

Effect of heat denaturation on beta-lactoglobulin-induced gastrointestinal sensitization in rats: Denatured β LG induces a more intensive local immunologic response than native β LG

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Beta-lactoglobulin (β LG) is one of the first foreign antigens encountered by a newborn child, and it is the major allergen causing cow's milk allergy. Heat denaturation causes changes to the protein structure, but the significance of heat-induced changes for immunogenicity or allergenicity is not known. To clarify how heat denaturation affects allergenicity and immunogenicity, we immunized Hooded-Lister rat pups with intra-peritoneal injections of native or heat-denatured β LG at days 43 and 62 after birth. The animals were then fed native and denatured milk products twice weekly from 73 to 101 days of age with a feeding tube, after which they were allowed cheese and milk *ad libitum*, until they were killed on day 131. Total immunoglobulin (Ig)E and β LG-specific IgG₁ and IgG_{2a} levels were determined from serum samples. Spontaneous interleukin-4 (IL-4) and interferon- γ (IFN- γ) production was measured from duodenal specimens, and specimens of gastrointestinal mucosae were studied for the presence of inflammatory cells. The rats immunized with native β LG had higher levels of total serum IgE than the unimmunized controls or the rats immunized with heat-denatured β LG, while heat-denatured β LG induced a significantly more intensive mononuclear inflammatory cell and eosinophil infiltration in the gastroduodenal mucosa. The β LG-specific IgG antibody and IL-4 and IFN- γ responses were similar in the two groups of immunized animals. Hence, denaturation modifies the immunogenic and allergenic properties of β LG. Heat-denatured β LG induces a more intensive local reaction in the gastrointestinal mucosa, while there is some evidence for enhanced systemic allergic sensitization by native β LG.

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Recent studies indicate that hypersensitivity reactions against cow's milk (CM) proteins affect up to 2.4% of infants (1), thus making cow's milk allergy (CMA) the most common food allergy in this age-group. Milk allergy is not a clear-cut entity, and the symptoms vary (2). This

variation may be related to differences in the dominant types of hypersensitivity reactions involved. The immediate (type I) reaction based on immunoglobulin (Ig)E antibodies is considered to be the most common, at least in infants. The reaction may take place on the mucosa or in

the circulation, and it is manifested soon after exposure. Typically there is infiltration of eosinophilic leucocytes to the reaction site and into the peripheral blood.

A cell-mediated reaction (type IV) has also been proposed as one of the central mechanisms in food allergy (3). This reaction type may be more important for the development of local lesions in the small intestinal mucosa, referred to as cow's milk protein enteropathy which, in its most severe forms, morphologically mimics coeliac disease (4).

The detection of milk protein-specific IgG antibodies (5) and duodenal lymphoid hyperplasia (6), suggesting local production of IgG class antibodies, in children with CMA indicates that a type III reaction might also be involved. The clinical manifestations resulting from such reactions may be local intestinal and/or general, and the latency (with respect to the ingestion of the relevant food allergen) lasting from hours to days, or even weeks (7).

Beta-lactoglobulin (β LG) is the major protein of bovine whey and the most important milk protein causing CMA (8). Along with several other allergens of animal origin, β LG belongs to the protein family of lipocalins (9,10).

During the dairy processing of liquid milk products, different heat treatments, such as pasteurization, cause changes (denaturation) in the native structure of β LG. This might alter the allergenic properties of β LG by changing the digestibility of the protein (11), and conformational alterations may expose new antigenic sites with different allergenic properties (12). The importance of the denaturation of whole β LG molecules in the pathogenesis of milk allergy has not, however, received much attention.

Animal models of food allergy have mainly focused on the detection of systemic IgE responses (13,14), or mediator release and

increased mucosal permeability after an antigen challenge (15). Structural or immunologic features of the local response in the gastrointestinal tract have not been studied in detail. In view of the variability of the morphological and clinical patterns of food allergy (FA) in humans, an analysis of local reactions in animal models might conceivably correlate better to the conditions in humans and provide an improved understanding of the pathogenic mechanisms in FA.

The purpose of the present study was to evaluate the significance of the heat denaturation of β LG on immune responses and mucosal structure in an animal model. After parenteral immunization and low-dose chronic exposure to native or heat-denatured β LG, we analyzed the systemic effects and the local response in the gastrointestinal tract.

