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Research paper

Hydrolyzed whey protein prevents the development of food allergy to β -lactoglobulin in sensitized mice

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ABSTRACT

Food allergy is an adverse immune response to dietary proteins. Hydrolysates are frequently used for children with milk allergy. However, hydrolysates effects afterwards are poorly studied. The aim of this study was to investigate the immunological consequences of hydrolyzed whey protein in allergic mice. For that, we developed a novel model of food allergy in BALB/c mice sensitized with alum-adsorbed β -lactoglobulin. These mice were orally challenged with either whey protein or whey hydrolysate. Whey-challenged mice had elevated levels of specific IgE and lost weight. They also presented gut inflammation, enhanced levels of SIgA and IL-5 as well as decreased production of IL-4 and IL-10 in the intestinal mucosa. Conversely, mice challenged with hydrolysate maintained normal levels of IgE, IL-4 and IL-5 and showed no sign of gut inflammation probably due to increased IL-12 production in the gut. Thus, consumption of hydrolysate prevented the development of clinical signs of food allergy in mice.

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1. Introduction

Food allergy is an adverse immune response to dietary proteins affecting up to 1–3% of adults and 3–8% of infants in westernized countries where prevalence rates are currently increasing. Cow's milk is introduced into a child's diet early in life, making allergy to the milk proteins one of the most common food allergies in early childhood. Major allergens characterized in cow's milk are distributed among the casein and whey fractions and include α S1-casein and β -lactoglobulin (BLG), respectively [1–3]. Although immunoglobulin E (IgE) antibodies against all major milk proteins have been found in blood sera of allergic subjects, BLG is considered to be the dominant cow's milk allergen [4].

The pathophysiology of cow's milk allergy involves IgE-mediated type I hypersensitivity and T cell-mediated delayed-type hypersensitivity reactions. Symptoms involve skin,

gastrointestinal and respiratory tracts and even systemic anaphylaxis may occur [5,6]. There is no cure for food allergies so far. The main treatment strategy for most food allergies is based on allergen avoidance, which may present potential adverse nutritional effects related to inadequate growth, neurological development and cardiovascular health [7]. Thus, hypoallergenic formulas using cow's milk hydrolysates are a good alternative for either infants at high risk or infants diagnosed with cow's milk allergy [8].

Hypoallergenic formulas are usually produced by enzymatic hydrolysis of different protein sources such as bovine casein/whey followed by further processing such as heat treatment and/or ultrafiltration. These hydrolyzed formulas are generally categorized as partial and extensive hydrolysates based on the degree of hydrolysis. Alternatively, they can be based on amino acid mixtures. Only pure amino acid mixtures are considered to be nonallergenic and even hypoallergenic products present residual allergenicity [9]. However, their allergy-preventive potential seems not only to depend on the degree but also on the process of hydrolysis. Both partially and extensively hydrolysed formulas can be used for primary prevention of allergy in infants at high atopic risk, while only extensively hydrolysed formulas are indicated for secondary prevention in patients with manifested cow's milk

Abbreviations: BLG, β -lactoglobulin; IgE, immunoglobulin E; SIgA, secretory immunoglobulin A; (DCs), dendritic cells.

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allergy [10]. For safety reasons, it is required that the sensitizing capacity of hypoallergenic formulas was tested in an animal model [11,12].

Therefore, the aims of this study were to develop an experimental murine model of food allergy to the cow's protein BLG that mimic the main clinical characteristics of human disease as well as to examine the allergenic and immunological properties of extensively hydrolyzed whey proteins using this model. In addition, we verified the effect of hydrolyzed whey protein in food aversion. This phenomenon was first described by Cara and coworkers using BALB/c mice sensitized to ovalbumin adsorbed in alum. As part of the clinical features of the allergic reaction, these mice developed aversion to the ingestion of saccharin-sweetened solutions containing the allergen [13]. The mechanisms involved in this behavioral change were traced to a strong activation of emotionally-related brain areas and were shown to be dependent on serum IgE antibodies [14]. To our knowledge, there was no information available on food aversion in BLG food allergy models or in the effects of hydrolyzed whey protein in this behavioral phenomenon.

2. Materials and methods

2.1. Animals

Male BALB/c mice at four weeks of age were obtained from the university animal facility (ICB/UFGM). Mice received water and food *ad libitum* throughout the experimental period. All procedures were in accordance with the ethical principles in animal experimentation, adopted by the Ethics Committee in Animal Experimentation of our institution (CETEA/UFGM).

2.2. Whey protein hydrolysate

Whey protein hydrolysate was obtained from Arla Food Ingredients, Denmark (Peptigen IF-3080). The degree of hydrolysis was 29%. Whey protein concentrate was obtained from Alibra, Brazil. The percentage of protein source in hydrolysate was 81.7% and from whey protein concentrate was 80%.

