β -LG alone or milk regimens) were used (data not shown). Significant differences were found, however, regarding the intensity of binding when the immunization regimens were compared ($P < 0.01$ for peptide sequences LL9 and GB32; $P < 0.001$ for LL10). The frequency of binding was also much increased after immunization using the milk-based regimen (Table 1). Exposure to β -lactoglobulin alone was less effective in inducing specific responses than a milk-based regimen especially with respect to the peptide sequences 21–40 and 41–60.

A smaller number of samples were also analysed for IgG recognition profiles using the same panel of synthetic peptides. No sequence was exclusively IgE-selective though differences were apparent between their recognition profiles (Table 2). In terms of comparative immunogenicity, there appeared to be sequences that were predominantly IgE- or IgG-reactive. IgE-dominant regions were the amino acid sequences 21–40 (GB32), 41–60 (LL9), 107–117 (β -LG2) and 148–168 (LL6), whereas the sequences 1–24 (β -LG5, LL7), 67–77 (β -LG1), 82–92 (β -LG4), 85–95 (β -LG6) and 117–127 (β -LG8) appeared more selective for IgG antibody recognition. However, such recognition was variable; in some individual samples peptides recognized by IgE and IgG antibodies were not the same whereas in other sera the same peptides were recognized by both classes of antibodies.

During the second phase of these studies, results from the testing of 31 positive sera samples were selected for comparison with human data at the INRA laboratories. Specific IgE responses against β -lactoglobulin, β -lactoglobulin-derived tryptic peptides and corresponding synthetic

peptides were determined at INRA in the same manner as had been performed with human sera [23]. For the direct ELISA assay immobilization of the peptide was by adsorption or in the case of small peptides, via streptavidin–biotin interactions, and bound antibodies revealed by the same anti rat-IgE monoclonal antibody labelled with the same acetyl cholinesterase (MARE-A ChE) as used in the BIBRA studies. The results found at INRA between the IgE-binding capacity of tryptic peptides and corresponding synthetic peptides were very similar (Tables 3a, 3b). The peptides most frequently recognized by the rat sera samples were those with sequences 21–40, 41–60, 102–124, 125–135 and 149–162.

Although the intensity and affinity of the IgE response to the peptide sequences used differed between the two laboratories, both found that the sequences recognized most frequently and recognized with greatest intensity were 21–40, 41–60 and 149–162. The sequence 102–124 corresponded with a weakly reactive region in the BIBRA studies whereas it was well recognized in the INRA laboratory. Additionally BIBRA found the sequences 60–79 and 74–84 to be recognized by rat IgE whereas INRA found peptide 125–135 to be a binding site. However, the series of synthetic peptides used in the two laboratories were not the same which made direct comparisons difficult.

Both the frequency as well as the intensity and affinity of the rat IgE response to tryptic immunoreactive peptides were found to be lower than the corresponding human response. Mean recognition by rat sera of the tryptic peptide 21–40 was 1.8% of the IgE response to whole

Table 1. Analysis of the effect of the two immunization regimens (sensitization to β -LG alone as opposed to semiskimmed milk followed by β -LG) on the intensity and frequency of rat IgE responses to synthetic peptides. Results were obtained using a direct ELISA and are expressed as percentage of the specific IgE response to native β -lactoglobulin

Table 2. Comparison of sera samples that have been investigated for both IgE- and IgG-reactive peptide sequences after immunization of Brown Norway rats using either β -LG alone or the milk-based regimen. Results were obtained using an acetylcholinesterase-based direct ELISA for IgE and using a horse radish peroxidase tracer for IgG

	β LG5 0–15	LL7 11–24	GB32 21–40	LL1 31–45	LL2 39–52	LL9 41–60	LL3 54–67	LL10 60–79	β LG1 67–77	β LG3 74–84
Number of samples tested	8	11	26	26	11	26	26	8	8	8
IgE & IgG reactive	0	1	3	1	2	6	7	5	0	5
IgE reactive only	0	0	11	2	0	11	8	1	0	3
IgG reactive only	5	7	7	12	8	5	7	1	6	0
Neither IgE nor IgG reactive	3	3	5	11	1	4	4	1	2	0