Materials and methods

Animal model

Conventionally outbred Hooded-Lister rats (Laboratory Animal Center, University of Oulu, Oulu, Finland) were housed in the Laboratory Animal Center, University of Oulu. The stock of rats was HsdOla:LH.

The schedule of the immunization and feeding of animals is presented schematically in Fig. 1. For immunization, native β LG containing the variants A and B (Sigma Chemical Co. St Louis, MO, USA) was further purified using anion-exchange chromatography (16). Denaturation of β LG was accomplished by heating the solution of native β LG for 30 min at 90°C immediately before immunization. This heat treatment induces reasonably consistent denaturation (17). The animals ($n=8-12$ in each group) were injected twice, intra-peritoneally, with 10 μ g of either native or heat-denatured β LG and 0.5 ml

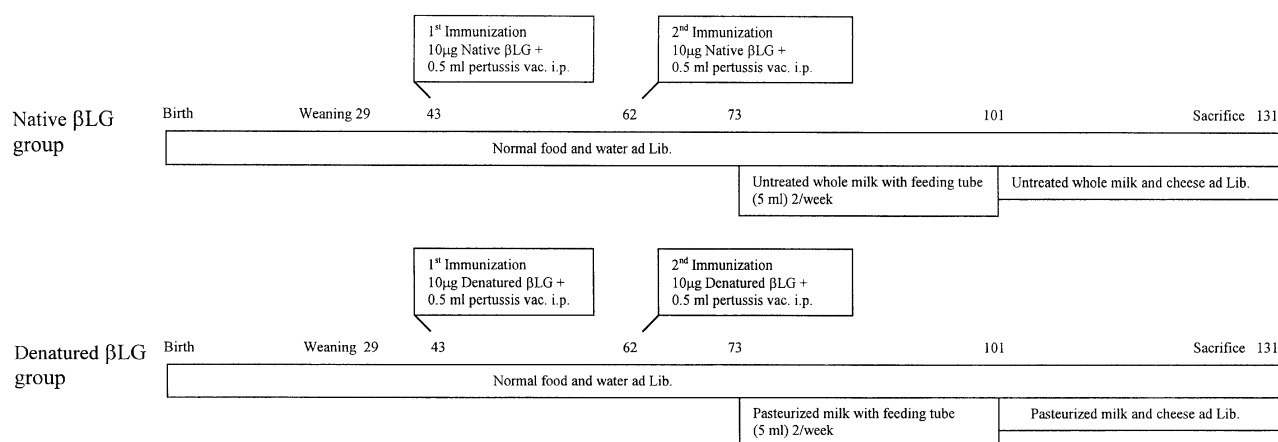


Fig. 1. Summary of the immunization procedure and the diets of the experimental animals.