2.3. Mice sensitization and oral challenge

Mice from control group were sensitized by an intraperitoneal (i.p.) injection of 0.2 ml saline (0.9%) containing 1 mg $\text{Al}(\text{OH})_3$ as adjuvant while mice from sensitized groups received i.p. injection of 0.2 ml saline (0.9%) containing adjuvant and 20 μg BLG (Sigma, St. Louis, MO, USA). Fourteen later, sensitized mice received an i.p. booster immunization with saline containing 20 μg soluble BLG and control mice received only i.p. saline. Seven days thereafter, mice were orally challenged with either 20% whey protein solution or 20% whey protein hydrolysate in the drinking bottle as their only source of liquid for a period of 7 days (Fig. 1A). Solutions were replaced daily.

2.4. Clinical evaluation and tissue collection

Body weight was determined weekly throughout the experiment and daily during allergen challenge. By using disease activity index (DAI) [15], the body weight and stool were scored every day after antigen challenge (day 0–7 post challenge) as follows: body weight score (zero – no weight loss; one – 1–5% weight loss; two – 6–10% weight loss; three – 11–15% weight loss; four – above 15% weight loss); stool viscosity (zero – normal; two – fluffy; four – diarrhea); blood presence in stool (zero – normal; two – apparent blood). During the oral challenge period, whey protein solution

consumption was assessed daily by weighting the remaining solution and comparing it with the weight obtained in the previous day. Before starting the oral challenge (Day 0) and 7 days thereafter, mice were anesthetized by i.p. injection of 10 mg/kg xylazine and 100 mg/kg ketamine hydrochloride and blood samples were obtained from all groups. Mice were then euthanized under deep anesthesia and epididymal fat as well as small intestine were collected.

2.5. Serum antibody measurement

Serum samples for measuring anti-BLG IgE antibodies were obtained from all mice 7 days after the last oral antigen exposure. Anti-BLG IgE were measured by capture-ELISA using plates coated with rat anti-mouse IgE. Fifty μl total serum, biotinylated BLG, and HRP-labeled streptavidin were added to the wells. Reaction developed by adding H_2O_2 in the presence of orthophenylenediamine (OPD, Sigma) as previously described [16]. Results are reported in arbitrary units using a positive reference serum (1000 U).

2.6. Histological analysis

After 7 days of oral challenge, mice were sacrificed by cervical dislocation. Tissue samples from the proximal jejunum were taken for histological analysis. They were fixed in 10% formalin in PBS, embedded in paraffin and cut into 5 μm thick sections. Sections were stained with hematoxylin-eosin (HE) and then evaluated by light microscopy (Olympus BX41). The intestinal mucosa was submitted to morphometric analysis using the Image J software (National Institutes of Health, Bethesda, Maryland, USA). Images were captured using a digital camera coupled to a microscope (Olympus BX41). Ten fields from HE-stained sections were randomly chosen at $10\times$ ($871.392 \mu\text{m}^2/\text{field}$) for villus height and crypt depth measurement. Data were reported as villus and crypt size measured in μm . Crypt length was considered as the distance from the bottom to the opening of crypt with intact columnar epithelia. Ten fields from HE-stained sections were randomly chosen at $40\times$ ($53.333 \mu\text{m}^2/\text{field}$) to count the number of eosinophils, and data are reported as number of eosinophils/field [17].

2.7. Secretory IgA

Small intestine was rinsed with 10 mL of cold PBS. Intestinal lavages were centrifuged at 12,000g for 20 min at 4°C , and levels of secretory IgA in the supernatants were determined by ELISA as previously described [18].

2.8. Measurement of proximal jejunum cytokines

Proximal jejunum samples were weighed and homogenized in PBS containing 0.05% Tween-20, 0.1 mM phenylmethylsulphonyl fluoride, 0.1 mM benzethonium chloride, 10 mM EDTA and 20 KIU Aprotinin A using a tissue homogenizer (100 mg tissue/ml buffer). Suspensions were centrifuged at 12,000g for 20 min at 4°C and the supernatants were transferred to microtubes and stored at -80°C until analysis. Concentrations of IL-4, IL-5, IL-10 and IL-12 were measured by enzyme-linked immunosorbent assay (ELISA) as previously described [18]. Briefly, supernatants were added to microtitre plates (Nunc), previously coated with purified monoclonal antibodies reactive to IL-4, IL-5, IL-10 and IL-12 (BD Bioscience). Standards and samples were added and incubated overnight at 4°C . Biotinylated monoclonal antibodies against cytokines were added and incubated for 1 h at room temperature, after which peroxidase-labelled streptavidin (Sigma) was added. A color reaction was developed at room temperature with 100 μL /well of orthophenylenediamine (1 mg/ml) and 0.04% H_2O_2