	β LG4 82–92	β LG6 85–95	β LG2 107–117	β LG8 117–127	LL8 120–135	PEP1 133–143	β LG9 140–150	LL6 148–162
Number of samples tested	8	8	8	8	11	8	8	26
IgE & IgG reactive	0	0	0	0	3	2	1	6
IgE reactive only	0	0	7	0	1	1	4	6
IgG reactive only	5	7	1	7	4	4	0	4
Neither IgE nor IgG reactive	3	1	0	1	3	1	3	10

β -lactoglobulin compared with 4.3% for human sera, 1.3% compared with 10% for peptide 41–60 and 2.2% compared with 9.5% for peptide 149–162. The frequency of recognition was 50% in rat sera compared with 65% in human sera for peptide 21–40, 64% compared with 93% for peptide 41–60 and 64% compared with 89% for peptide 149–162 [17].

Discussion

Several studies have investigated the antigenic regions of isolated milk proteins using sera from immunized rabbits or mice [24,25] but we believe few studies have been carried out analysing the binding sites in the β -lactoglobulin molecule using sera from sensitized inbred Brown Norway

Table 3. The results obtained at INRA on the IgE binding capacity of 31 samples of rat sera by direct ELISA on immobilized (a) tryptic and (b) synthetic peptides expressed as percentage of the specific IgE response to native β -lactoglobulin

<i>Tryptic peptides</i>											
Rat serum	β -LGB										
BIBRA	(mOD)	1–8	9–14	25–40	41–60	78–83	84–91	92–100	102–124	125–135	149–162
Mean	1623	0.1	0.2	1.8	1.3	0.0	0.2	1.0	0.7	0.4	2.2
s.d.	554	0.4	0.5	2.4	1.4	0.1	0.4	2.6	0.8	0.5	4.5
Max.	2764	1.6	1.8	8.0	4.8	0.6	1.7	13.0	2.6	1.3	22.0
Min.	275	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0/0	0.0
Frequency	100	13.0	17.0	50.0	64.0	8.0	12.5	42.0	50.0	42.0	57.0

<i>Synthetic peptides</i>									
Rat serum	β -LGB	LL7	LL1	LL2	LL3	LL4	LL5	LL8	LL6
BIBRA	(mOD)	11–24	31–45	39–52	54–67	91–104	107–120	120–135	148–162
Mean	1623	0.3	1.5	0.7	2.5	0.9	0.6	1.0	2.8
s.d.	554	0.5	3.0	1.2	4.0	2.3	0.7	1.7	5.3
Max.	2764	1.7	16.0	4.5	17.0	12.0	3.0	7.0	25.0
Min.	275	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Frequency	100	32.0	52.0	32.0	61.0	39.0	52.0	45.0	61.0

rats. High IgE responder strains such as the BN rat have been used in a number of laboratories for investigating aspects of allergic responses [18,26–28]. Furthermore the IgE response in the BN rat to the complex mixture of allergens present in milk has previously been shown to have similar antibody specificities to that found in humans [13].

These present studies have further validated the use of the BN rat as a model for the identification of potentially allergenic food constituents. The question as to whether a similar epitope-specific profile is produced following oral administration has not yet been investigated. Our major aim in these studies has been to establish an animal model for predicting potentially allergenic protein components in the diet. These studies, taken together with our previous work demonstrating that allergens recognized by parenterally sensitized BN rats are consistent with those recognized in sera from cow's milk-allergic subjects, have enabled us to rank food proteins in terms of their inherent allergenic potential and to apply the toxicological concept of dose–response to an essentially immunological phenomenon [29,30].

We would like to emphasize that the results achieved were made possible by the adaptation of a sensitive ELISA assay using antirat IgE-labelled with acetylcholinesterase [20], which allowed determination of anti-peptide rat IgE in a similar fashion to that of human IgE [31], as well as the availability of a large number of purified synthetic peptides covering the complete amino acid sequence of the monomer of bovine β -lactoglobulin as synthetic peptides, including some overlapping synthetic peptides.

