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Soybean glycinin- and β -conglycinin-induced intestinal immune responses in a murine model of allergy

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Glycinin and β -conglycinin are major soybean allergens involved in food hypersensitivity. The study was aimed to investigate the soybean protein-induced intestinal immune responses and its possible mechanism. Balb/c mice were sensitised intragastrically with glycinin and β -conglycinin without an adjuvant for five weeks. Results showed that the sensitised mice displayed diarrhoea symptoms and jejunal morphological changes, including decreased villous height and thickened crypt depth. Both the histamine and immunoglobulin A levels in jejunum were increased in soybean allergen-sensitised mice. Moreover, the number of IgA+ B cells and CD4+ T cells in the jejunal lamina propria of sensitised mice were significantly increased. The expression levels of interleukin-4 and interferon- γ were increased, whereas the levels of IL-10 and transforming growth factor- β (TGF- β) were decreased in the jejunum mucosa. In conclusion, glycinin and β -conglycinin induce a mixed Th1/Th2 immune response. The suppression of IL-10 and TGF- β may play important roles in the development of intestinal allergic reactions.

Keywords: glycinin; β -conglycinin; Balb/c mice; mucosal immune response; cytokine expression

1. Introduction

Soybean protein is a major source of dietary protein in food and feed industry because of its nutritional properties (Golubovic, van Hateren, Ottens, Witkamp, & van der Wielen, 2005; Hancock, Cao, Kim, & Li, 2000). However, allergic reactions induced by soybean protein are common in both human (Foucard & Yman, 1999) and farm animals (Dreau, Lalles, Philouzerome, Toullec, & Salmon, 1994; Guo, Piao, Cao, Ou, & Li, 2008). Glycinin and β -conglycinin are the two major storage proteins in soybean, constituting approximately 40% of the total seed protein (Friedman & Hilleman, 2001). Food allergy caused by the ingestion of glycinin and β -conglycinin has been documented in many reports, which ranged from mild gastrointestinal distress to suspected anaphylaxis (Dreau et al., 1994; Helm et al., 2000; Sun, Li, Dong, Qiao, & Ma, 2008a).

Several animal models have recently been built to investigate the possible mechanisms of these clinical symptoms (Guo et al., 2008; Sun et al., 2008a, 2008b; Zhao, Qin, Sun, Zhang, & Wang, 2010). The gastrointestinal mucosa is important

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in inducing the allergic responses to food proteins (Bannon, 2004). Previous studies have demonstrated that soybean protein contributes to disorder of immunological functions and intestinal damage in piglets (Hao, Zhan, Guo, Piao, & Li, 2009; Huang, Xu, Yu, Gao, & Liu, 2010) and rats (Guo et al., 2008). A combined T-helper cell (Th)1/Th2 response of the intestinal mucosa has been observed in glycinin-sensitised piglets (Hao et al., 2009). However, the relationship between immune responses and soybean protein-induced food allergy in the gut mucosal system is still not fully understood. It is also not obvious whether the differentiation of gut mucosal lymphoid cells involves in the immune responses induced by soybean allergens.

The objective of the current study was to investigate the intestinal immune responses induced by glycinin and β -conglycinin in a mice model established in our previous study (Liu, Feng, Xu, Wang, & Liu, 2008). Mice were gavaged with purified glycinin or β -conglycinin to prevent the influence of other allergenic components and were subjected to the assessment of diarrhoea. Levels of jejunal immunoglobulin A (IgA) and histamine, differentiation of intestinal lymphocytes and expression profile of cytokines were also measured for evaluation. Our study may provide valuable information in the study of soybean protein-induced allergy.