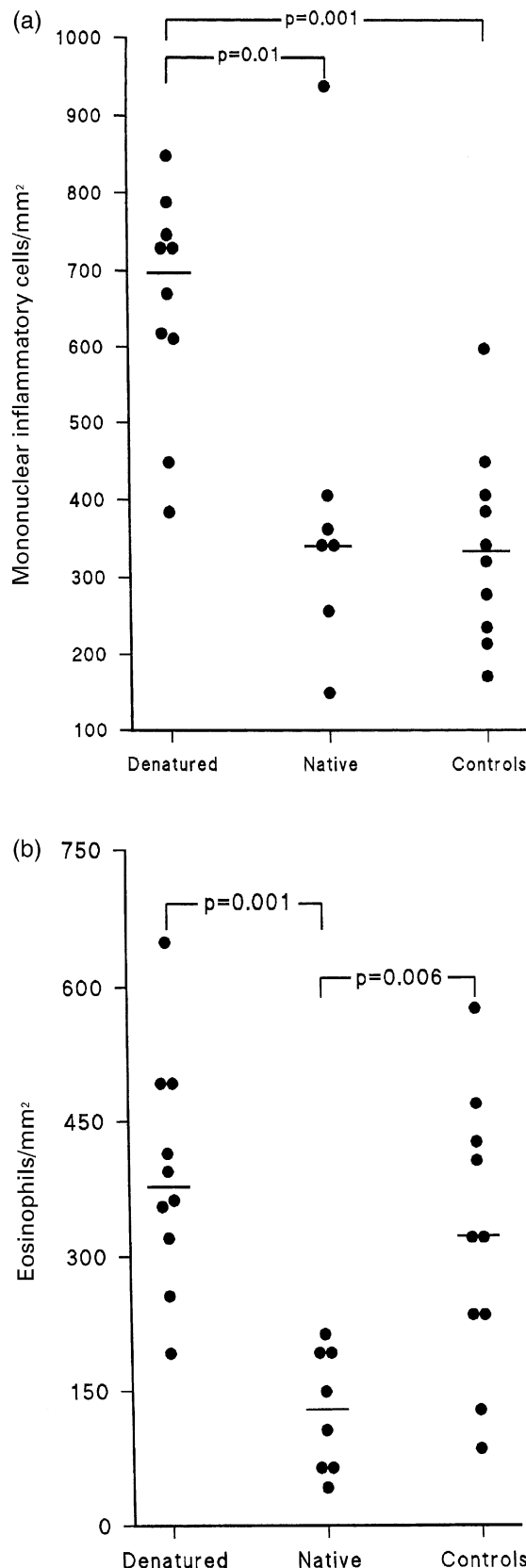


Fig. 2. Density of mononuclear inflammatory cells (a) and eosinophils (b) in gastric antral mucosa in untreated controls and in rats sensitized with native or heat-denatured β -lactoglobulin (β LG).

of pertussis vaccine, containing whole inactivated bacteria (Public Health Institute, Helsinki, Finland), at 43 and 62 days of age. Starting after the second immunization, during days 73–101, the pups injected with the denatured β LG were fed 5 ml of pasteurized milk with a feeding tube twice a week, while those injected with native β LG received unprocessed milk. During days 102–130, the sensitized rats were allowed *ad libitum* normal animal food [Rat and mouse No3, RM3 (E) Breeding expanded; SDS Special diet services, Witham, Essex, UK], water, cheese and pasteurized (denatured β LG group) or unprocessed (native β LG group) milk. The control animals ($n=10$) were not immunized and were fed normal animal food and water *ad libitum*. On day 131, the rats were killed using ketamine anaesthesia.

The study was approved by the Ethical Committee for Animal Studies, University of Oulu.

Histopathology

On day 131, tissue samples were taken from the antrum of the stomach, duodenum, proximal jejunum (≈ 5 cm from the pylorus), and colon. The specimens were fixed in 10% neutral-buffered formalin and embedded in paraffin. Sections were cut at 5- μ m and stained with hematoxylin and eosin. All cell counts were made by one of the investigators (J.R.) blinded to the treatment data and other results.

Epithelial cells, intraepithelial lymphocytes, and eosinophils were counted (total 300 cells) in the specimens obtained from the duodenum and jejunum. The results were expressed as numbers of intra-epithelial lymphocytes or eosinophils per 100 epithelial cells.

The densities of mononuclear inflammatory cells and eosinophils were counted separately in the glandular deep-lamina propria and foveolar (superficial) lamina propria of antral mucosa, in the villous lamina propria of duodenal mucosa, and in the inter-cryptal glandular lamina propria of colon mucosa. Using a $\times 40$ objective (in gastric mucosa) or a $\times 100$ objective (in intestinal mucosa) and an eyepiece with a graticule of 20×20 squares, at least three random fields of lamina propria were counted from each specimen and the mean was calculated. The results of the cell counts are expressed as number of cells/0.001 mm². The foveolar lamina propria of antral mucosa contained too few cells to permit inter-group comparisons.