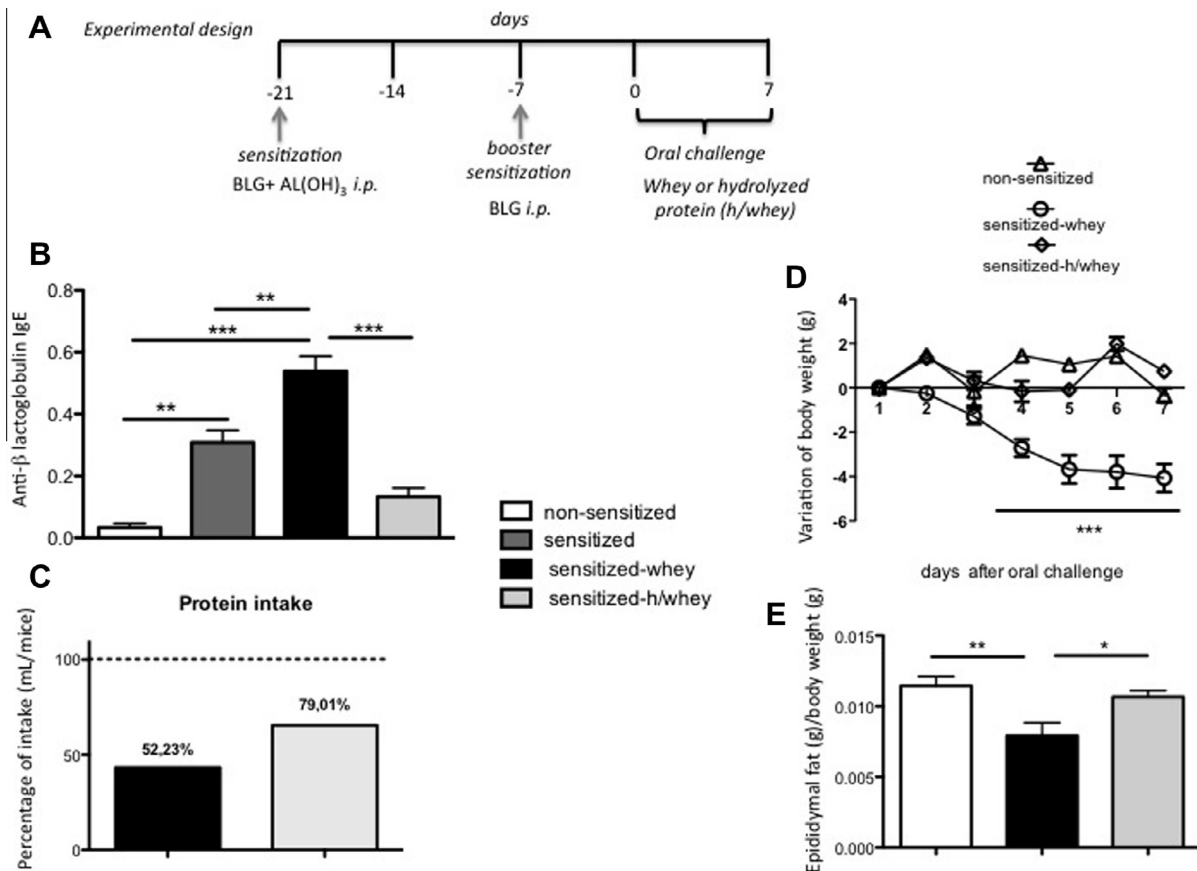


Fig. 1. Oral challenge with extensively hydrolyzed whey protein reduced signs of food allergy. (A) Sketch of the experimental protocol used in our study. Mice from sensitized groups received 0.2 ml saline (0.9%) by i.p. injection with adjuvant and 20 μ g β -lactoglobulin (BLG). Two weeks later, sensitized mice received a booster sensitization by i.p. injection of saline containing 20 μ g BLG and mice from control group (non-sensitized) received only saline. One week afterwards, drinking bottles containing tap water were replaced by bottles containing either 20% whey protein solution (sensitized-Whey) or whey protein hydrolysate (sensitized-h/whey) for a period of one week. (B) anti- β -lactoglobulin IgE levels were measured in sera of mice after oral challenge. (C) Whey protein solution intake was assessed every day during oral antigenic challenge and data was represented as percentage of average solution consumption (mL/mice): sensitized group/non-sensitized whey. Dashed line represents average consumption of mice from the non-sensitized group. (D) Body weight was assessed weekly before antigenic challenge and daily afterwards. (E) Epididymal fat was collected, weighted and correlated to body weight. All data, except protein intake, were represented as means $\pm$ SEM of ten mice per group. $p < 0.05$. ** $p < 0.01$, *** $p < 0.001$ ANOVA-Tukey.

substrate in sodium citrate buffer. Reaction was terminated by the addition of 20 mL/well of 2 N H₂SO₄. Absorbance was measured at 492 nm by an ELISA reader (Bio-Rad Model 450 Microplate Reader).