2. Materials and methods

2.1. Preparation of soybean protein

Purified soybean glycinin and β -conglycinin were offered by Prof. Shuntang Guo of the Food Institute of China Agricultural University (proteins were purified by fractional salting-out method with sodium and potassium salts of different pH, Patent No. 200410029589.4, China) and were used without further purification. The contents of glycinin and β -conglycinin were assayed by a protein assay kit (Product No. 500-0001, Bio-Rad, Hercules, USA) according to the manufacturer's instructions. The purity of protein was measured by sulphate polyacrylamide gel electrophoresis. Gels stained with Coomassie Brilliant Blue R-250 were scanned and analysed by Quantity One™ Software (Bio-Rad, Hercules, USA). The result confirmed that the samples contained more than 95% glycinin or β -conglycinin (Liu, Feng, Xu, Wang, & Liu, 2008). No endotoxin could be detected by the method of Hao et al. (2009), which suggested that the protein samples were not contaminated.

2.2. Mice sensitisation and experimental protocol

Mice were sensitised with glycinin or β -conglycinin according to our previous study (Liu et al., 2008). Briefly, thirty second-generation female Balb/c mice (six-week-old, body weight, 22.0 ± 2.41 g) were obtained from Zhejiang Chinese Medical University (Hangzhou, China). Mice were kept in stainless-steel wire cages (1 mice/cage) with soy-free feed and water *ad libitum*. Cages were put in a specific pathogen-free room maintained at $22 \pm 3.1^\circ\text{C}$, with a relative humidity of 55–65% and controlled lighting (10–12 h/day).

Mice were divided into three groups ($n = 10$) and sensitised by an intragastric administration of 1 mg of glycinin or β -conglycinin [per mouse, 1.0 ml of a filter-sterilised solution containing 1 mg/ml glycinin or β -conglycinin, dissolved in

phosphate buffered saline solution (PBS), pH 7.4] for 35 days without the use of an adjuvant. Control animals received an equal volume of sterile saline. The daily oral administration was performed using a 10-gauge stainless-steel animal feeding needle at 9 a.m. and was finished within 30–60 min by two assistants. Faecal samples were daily collected and immediately classified to determine the cases of diarrhoea. Three hours after the last sensitisation on day 35, all mice were anaesthetised and killed. Small intestinal tissue from the middle part of the jejunum was thoroughly flushed with saline to remove the intestinal contents and subsequently cut into 2 cm sections. One part of the samples was fixed in 4% paraformaldehyde/0.1 mol/l PBS, pH 7.2–7.4 at room temperature for 24 h and embedded in paraffin before cutting into 5 μ m-thick sections. The remaining segments of the jejunum were quickly frozen in liquid nitrogen and stored at - 80°C until needed for histamine, IgA and cytokines analysis. The experimental procedures followed the guidelines of the Animal Care and Use Ethics Committee of Zhejiang University (Hangzhou, China).

2.3. Diarrhoea evaluation

Mice faeces were classified by the methods of Sun et al. (2008b) and recorded daily throughout the entire experimental period.

2.4. Histology

Intestinal samples were prepared according to previously described histological procedures (Nakajima-Adachi et al., 2006). Jejunal sections of 5 μ m thickness were stained using haematoxylin and eosin. Villus height and crypt depth were determined at a 40 $\times$ magnification under a light microscope (BX-51, Olympus, Japan). Only villi fully attached to the lamina propria and with well-defined tips were measured. Ten villus heights and crypt depths of each sample were recorded from each section on randomly selected microscopic fields.

2.5. Determination of intestinal histamine level

The histamine level in the jejunum was measured by high-performance liquid chromatography (e2695, Waters, USA) according to Sun et al. (2008a). Standard sample of histamine was purchased from Sigma (St. Louis, USA). The data were analysed by integration software (Empower2™, Waters, USA).