In the duodenal samples, the number of lymphoid follicles in the whole sample area was

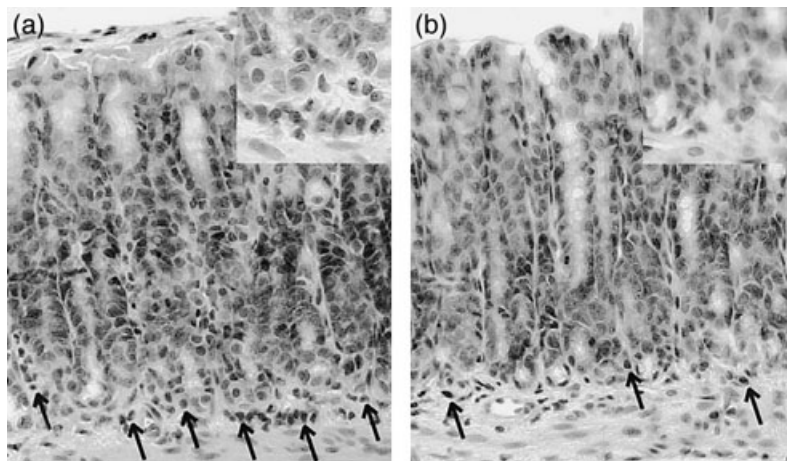


Fig. 3. Photomicrographs of rat gastric antral mucosa. Rats immunized with heat-denatured β -lactoglobulin (β LG) (a) show increased density of inflammatory cells (arrows) in the lower lamina propria compared to rats immunized with native β LG (b). Insets show details of the glandular deep-lamina propria of gastric mucosa.

counted and expressed as number/mm². A lymphoid follicle was defined as a dense, roundish collection of lymphocytes. Both lymphoid follicles with and without germinal centres were counted.

Serum immunoglobulin E levels

Blood samples were obtained through cardiac puncture on day 131. Serum was separated and frozen at -20°C . An enzyme-linked immunosorbent assay (ELISA) was developed for the measurement of rat serum IgE concentrations and validated by measuring samples with known concentrations of rat IgE and by confirming inter-assay reproducibility. Costar 3590 micro-

titer plates (Life Technologies AB, Täby, Sweden) were coated with 200 μl /well of 0.25 $\mu\text{g}/\text{ml}$ monoclonal mouse anti-rat IgE antibody (Clone MARE-1; Biosource Int., Camarillo, CA, USA) diluted in phosphate-buffered saline (PBS) (Life Technologies AB, Täby, Sweden), pH 7.4. The plates were incubated at 37°C in a moist chamber for 2 h and then at 4°C for 3 h. The plates were washed four times with PBS-Tween-20 (Life Technologies AB) and blocked with a solution of 0.5% human serum albumin (HSA) (Hoechst-Behring, Frankfurt, Germany) in PBS-Tween-20 (PHT) and incubated in a moist chamber at 37°C for 3 h and then at 4°C overnight. The plates were used immediately after coating. The following incubations took place in a moist chamber at

Table 1. Quantitation of mucosal inflammation in untreated controls and in rats immunized with heat-denatured or native β -lactoglobulin (β LG)

	Denatured β LG	Native β LG	Controls
Antrum			
Mononuclear inflammatory cells	0.7 (0.4–0.8)*	0.3 (0.2–0.9)*	0.3 (0.2–0.6)*
Eosinophils	0.4 (0.2–0.7)†	0.1 (0.04–0.2)†	0.3 (0.09–0.6)†
Duodenum			
Mononuclear inflammatory cells	6.4 (5.3–8.0)	7.6 (5.9–9.3)	6.1 (4.8–10.1)
Eosinophils	0.5 (0.09–1.6)	0.3 (0.09–0.7)‡	0.9 (0.2–1.5)‡
Intraepithelial lymphocytes	12.5 (2.4–21.1)	7.9 (4.9–16.7)§	16.2 (8.8–26.4)§
Intraepithelial eosinophils	0.8 (0–1.1)	0.0 (0–1.5)	1.0 (0–1.9)
Lymphoid follicles	0.0 (0–0.32)	0.13 (0–0.32)	0.21 (0–0.46)
Jejunum			
Mononuclear inflammatory cells	8.2 (5.8–10.4)	7.5 (6.0–8.7)	7.1 (5.7–9.6)
Eosinophils	0.7 (0.2–1.0)	0.8 (0.4–1.2)	0.9 (0.5–1.1)
Intraepithelial lymphocytes	15.4 (4.2–25.1)	15.5 (9.6–21.3)	17.9 (8.9–31.1)
Intraepithelial eosinophils	0.8 (0.3–2.0)	1.0 (0–1.6)	1.2 (0.4–2.2)
Lymphoid follicles	0.15 (0–0.4)	0.03 (0–0.21)¶	0.17 (0–0.5)¶
Colon			
Mononuclear inflammatory cells	0.4 (0.2–1.0)	0.5 (0.4–0.7)	0.4 (0.2–0.8)
Eosinophils	0.07 (0.04–0.2)	0.07 (0–0.5)	0.1 (0.04–0.3)

Data are expressed as median (range).