2.9. Statistical analysis

Results were expressed as the mean $\pm$ SEM, as indicated in the figure legends. Kolmogorov–Smirnov test confirmed normal distribution of samples. Significance was determined by the ANOVA-Tukey and Student *t*-tests (for protein intake), with $P < 0.05$ defining significance as compared to the control group.

3. Results

3.1. Oral challenge with extensively hydrolyzed whey protein prevented the development of food allergy signs in β -lactoglobulin sensitized mice

IgE has a critical role in the allergic response and in the resulting avoidance to consume the allergen (aversion phenomenon) [14]. Continuous ingestion of whey protein in the bottle during 7 days by sensitized mice resulted in a significant increase in

specific IgE production when compared to non-sensitized and sensitized groups. However, oral challenge with hydrolyzed whey prevented specific IgE production enhancement (Fig. 1B). In order to follow the development of antigen aversion, analysis of protein intake was performed daily during the 7 days of continuous oral challenge. Sensitized mice that were challenged with whey consumed a lower percentage of protein solution (52.23%) when compared to non-sensitized mice (dashed line in Fig. 1C) challenged with whey suggesting that they developed aversion to the allergen. On the other hand, mice that were challenged with hydrolyzed whey consumed about 79% of solution (Fig. 1C). Weight loss is an important clinical feature of experimental food allergy [7]. Four days after allergen ingestion started, sensitized mice that were challenged with whey continuously lost weight until day 7. Mice challenged with hydrolysate did not present any weight loss (Fig. 1D). Similarly, a marked reduction of epididymal adipose tissue mass in mice challenged with whey protein solution was observed. Hydrolysate solution also prevented epididymal adipose tissue reduction (Fig. 1E). In addition, ingestion of hydrolysate instead of whey solution protected mice from the development of clinical signs of allergy as measured by a disease activity index (DAI) that includes diarrhea, rectal bleeding and weight loss (Fig. 2A).