In the rat sera samples analysed by INRA, the IgE-binding capacity was very similar, whether the fragments used were tryptic peptides or the corresponding synthetic peptides. However, of much greater interest was the demonstration that the most frequently recognized peptides by human and rat IgE were mainly the same, namely the amino acid sequences 21–40, 41–60, 102–124 and 148–162. It should be noted that peptide 125–135 was also well recognized by rat IgE whereas it corresponded with a rather weakly immunoreactive region of β -lactoglobulin for human IgE, and that in the BIBRA studies the synthetic sequence 74–84 (β LG3) was frequently recognized by rat IgE. Unlike the human situation, however, aliquots from a single food (milk) or protein (β -lactoglobulin) were used in the laboratory sensitization regimens which could have resulted in increased recognition of some regions.

During the course of our studies it became evident that if the BN rats were sensitized with milk (which contains about 10% β -lactoglobulin) followed by β -lactoglobulin, higher IgE titres were induced than if the animals were sensitized to β -lactoglobulin alone [17]. We therefore considered it important to ascertain whether the different immunization regimes resulted in qualitative differences

in the IgE response to peptides. Indeed, the regimes used to induce specific IgE responses in the BN rats were shown to affect both the frequency and intensity of recognition of the immunoreactive regions. When milk-based immunization regimens were used to induce responses they showed an increased capacity to induce β -lactoglobulin-specific IgE compared with when a purified β -lactoglobulin preparation alone was used. Sequences 21–40 and 41–60 were especially dependent on the context in which β -lactoglobulin was presented. It is interesting to note that both sequences could be visualized as exposed loops on the surface of the protein molecule [32], and may therefore more easily be carried to the immune system in a fat emulsion [19]. Such observations give credence to the belief that other constituents within the food may play a role in inducing allergenic responses.

A further question raised has been whether the allergenic epitopes identified in β -lactoglobulin are contained within or different from those regions recognized by IgG antibodies. Comparisons of IgE and IgG recognition of β -lactoglobulin-derived synthetic peptides on a small number of samples suggested that the immunoreactive regions recognized by IgE and IgG were not always the same. This suggests that the allergenic sites of β -lactoglobulin did not always correspond with the antigenic determinants. However, although some sequences appeared predominantly IgE or IgG reactive, recognition was variable and the number of remaining sera samples too small for any conclusions to be drawn.

As demonstrated in these studies there appears to be a good correlation between immunoreactive peptides (epitopes) in β -lactoglobulin identified in sera of milk-allergic patients with those identified in sensitized rats [17]. This provides evidence that the immune system of the BN rat and the human — at least in the case of milk allergens — is recognizing similar protein allergens and indeed, at the molecular level, similar peptide epitopes. The route of immunization applied during this study was intraperitoneal administration and not ingestion which supports the concept that enzymatic digestion in the gastrointestinal tract did not affect the processing and presentation of the immunoreactive determinants of β -lactoglobulin.

Manifestations of allergy to β -lactoglobulin were not monitored during these studies but earlier work at BIBRA, where rats were parenterally sensitized to milk proteins then challenged orally with the allergen showed that levels of serum RMCPII were raised from 5 to 30 μ g/mL, thus demonstrating local mucosal mast cell involvement [30].

The intensity and affinity of the rat IgE responses were found to be lower than the corresponding human responses. This suggests that the intraperitoneally-sensitized rat IgE response may be more specific towards conformational

epitopes while the corresponding response of orally sensitized humans would be more directed toward linear epitopes [24].

Nevertheless we are confident that at least three major human IgE epitopes of β -lactoglobulin have been recognized by the rat IgE sera and that the Brown Norway rat can, under appropriate conditions, be useful in screening for the potential allergenicity to humans of different food components.