Cells were counted in different parts of the gastrointestinal tract. Cell density was counted separately in the lamina propria (cells/0.001 mm²) and the surface epithelium (intraepithelial cells/100 epithelial cells). The number of lymphoid follicles refers to the number of follicles/mm².

* $p = 0.01$ denatured/native; $p = 0.001$ denatured/control.

† $p = 0.001$ denatured/native; $p = 0.006$ native/control.

‡ $p = 0.009$ native/control; § $p = 0.01$ native/control.

¶ $p = 0.037$ native/control.

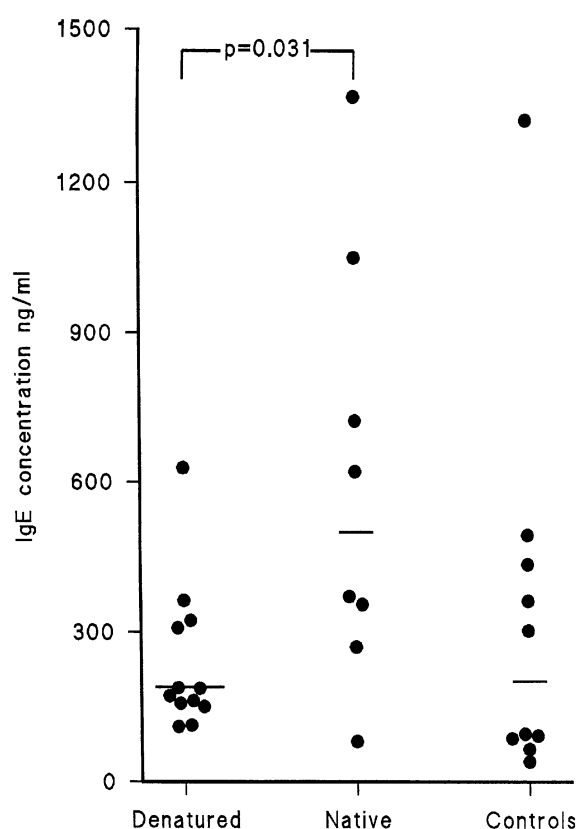


Fig. 4. Serum total immunoglobulin E (IgE) levels in untreated controls and in rats sensitized with native or heat-denatured β -lactoglobulin (β LG).

37°C for 1 h, followed by four washes with PBS–Tween-20. At each of the following steps, the added volume of reagent was 100 μ l/well. Rat sera diluted 1 : 25 in PHT or PHT without serum (blanks) were placed in duplicate wells. Biotin-conjugated monoclonal mouse anti-rat IgE (clone MARE-1; Biosource Int.), at a concentration of 0.25 μ g/ml, and then horseradish peroxidase-conjugated streptavidin (Streptavidin poly HRP; CLB, Amsterdam, the Netherlands) diluted 1:10,000 in high-performance ELISA

buffer (HPE; CLB), were added. Substrate to HRP [3,3', 5,5'-Tetramethyl-Benzidine (TMB); Sigma] was added and incubated at room temperature. After 30 min, the reaction was terminated by adding 1.8 M H_2SO_4 . The optical densities (OD) were read at 450 nm in an Anthos htIII ELISA reader (Anthos Labtec Instruments, Salzburg, Austria). OD values of the blanks were subtracted from those of the samples.

Specific IgG₁ and IgG_{2a} antibodies to ovalbumin and β -lactoglobulin

ELISAs for the measurement of β LG/OVA-specific IgG₁ and IgG_{2a} antibodies were also produced. Costar 3590 microtiter plates were coated with 200 μ l/well of 25 μ g/ml OVA (Sigma) or 10 μ g/ml β LG (Sigma) diluted in PBS. The coating antigen was OVA in the standard lanes and β LG for the samples. The plates were incubated at 37°C in a moist chamber for 2 h and then at 4°C for 3 h. The plates were washed four times with PBS–Tween-20, blocked with PHT, and incubated in a moist chamber at 37°C for 3 h and at 4°C overnight.