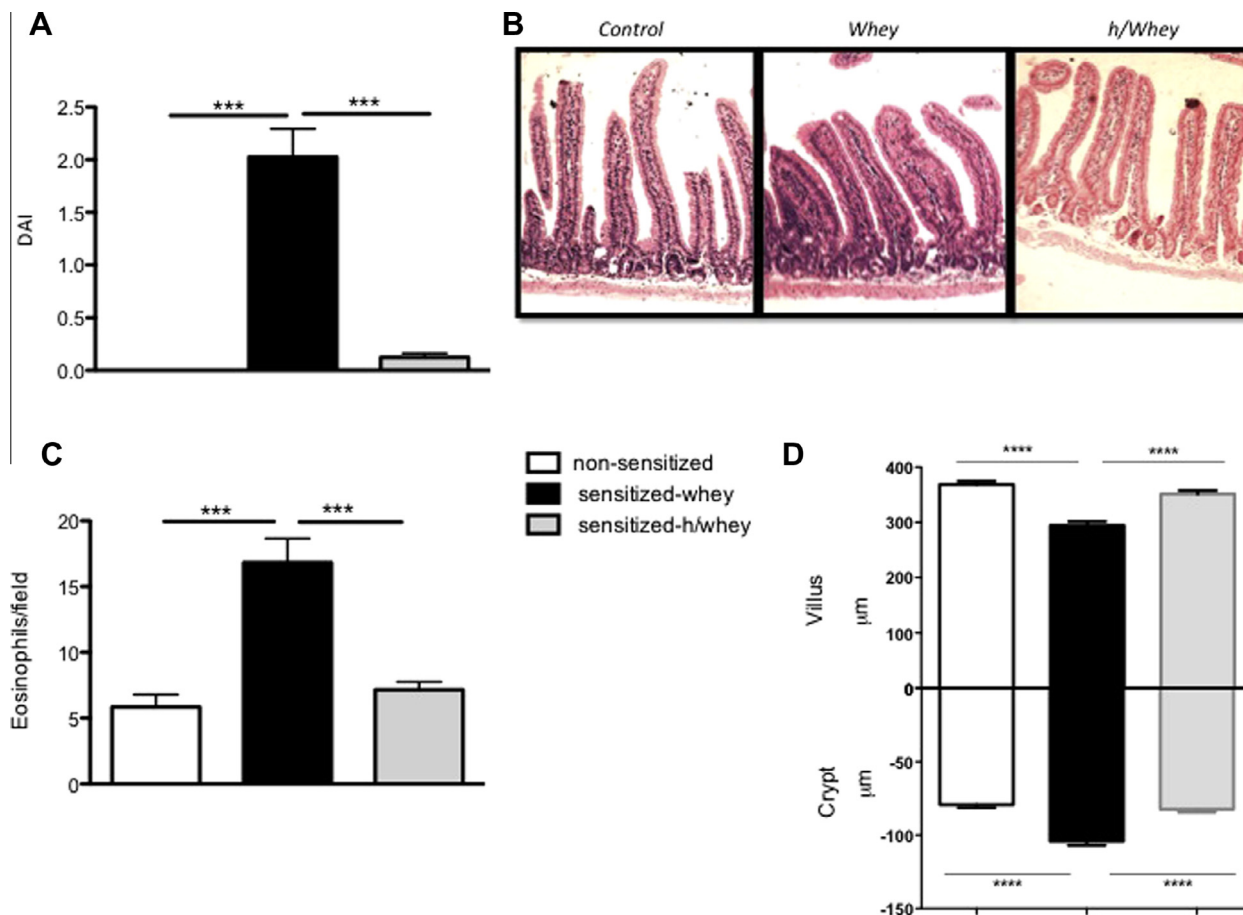


Fig. 2. Oral challenge with extensively hydrolyzed whey protein prevented intestinal inflammation in sensitized mice. (A) Disease activity index (DAI) was scored as detailed in methods. (B) Representative photomicrographs of hematoxylin–eosin stained proximal jejunum tissue of mice sensitized or not after 7 days of oral challenge. (C) Number of eosinophils per field. (D) villus/crypt ratio. Data were represented as means $\pm$ SEM of ten mice per group. Legend: Whey: whey protein solution or h/whey: hydrolyzed whey protein solution. *** p < 0.001 ANOVA–Tukey.

3.2. Oral challenge with extensively hydrolyzed whey protein prevented intestinal inflammation in β -lactoglobulin sensitized mice

Ingestion of whey solution induced submucosal edema and increased inflammatory cell infiltration in the gut of sensitized mice (Fig. 2B). As part of this inflammatory reaction, there was a 10-fold increase in the number of eosinophils in the gut mucosa of whey-challenged but not of hydrolysate-challenged mice when compared to control group of non-sensitized mice (Fig. 2C). Size of villus and crypt is one of the most sensitive parameters to intestinal inflammation and it is altered in children that are allergic to cow's milk [25]. Indeed, allergic whey-challenged mice had altered villus and crypt size whereas hydrolysate-challenged animals had a size similar to the one found in non-sensitized group (Fig. 2D). Thus, oral challenge with whey hydrolysate prevented the development of intestinal inflammation as well as the local accumulation of eosinophils.

3.3. Oral challenge with extensively hydrolyzed whey protein prevented the increase in the intestinal levels of secretory IgA (sIgA)

Since sIgA contributes to the barrier function and it is involved in immunological homeostasis [19], we measured levels of this immunoglobulin in small intestinal lavage fluids. sIgA levels were augmented in allergic mice following local inflammation. Oral challenge with hydrolysate instead of whey protein prevented such augment (Fig. 3).

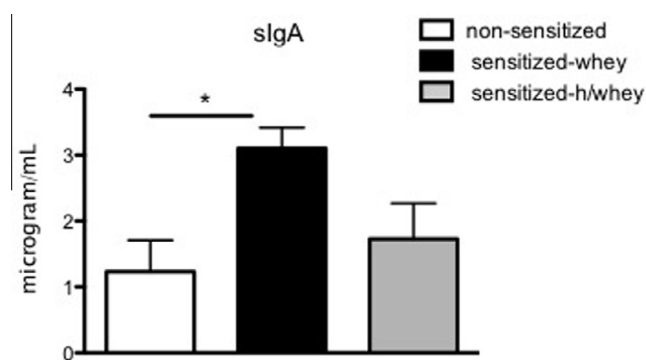


Fig. 3. Oral challenge with extensively hydrolyzed whey protein prevented secretory IgA (sIgA) augment. sIgA levels were measured in small intestine lavage by ELISA as described in methods. Data were represented as means $\pm$ SEM of ten mice per group. Legend: Whey: whey protein solution or h/whey: hydrolyzed whey protein solution. * p < 0.05, ANOVA–Tukey.

3.4. Oral challenge with extensively hydrolyzed whey protein enhanced IL-12 in gut mucosa of β -lactoglobulin sensitized mice

In order to determine whether whey hydrolysate could modulate cytokine production in the site of inflammation, we measured the levels of IL-4, IL-5, IL-10 and IL-12 in proximal jejunum tissues. Oral challenge with whey protein promoted reduction in the levels of IL-4 as well as IL-10 and induced an increase in IL-5 production

in allergic mice. Whey protein hydrolysate had no effect on the levels of either IL-4 or IL-5 in sensitized mice. However, production of the anti-inflammatory cytokine IL-10 diminished and the pro-inflammatory IL-12 production was increased in mice that ingested hydrolyzed protein (Fig. 4).