2.6. Immunohistochemistry

Intestinal tissue sections (5 μ m thick) were dewaxed in xylene and rehydrated by graded ethanol (70%, 80%, 95% and 100%). Sections were heated at 100°C in Tris–HCl buffer with pH 9.0 for 100 s, and then washed 3 $\times$ 5' (3 times, 5 min each) with PBS. Endogenous peroxidase activity was depleted with 3% hydrogen peroxide followed by washing for 15 min in PBS (5 min $\times$ 3 times). The sections were incubated overnight at 4°C with rat monoclonal antibodies, directed anti-mouse IgA (BD Pharmingen™, Franklin Lakes, USA) diluted 1:100, anti-mouse cluster of differentiation (CD)4 (Dako, Glostrup, Denmark) diluted 1:50, anti-mouse CD8

(Dako) diluted 1:200 in PBS containing 0.05% bovine serum albumin, and PBS as negative control. After washing for 15 min in PBS (5 min $\times$ 3 times), the sections were subsequently incubated with horseradish-peroxidase-labelled goat anti-rat IgG (Dako, Glostrup, Denmark) for 40 min at 37°C. Sections were washed again in PBS and incubated in liquid diaminobenzidine solution (Dako) for 3 min for the colour reaction. Sections were then counterstained with Harris' haematoxylin for 1 min, dehydrated by a graded series of alcohols, cleared with xylene, and finally covered. Lymphocytes stained for IgA+, CD4+ and CD8+ in the lamina propria were counted by using an image analysis system (Canon, Cambridge, England) at five randomly selected areas (about 0.04 mm² each). Brownish-yellow stained lymphocytes were recognised as positive cells. The immune cells were expressed as cells/mm² tissue. All the counting were performed and confirmed by a single observer.

2.7. Detection of intestinal IgA

About 0.5 g of jejunal mucosal samples was resolved in 1 ml of PBS, centrifuged at 5000 g for 15 min at 4°C after homogenisation. The supernatant was recovered and the protein content was quantified by a protein assay kit (500-0001, Bio-Rad, Hercules, USA).

IgA levels were detected by ELISA. Ninety-six-well polystyrene ELISA plates (Corning, New York, USA) were coated with 50 μ l/well of goat anti-mouse IgA (Sigma, St. Louis, USA) and washed three times with PBS. Supernatants (100 μ l) from washings of intestine were plated in serial twofold dilutions at 37°C for 1 h and washed five times. Wells were then incubated with a horseradish peroxidase-conjugated anti-mouse IgA (Sigma, St. Louis, USA) and 0.5 mg/ml O-phenylenediamine dihydrochloride for detection of the absorbance at 500 nm. Purified mouse IgA (Sigma, St. Louis, USA) was used as the standard and all samples were accessed for a minimum of three times. The results are expressed as the amount of IgA (μ g) in 1 mg of total protein from intestinal mucus homogenate.

2.8. Quantification of cytokines interleukin-4, interleukin-10, interferon- γ and transforming growth factor- β

Total jejunal RNA was isolated using the Trizol reagent (Gibco BRL, Scotland, UK) according to the manufacturer's instructions. The complementary DNA was synthesised from 1 μ g of total RNA in a 20 μ l reaction mix by MultiScribe reverse transcriptase and random hexamers (Applied Biosystems, Carlsbad, USA) according to Vaali et al. (2006). The primers of interferon- γ (IFN- γ) (forward primer: TCA AGT GGC ATA GAT GTG GAA GAA; reverse primer: TGG CTC TGC AGG ATT TTC ATG), interleukin-4 (IL-4) (forward primer: ACA GGA GAA GGG ACG CCA T; reverse primer: GAA GCC CTA CAG ACG AGC TCA), IL-10 (forward primer: GGT TGC CAA GCC TTA TCG GA; reverse primer: ACC TGC TCC ACT GCC TTG CT) and transforming growth factor- β (TGF- β) (forward primer: AGA GGT CAC CCG CGT GCT AA; reverse primer: TCC CGA ATG TCT GAC GTA TTG A) were according to Zaheer et al. (2007). The real-time quantitative PCR was performed with the ABI PrismTM 7500 sequence detection system using SYBRTM green fluorescence (Applied Biosystems, Warrington, UK).