Acknowledgements

We would like to thank Linda Law, Clare Mills and M. R. A. Morgan (Institute of Food Research, Norwich, UK) for the gifts of synthetic peptides LL7, GB32, LL1, LL9, GB39, LL3, LL10, LL4, LL8 and LL6 and Samantha Williams, R. A. Badley and P. Davis (Unilever Research, Colworth, UK) for peptides β -LG5, β -LG1, β -LG3, β -LG4, β -LG6, β -LG7, β -LG8, Pep1 and β -LG9. We would also like to thank Gary Matthews and Mary Scott for expert assistance.

This project was supported by the Commission of the European Union Agro-Industrial Research Programme, AIR2-CT94-0970, and the UK Ministry of Agriculture, Fisheries and Food to whom our thanks are due.

References

- Host A, Halken S. A prospective study of cows milk allergy in Danish infants during the first 3 years of life. *Allergy* 1990; 45:587-96.
- Wüthrich B, Johansson SGO. Allergy to cheese produced from sheep's and goat's milk but not to cheese produced from cow's milk. *J Allergy Clin Immunol* 1995; 96:270-3.
- André F, André C, Colin L, Cacaraci F, Cavagna S. Role of new allergens and of allergen consumption in the increased incidence of food sensitisation in France. *Toxicology* 1994; 93:77-83.
- Ortolani C, Pastorello EA, Farioli L et al. IgE-mediated allergy from vegetable allergens. *Ann Allergy* 1993; 74:470-6.
- Challacombe SJ. Investigation of the secretory and systemic immune responses to ingested materials in animal models. In: Miller K, Nicklin S, eds. *Immunology of the Gastrointestinal Tract*, Vol II. Boca Raton, FL, USA: CRC Press, 1987:99-124.
- Dahlgren UIH, Wold AE, Hansen LA, Midtvedt T. Expression of dietary protein in *E. Coli* renders it strongly antigenic to gut lymphoid tissue. *Immunology* 1991; 73:394-7.
- Atkinson HAC, Johnson IT, Gee JM, Grigoriadou F, Miller K. Brown Norway rat model of food allergy: effect of plant components on the development of oral sensitization. *Food Chem Toxicology* 1996; 34:27-32.
- McLaughlan P, Anderson KJ, Widdowson EH, Combs RA. Effect of heat on the anaphylactic-sensitising capacity of cow's milk, goat's milk and various infant formulae fed to guinea-pigs. *Arch Dis Child* 1981; 56:165-71.
- Placenti GL, Bertolini A, Spezia E, Piscione T, Boner AL. Ability of a new infant formula prepared from partially hydrolysed whey to induce anaphylactic sensitisation; evaluation in a guinea pig model. *Allergy* 1994; 49:361-4.
- Holt P, Turner KJ. Persistence of IgE secreting cells that are refractory to T-cell control. *Int Arch Allergy Appl Immunol* 1985; 77:54-6.
- Bazin H, Platteau B. Production of circulating reaginic (IgE) antibodies by oral administration of ovalbumin to rats. *Immunology* 1976; 30:679-84.
- Knippels LMJ, Penninks AH, Spanhaak S, Hoabens GF. Oral sensitization to food proteins: a Brown Norway rat model. *Clin Exp Immunol* 1998; 28:368-75.
- Atkinson HAC, Miller K. Assessment of Brown Norway rat as a suitable model for the investigation of food allergy. *Toxicology* 1994; 91:281-8.
- Jarrett EE, Haig DM, McDougall W, McNutty E. Rat IgE production. II. Primary and booster reaginic antibody responses following intradermal or oral immunisation. *Immunology* 1976; 30:671-7.
- Hoffman A, Jamin A, May S, Hausteine D, Vieths S. A new in vitro model for testing of food allergens: allergen specific mediator release of passively sensitised rat basophil leukemia cells. *Food Agric Immunology* 1997; 9:309-25.
- Wal J-M, Bernard H, Creminon C, Hamberger C, David C, Pettre G. Cow's milk allergy: the humoral immune response to eight purified allergens. In: McGhee J, Mestecky J, Thaskalova H, Steizl J, eds. *Advances in Experimental Medicine and Biology: Recent Advances in Mucosal Immunology*. New York, NY, USA: Plenum Press, 1995:878-81.
- Sélo I, Clément G, Bernard H et al. Allergy to bovine β -lactoglobulin: specificity of human IgE to tryptic peptides. *Clin Exp Allergy* 1999; 29:1055-63.
- Miller K, Matthew GS, Smyth M, Fitzgerald R. The establishment of an animal model for the screening of food products with potentially allergenic constituents. *Biochem Soc Transactions* 1997; 27:376S.
- Ju HR, Matsuura I, Yamada K, Sugano M, Imaizumi K. Systemic release of mucosal mast-cell protease in primed Brown Norway rat after feeding with β -lactoglobulin. *Biosci Biotech Biochem* 1995; 59:771-5.
- Matthews GS, Wal J-M, Creminon C, Miller K. Acetylcholinesterase-based enzyme-linked immunosorbent assay (ELISA) for the determination of antigen-specific rat IgE. *Food Agric Immunology* 1997; 9:97-106.
- Grassi J, Frobert Y, Pradelles P et al. Production of monoclonal antibodies against interleukin-1a and 1b. Development of two enzyme immunometric assays (EIA) using acetylcholinesterase and their application to biological media. *J Immunol Methods* 1989; 123:1170-3.
- Pradelles P, Grassi J, Maclouf J. Enzyme immunoassay of eicosanoids using acetyl cholinesterase as a label: an alternative to radio immunoassay. *Anal Chem* 1985; 57:1170-3.
- Sélo I, Negroni L, Creminon S, Grassi J, Wal J-M. Preferential labelling of α -amino N terminus groups in peptide by biotin: an application for coating procedures in enzyme immunoassays. *J Immunol Methods* 1996; 199:127-38.