The following incubations took place in a moist chamber at 37°C for 1 h, followed by four washes with PBS–Tween-20. At each of the following steps, the added volume of reagent was 100 μ l/well. Rat sera diluted 1 : 10 in PHT or PHT without serum (blanks) were added to duplicate wells. The reference serum, a rat serum known to contain antibodies to OVA, in a dilution range of 1 : 800–1 : 51,200, was used. Monoclonal mouse anti-rat IgG₁ (clone MARG1-2; Biosource Int.; 3 μ g/ml) and IgG_{2a} (clone MARG2a-1, Biosource Int.; 1 μ g/ml) antibody followed by alkaline phosphatase (ALP)-conjugated rabbit anti-mouse IgG (rabbit anti-mouse IgG ALP conjugate; Sigma; 1 : 5000 for IgG₁ and 1 : 2500 for IgG_{2a}) were added. A substrate to ALP (para-nitrophenyl

Table 2. Measurement of serum total immunoglobulin (IgE), β -lactoglobulin (β LG)-specific IgG₁ and IgG_{2a}, and spontaneous secretion of interferon- γ (IFN- γ) or interleukin-4 (IL-4), from duodenal mucosa in controls and in animals immunized with heat-denatured or native β LG

	Denatured β LG	Native β LG	Controls
IgE (ng/ml)	180 (110–630)*	500 (80–1370)*	200 (40–1320)
IgG ₁ (OD)	0.03 (0.00–0.06)	0.021 (0.01–0.06)†	0.03 (0.02–0.09)†
IgG _{2a} (OD)	0.06 (0.03–0.18)	0.06 (0.04–0.11)	0.09 (0.06–0.17)
IL-4 (pos./tot.)	55% (5/9)	36% (3/8)	70% (7/10)
IL-4 (pg/ml)	7.7 (0–51)	0 (0–101.4)	9.3 (0–34)
IFN- γ (pos./tot.)	55% (5/9)	36% (3/8)	10% (1/10)
IFN- γ (pg/ml)	28.1 (0–282)‡	0 (0–158.8)	0 (0–88.5)‡

* $p = 0.031$ denatured/native.

† $p = 0.037$ native/controls.

‡ $p = 0.038$ denatured/controls (Kruskal–Wallis).

Data are expressed as median (range). For IFN- γ and IL-4 the proportion of cases with detectable secretion (pos./tot.) are indicated, and for IgG the optical density (OD) measurements are given.

phosphate (pNPP), 200 µl/well; Sigma) was added, and the reaction was incubated at room temperature. After 30 min, the reaction was terminated by the addition of 3 M NaOH (50 µl/well). The ODs were read at 405 nm in an Anthos htIII ELISA reader (Anthos Labtec Instruments). OD values of the blanks were subtracted from those of the samples.

Measurement of interferon- γ and interleukin-4

For the evaluation of spontaneous secretion of IFN- γ and IL-4, fresh specimens taken from the duodenum on day 131 were weighed and placed immediately in cell culture medium (RPMI, 0.5 ml) and incubated at 37°C for 5 h. The supernatants were frozen at -70°C. Secreted IFN- γ and IL-4 were measured using ELISAs (Quantikine M murine IFN- γ , RIF00; IL-4, R4000; R & D Systems, Abingdon, Oxon, UK) according to the manufacturer's instructions but with minor modifications. Volumes of 100 µl/well of sample and standards were incubated at room temperature for 2 h. After five washes with washing solution, conjugate (100 µl for IL-4 and 120 µl for IFN- γ) was added and incubated at room temperature for 2 h. After five washes, 100 µl of substrate was added and incubated at room temperature for 30 min. The reaction was stopped by using stopping solution at 100 µl/well. The OD was read at 450 nm in a Labsystems ascent ELISA reader (Labsystems, Helsinki, Finland). The blanks were subtracted from the sample ODs.

Statistics

Statistical analysis was performed using SPSS (version 9.0; SPSS Inc., Chicago, IL, USA). Groups were compared using the non-parametric Kruskal–Wallis test or chi-square test for dichotomous variables.