The standard PCR conditions were used as described by Cardoso et al. (2008). Relative values of expression were normalised using 18S rRNA (forward primer: TAA GTC CCT GCC CTT TGT ACA CA; reverse primer: GAT CCG AGG GCC TCA CTA AAC) as an endogenous internal standard and calculated using the formula $x = 2^{-\Delta\Delta C_t}$, where x = fold difference in amount of starting material between two groups (allergen-sensitised group versus non-sensitised group), $\Delta\Delta C_t = \Delta E - \Delta C$, and $\Delta E = C_{t_{exp}} - Ct18S_{exp}$, $\Delta C = C_{t_{control}} - Ct18S_{control}$. 'Ct' stands for threshold cycle that is the fractional cycle number at which the amount of amplified target reaches a fixed threshold. Negative controls without RNA were also performed and analyses were determined in triplicate.

2.9. Statistical analysis

All data were expressed as the mean $\pm$ SEM and analysed by the general linear model of SAS system (SAS Institute, Cary, USA). Polynomial contrasts were performed using Turkey's post test. Values of $p < 0.05$ were considered as significant.

3. Results

3.1. Diarrhoea occurrence in Balb/c mice sensitised with soybean glycinin and β -conglycinin

To test if the oral administration of glycinin and β -conglycinin resulted in clinical manifestation of allergy, mice were checked daily for the occurrence of diarrhoea. Three of the glycinin-sensitised mice and four of the β -conglycinin-sensitised mice showed diarrhoea symptoms (pasty, semi-liquid, and liquid feces). The symptom appeared two days after oral sensitisation and lasted for less than five days. Control mice dosed with saline failed to present any symptoms.

3.2. Morphological structure and histamine content in the jejunum of mice sensitised with soybean proteins

To investigate the effects of glycinin and β -conglycinin on the small intestine after oral sensitisation, changes in the morphologic structure of the jejunum were determined (Table 1). Noticeable histological changes were observed with decreased villous height ($p < 0.01$) and thickened crypt depth ($p < 0.01$) in the glycinin- and β -conglycinin-sensitised mice compared to the control. The jejunal histamine levels

Table 1. Effects of soybean glycinin and β -conglycinin on jejunum morphology and histamine concentration in Balb/c mice.

	Saline	Glycinin	β -conglycinin	SEM	p -Value
Villus height (μ m)	332.6 ^a	266.7 ^b	278.3 ^b	5.55	<0.01
Crypt depth (μ m)	99.5 ^b	137.5 ^a	132.2 ^a	3.23	<0.01
Histamine content (μ g/g)	5.59 ^b	8.53 ^a	8.41 ^a	0.26	<0.01

Note: Different superscripts indicate significant differences between treatments ($p < 0.05$). Each value is the mean of data from 10 mice per group; SEM, standard error of the mean.

were increased ($p < 0.01$) in mice sensitised with glycinin and β -conglycinin compared with the saline-treated mice.

3.3. Differential accumulation of lymphocytes in the jejunum

To have a quantitative characterisation of the allergic reactions induced in the damaged intestinal tissue, the number of IgA+, CD4+ and CD8+ lymphocytes in the jejunal lamina propria were counted. Numbers of IgA+ B cells and CD4+ T cells were increased ($p < 0.01$) in the mice sensitised with glycinin or β -conglycinin (Figures 1 and 2). However, the number of CD8+ T cells were not affected ($p > 0.05$) in glycinin- and β -conglycinin-sensitised mice.