4. Discussion

In this study, we aimed two goals. First we developed a novel model of food allergy to a common allergen present in whey proteins of cow's milk that reproduced the main signs of the human disease [20]. Then, we tested the effects of ingestion of intact versus hydrolyzed whey in already sensitized mice. Despite the fact that whey protein concentrate and hydrolyzed whey protein came from different companies, the amount of protein in the products were very similar (around 80%). In addition, both were submitted to microbiological test. Thus, it is plausible to assume that there was no interference in the results due to the origins of the products tested.

Our mouse model of food allergy to BLG displayed the main features already described for other food allergy models, namely the one described by Saldanha and coworkers [17] using the hen's egg protein ovalbumin. Allergic mice sensitized with BLG and challenged with whey (which contains the sensitized antigen), had high levels of serum anti-BLG IgE, and presented clinical as well as histological signs of intestinal inflammation. In addition, they showed a reduction in length of villus and crypt in the intestine. One previously work described a significant decrease in the villus/crypt ratio in jejunal biopsy of children with eczema due to food allergy [21]. According to this, Gómez-Chávez and colleagues found partial atrophy of the villi in the jejunal mucosa of children with allergy to cow's milk [22]. Thus, our model has inflammatory parameters similar to the ones found in human disease.

Allergic mice also presented aversion to the allergen, which was measured by the intake of the antigen-containing solution. Immunologically induced taste aversion is an important component of food allergy as documented before and it is related to IgE responses [14,23]. In the present study, we showed that sensitized mice challenged with whey protein concentrate drank 50% less solution when compared to non-sensitized mice challenged with whey. Thus, the food aversion phenomenon was observed also in our model using BLG as an allergen. Mice that were challenged with hydrolysate drank almost 80% of the solution when compared to non-sensitized mice challenged with whey protein concentrate. Thus, reduction in consumption in this group was less prominent than the one observed in mice from the sensitized and whey-challenged group. It is noteworthy that the degree of food aversion (avoidance of antigen consumption) in both groups of sensitized mice was related to the specific IgE levels found in these mice as already described by Basso and coworkers using the ovalbumin model of food allergy [14].

Since BLG is a major allergenic component of cow's milk, this protein has been already used for sensitization in mouse models of allergy. Most of these studies, however, used mice that were only sensitized with native BLG adsorbed in either alum [24] or incomplete Freund's adjuvant intraperitoneally [25] to produce high levels of IgE. Some studies from the same group have used challenges with BLG either by intranasal route to evaluate respiratory allergic reaction [24,26] or by a single gavage to evaluate serum inflammatory mediators [24]. However, previous studies did not examine gut inflammation and food aversion as allergic inflammatory parameters.

We also demonstrated that extensively hydrolyzed whey protein displayed hypoallergenic properties during oral challenge. Oral challenge with hydrolysate maintained the anti-BLG IgE levels

similar to the values found in sensitized mice. Therefore, that oral treatment with hydrolysate did not reduce sensitization, but it induced a state of stabilization of the specific antibody response. A previous study by Verdolin and coworkers [27] reported a similar result of oral administration of ovalbumin to mice that have been immunized afterwards with ovalbumin adsorbed in alum. Ovalbumin fed mice had variable levels of specific IgE after immunization but these levels stayed stable after several boosters with the same antigen whereas saline fed mice presented progressively higher levels of IgE during the course of sequential boosters. Stabilization seems to be a variation of oral tolerance phenomenon although it is not clear, by this previous study, whether the mechanisms involved in both events are the same.

Since IL-10 is an important cytokine involved in oral tolerance induction and in the maintenance of maintain gut homeostasis [18], local levels of this cytokine were measured in the proximal jejunum of allergic mice. There was a reduction in IL-10 production in both allergic and hydrolysate-fed mice.

To test whether the decrease in the Th2 response observed in hydrolysate-fed mice was associated with a skewed immune response, the Th1 cytokines IFN- α and IL-12 was evaluated. Levels of IFN- α were not detected in jejunum from any group of mice, but we found enhanced levels of IL-12 in proximal jejunum of mice treated with hydrolyzed protein. IL-12 is an important cytokine for the differentiation of Th1 cells [28]. Duan and coworkers also found that hydrolysates of BLG significantly enhanced production of IFN- α secretion by murine splenocytes of mice orally treated with hydrolysates before sensitization [29]. Therefore, our data suggest that hydrolysates prevented the enhancement in IgE production in sensitized mice and this effect was associated with up-regulation of Th1 cytokines such as IL-12.