- 24 Kaminogawa S. Food allergy, oral tolerance and immunomodulation — their molecular and cellular mechanisms. *Biochem* 1996; 60:1749–56.
- 25 Kim SM, Enomoto A, Hachimura S, Yamauchi K, Kaminogawa S. Serum antibody response elicited by a casein diet is directed to only limited determinants of a S1-casein. *Int Archs Allergy Immunol* 1993; 101:260–5.
- 26 Miller K, Nicklin S. Mechanisms of food intolerances. Development and use of experimental animal models. In: Walker R, Quattrucci E, eds. *Nutritional and Toxicological Aspects of Food Processing*. London: Taylor & Francis, 1988, 351–60.
- 27 McMenamin C, Schon-Hegrad M, Oliveo J, Girn B, Holt PG. Regulation of IgE responses to inhaled antigens: cellular mechanisms underlying allergic sensitization versus tolerance induction. *Int Arch Allergy Appl Immunol* 1991; 94:78–82.
- 28 McMenamin C, Holt PG. The natural immune response to inhaled soluble protein antigens involves major histocompatibility complex (MHC) class I-restriction CD8⁺ T cell mediated but MHC class II-restricted CD4⁺ T cell-dependent immune deviation resulting in selective suppression of immunoglobulin E production. *J Exp Med* 1993; 178:889–99.
- 29 Atkinson H, Meredith C. Comparison of the inherent allergenicity of a range of dietary proteins using the Brown Norway rat. *Human Exp Toxicol* 1998; 17:500.
- 30 Miller K, Laugee J, Meredith C. The monitoring of the effects of food components on immunoreactivity in experimental animals. *Allergy* 1998; 53:35–7.
- 31 Wal J-M, Bernard H, Yvon M et al. Enzyme immunoassay of specific human IgE to purified cows milk allergens. *Food Agric Immunology* 1995; 7:175–87.
- 32 Williams SC, Badley RA, Davies RJ, Puijk WC, Meloen RH. Detailed epitope mapping of bovine beta lactoglobulin. *Biochem Soc Trans* 1997; 25:161S.