Results

To determine whether heat denaturation had any effect on the immunogenic or allergenic properties of β LG, rats were sensitized with either native or heat-denatured β LG when $\approx$ 6 and 9 weeks of age and then fed with the respective antigens, while the control group was not immunized and was fed with normal food pellets. After $\approx$ 13 weeks the rats were studied to evaluate whether these regimens had caused any differences in the structure of the gastrointestinal mucosa, serologic response or local lymphokine secretion.

Morphology of the gastrointestinal tract

The intensity of the mucosal inflammatory cell reaction was analyzed in the different parts of the gastrointestinal tract. The lamina propria in the glandular zone of antral mucosa obtained from the rats immunized with heat-denatured β LG contained a greater number of mononuclear inflammatory cells than the corresponding tissue of rats immunized with native β LG ($p = 0.01$) or in the untreated controls ($p = 0.001$; Table 1, Fig 2a, and Fig. 3a, 3b). The density of eosinophils was higher in the rats immunized with denatured β LG than in those immunized with native β LG ($p = 0.001$), and similar to that of the controls (Table 1, Fig. 2b), while controls had a greater number of eosinophils than rats immunized with native β LG ($p = 0.006$). There was a significant correlation between eosinophil and lymphocyte infiltrations of the lamina propria in the antral mucosa samples ($r = 0.60$, $p < 0.001$).

The cell counts in the duodenum were overall similar in the three groups of rats. The density of intraepithelial eosinophils tended to be higher in the animals immunized with heat-denatured β LG ($p = 0.09$) as compared to those immunized with native β LG. In addition, as shown in Table 1, the densities of duodenal intraepithelial lymphocytes ($p = 0.01$) and lamina propria eosinophils ($p = 0.009$) were significantly higher in the controls than in rats immunized with native β LG. The densities of eosinophils and mononuclear inflammatory cells in the jejunum and colon were similar in the three groups. The number of lymphoid follicles in the jejunum was significantly higher in the controls as compared to the native β LG group ($p = 0.037$; Table 1).

Local cytokine production

Spontaneous secretion of IFN- γ and IL-4 from duodenal samples was low during the 5-h incubation period (Table 2), and there was no significant difference in the number of animals with detectable secretion between the immunized groups. However, median secretion of IFN- γ was higher in rats immunized with heat-denatured β LG as compared with controls ($p = 0.038$; Table 2). The IFN- γ concentrations did not correlate with the cell densities in the mucosal samples from any of the four regions. The IL-4 concentrations, on the other hand, correlated with the density of mononuclear inflammatory cells in colonic mucosa ($r = 0.63$, $p = 0.002$).

Serum IgE, IgG₁ and IgG_{2a} levels

The rats immunized with native β LG had a higher total IgE concentration in serum than the rats immunized with heat-denatured β LG (Table 2, Fig. 4) ($p = 0.031$). The density of intraepithelial lymphocytes in the jejunal samples correlated with the serum IgE levels ($r=0.39$, $p = 0.03$).

The levels of β LG-specific IgG₁ and IgG_{2a} were low in all groups and, in some cases, below the measurement range of the assay, making the conclusion about specific IgG response unsatisfactory. IgG_{2a} tended to be higher in the control group than in the group immunized with native β LG ($p = 0.037$), but no other significant differences were observed (Table 2). The IgG_{2a} concentrations correlated significantly with the jejunal intra-epithelial lymphocytes ($r=0.38$, $p=0.04$).

Discussion

Our findings suggest that native and heat-denatured β LG from bovine milk provoke different immunologic responses. Native β LG induced an increase in the total serum IgE, suggesting an IgE-mediated and systemic response, while heat-denatured β LG produced a more local response without evidence for an IgE-mediated systemic response. In accordance with this, heat-denatured β LG induced a stronger inflammatory response in mucosa than native β LG. This was most evident in antral mucosa, as shown by the increased numbers of mononuclear inflammatory cells and eosinophils. In the duodenal samples, a similar trend was found for eosinophils and intraepithelial eosinophils and lymphocytes. More distally in the jejunum and colon, no significant differences were seen in the two groups of immunized animals.