The immunomodulatory properties of different forms of BLG have been assessed using oral tolerance by intragastric administration of BLG in a mouse model of food allergy. In these studies, previous oral treatment of mice with native BLG inhibited more efficiently allergic sensitization than with hydrolysates of BLG produced by either by cyanogen bromide (CNBr) or trypsin. Conversely, synthetic peptides were inefficient for it even if covering 50% of the BLG sequence, demonstrating that the immunomodulatory potential requires the presence of all derived fragments of BLG as in the native protein. Denatured or hydrolyzed products were less efficient in inducing oral tolerance induction [24,30]. According to this, Fritsché and colleagues showed that partially hydrolyzed proteins are able to induce specific oral tolerance, whereas extensively hydrolyzed proteins are not [31]. In our work, we used extensively hydrolyzed formula, thus the mechanism seems to be not related to oral tolerance induction.

On the other hand, extensively hydrolyzed protein did not present residual peptides able to elicit an allergic response in sensitized mice. This could be due to a preferential uptake of peptides by epithelial cells instead of Peyer's patches M cells that deliver antigen directly to the Th2-driving dendritic cells sitting there [32]. Interestingly, a recent study using a cross-linked form of β -lactoglobulin (CL-BLG) in a food allergy model in BALB/c mice showed that cross-linking of BLG reduced its epithelial uptake but promoted sampling through Peyer's patches [32]. Differences in endocytosis by dendritic cells (DCs) and *in vitro* endolysosomal processing were observed between BLG and aggregated CL-BLG. Furthermore, CL-BLG primed DCs induced higher Th2 response *in vitro*. In addition, oral treatment of sensitized BALB/c mice with CL-BLG resulted in high levels of specific IgE. Therefore, it is plausible that hydrolyzed BLG may result in the exact opposite consequences driven by aggregated BLG inducing immune deviation towards Th1 responses and preventing elevation of Th2 cytokines as well as IgE production in sensitized mice.