Thermal modification involves mainly conformational changes, which may be associated with chemical modifications of the molecule, such as scrambling of disulfide bonds (18). These alterations may have an effect on allergenicity by either enhancing or decreasing digestibility and by hiding, destroying or disclosing antigenic epitopes. As B-cell epitopes are mostly conformational and dependent on the three-dimensional structure of antigen, while T-cell epitopes are linear, it is probable that denaturation would primarily alter the B-cell reactivity pattern. Reversibility of the thermal modification of β LG is related to temperature, with high-temperature treatment inducing more permanent changes (17). While a high-temperature (90°C)

denaturation protocol was used in the present study to achieve stable denaturation, it should be noted that the denaturation pattern after conventional pasteurization at 72°C may be different and therefore its effect on immunogenic properties may not be identical to that of the high-temperature denaturation.

There is some evidence to indicate that heat denaturation makes β LG more susceptible to pepsin hydrolysis (11,19) and might therefore decrease its allergenic properties by reducing the number of conformational B-cell epitopes. Supporting this notion, pepsin digestibility has been shown to have a significant, negative correlation with the allergenicity of β LG in a dog model (20). In the present study we were not able to analyze the effect of denaturation on the levels of specific anti- β LG IgE. However, an increased concentration of total serum IgE was induced by native β LG, but not by heat-denatured β LG. We speculate that IgE increase associated with native β LG might be similarly connected to a predominance of B-cell epitopes, while the absence of any IgE response, together with the local inflammatory cell reaction induced by heat-denatured β LG, might be connected with a predominance of T-cell epitopes.

Heat denaturation of β LG is associated with the appearance of at least one new epitope that is not present in the native state (12). At least six regions of human IgE binding have been identified in β LG (21). The same epitopes are recognized by rat IgE (14), supporting the validity of a rat model for mechanistic studies of human allergic disease. It is of interest that while most epitopes are targets for IgE, some are more selective for IgG (14). In our study, the absence of an IgE increase after immunization with heat-denatured β LG favors a T helper 1 (Th1)-predominant reaction. While Th1 activation is connected with the IgG response, the result would agree with the idea that heat denaturation exposes a relatively greater number of IgG targets. However, no significant differences in the titres of specific IgG antibodies were observed in our study. All IgG₁ and IgG_{2a} levels were low and close to the detection limit of our assay, thus making it difficult to reach a conclusion regarding differences in the specific IgG response. The reason for the lack of detectable IgG response to β LG remains unresolved, but could be related to the dosage or timing of the immunization.

The cytokine production, as evaluated by spontaneous secretion from the duodenal samples, was low in all groups and no differences between the two β LG-treated groups were observed. However, in comparison with controls,

animals immunized with heat-denatured β LG produced more IFN- γ , further suggesting the presence of some Th1 polarization in this group. The overall low level of cytokine secretion is in line with human studies, which have shown that even the allergic state in early infancy is associated with a poor Th1 (IFN- γ) and Th2 (IL-4) function, while the polarization of the response appears later (22).

Milk allergens in human patients are almost always heat-denatured proteins, as unprocessed milk is seldom available. Milk allergy in infants is usually associated with gastrointestinal symptoms, and specific milk protein antibodies of the IgG isotype are usually present, while IgE antibodies are less common (23). The number of individuals reacting merely locally in the mucosa may be higher than estimated, as clinical verification of such reactions is possible only by a mucosal biopsy.

The antrum of the stomach and the bulb of the duodenum were the predominant sites of mucosal infiltration of cells, while the more distal parts of the gastrointestinal tract showed no effects of the immunization procedures. These findings support previous observations in humans, which have shown that the lesions in milk-allergic infants are located in the antral mucosa (24), while school-age children with milk allergy show an increase of lymphoid tissue in the bulb of the duodenum (25).

In conclusion, modification of the β LG molecule by heating seems to alter its immunological properties in such a way that the heat-denatured protein induces a stronger local reaction in the foregut mucosa. The native protein induced a more generalized response, as ascertained by increased total IgE concentrations in the serum samples. The results suggest that the denaturation processes of CM proteins might have implications for the pathogenesis of gastrointestinal milk allergy in humans.

Acknowledgments

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