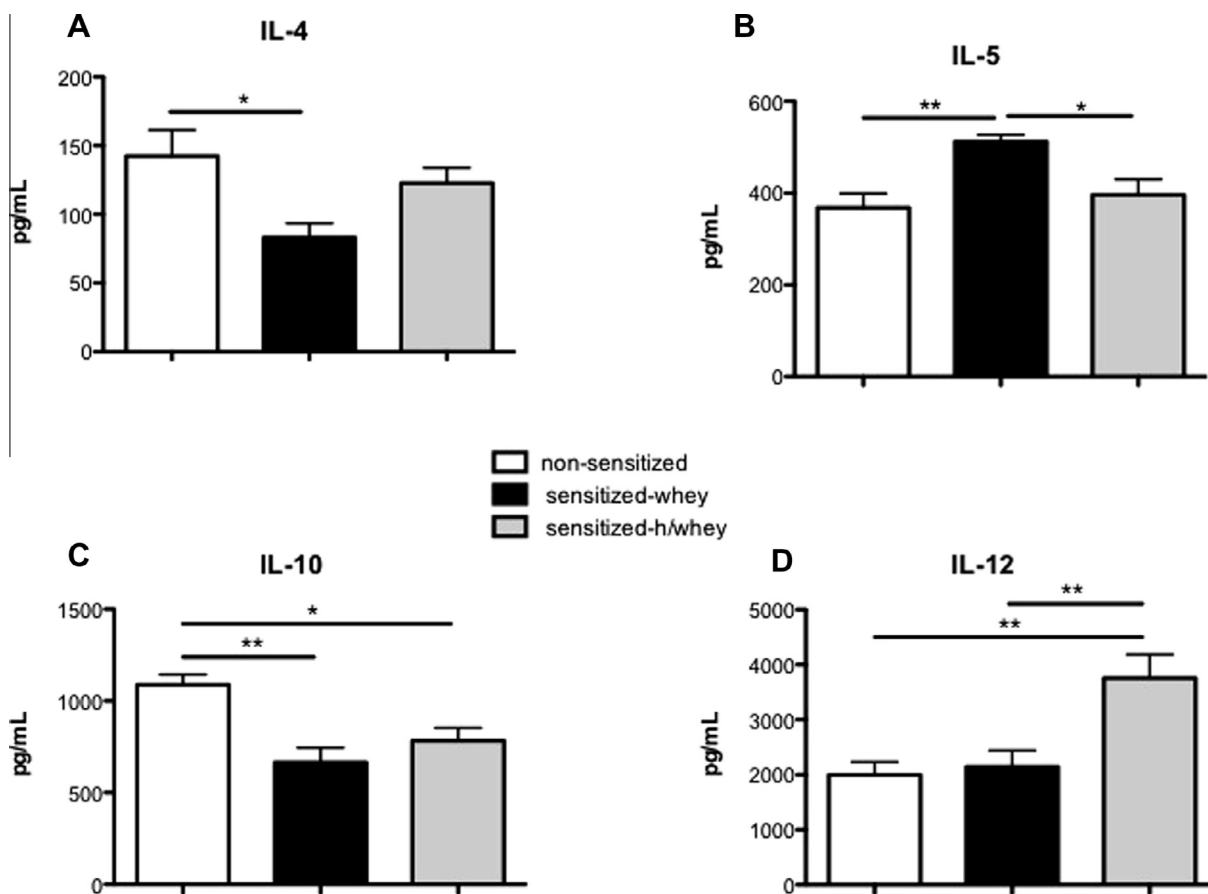


Fig. 4. Oral challenge with extensively hydrolyzed whey protein modulated cytokines in gut mucosa. (A) IL-4 (B) IL-5 (C) IL-10 and (D) IL-12 levels were measured in proximal jejunum by ELISA as described in methods. Data were represented as means $\pm$ SEM of ten mice per group. Legend: Whey: whey protein solution or h/whey: hydrolyzed whey protein solution. * $p < 0.05$. ** $p < 0.01$, ANOVA-Tukey.

IgE binds to mast cells via its high affinity receptor (Fc ϵ RI) leading to release of a large number of proinflammatory mediators and proteases into adjacent tissues [6]. Also, these activated mast cells produce Th2-type cytokines, including IL3, IL-5, and IL-13, leading to the accumulation of eosinophils [33]. Indeed, in our model, we found high levels of IL-5 in the proximal jejunum of allergic mice but not in mice treated with hydrolysate. Conversely, IL-4 levels were reduced in the proximal jejunum of allergy mice while the hydrolysate maintained IL-4 levels similar to the ones found in control group. Despite the reduction in IL-4 detected in jejunum, we found enhanced levels of IL-4 in mesenteric lymph node (data not shown). Thus, this could be due to the migration of IL-4-producing T cells to the lymphoid organs.

In addition, since hydrolysate did not enhance anti-BLG IgE levels, antigen aversion was also reduced in sensitized mice. Clinical effects of food allergy were also modulated in mice that ingested hydrolyzed whey. Allergic reaction to BLG developed with weight loss mostly due to loss of fat tissue as described previously in the ovalbumin-induced food allergy model [17] [34]. Hydrolysate prevented weight loss and reduction in adipose tissue mass improving the clinical signs of food allergy.

Inflammatory cell infiltration with high frequency of eosinophils was seen after 7 days of oral antigen exposure in allergic mice. In accordance with the reduced BLG-specific IgE production, hydrolyzed protein did not present either inflammation or eosinophil infiltration in the gut mucosa. Allergic mice but not hydrolysate-fed mice had reduced villus/crypt ratio.

In addition to many regulatory mechanisms that maintain gut homeostasis, SIgA is one of the protective mechanisms that act by antigen binding and exclusion [35]. In spite of the fact that food allergy may result in deficiency of several facets of the epithelial barrier function, including secretory IgA release into the gut lumen [36], we observed enhanced levels of SIgA in feces of BALB/c mice sensitized with BLG and challenge with the same antigen. Secretory IgA can also mediate a potent anti-inflammatory function following interaction with SIGNR1 on dendritic cells which induces immune tolerance via regulatory T cell expansion [37]. Therefore, this increase in the secretion of a protective immunoglobulin could be a regulatory mechanism triggered to counteract inflammation in the gut mucosa due to allergic reaction to BLG. Levels of SIgA enhanced in allergic mice suggesting that inflammation triggered this modulatory component of immune response. Oral administration of hydrolysate, on the other hand, had little inflammatory consequences and therefore was not accompanied by augmented levels of SIgA.

In this study we showed that hydrolyzed whey protein prevented intestinal inflammation as well as the development of clinical signs of food allergy in sensitized mice. Therefore, it seems that extensive hydrolysis decreases the ability of BLG to induce allergic inflammation. On the other hand, extensively hydrolyzed proteins did induce the modulatory mechanisms triggered by oral tolerance such as regulatory cytokines. Instead, stabilization of IgE production and reduction in allergic inflammation in sensitized mice were associated with immune deviation to Th1-like responses. Oral treatment with hydrolysate also maintained levels

of sIgA, IL-4 and IL-5 similar to the ones found in control group. Therefore, the whey hydrolysate tested was able to prevent not only the signs of inflammation but also changed immunological features typical of food allergy pathology. These results suggest that whey protein hydrolysates can be safely used by already sensitized children.

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