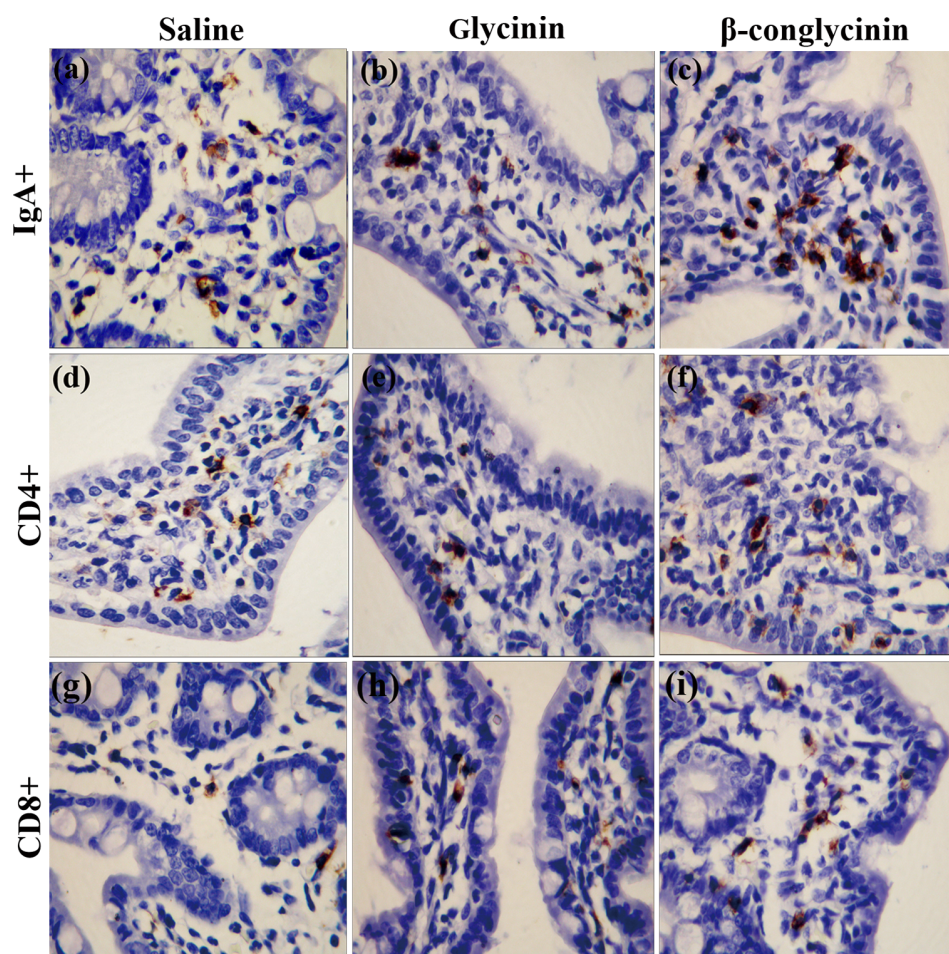


Figure 1. IgA+, CD4+ and CD8+ lymphocytes in the jejunal lamina propria. Stained sections were photographed at 400 $\times$ magnification. Positive cells were stained brownish-yellow. (a)–(c): IgA+ plasma cells in mice with saline (a), glycinin (b) and β -conglycinin (c); (d)–(f): CD4+ T cells in mice gavaged with saline (d), glycinin (e) and β -conglycinin (f); (g)–(i): CD8+ T cells in mice gavaged with saline (g), glycinin (h) and β -conglycinin (i).

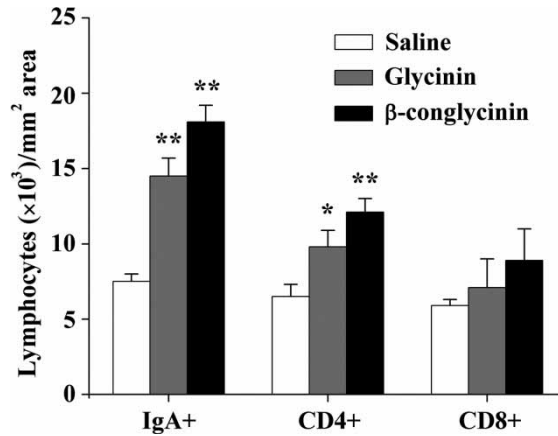


Figure 2. Number of IgA+, CD4+ and CD8+ lymphocytes per 1 mm² microscope field in jejunal lamina propria. The data are presented as mean $\pm$ SEM of 10 mice per group. Statistical evaluation was performed between soybean protein dosed mice and saline group. * represents $p < 0.05$ and ** represents $p < 0.01$ versus saline-dosed group.

3.4. Level of intestinal mucus IgA

The increase of IgA+ plasma cells in small intestine highlighted the role of IgA that participated in the pathogenesis of the food-induced intestinal inflammation. Figure 3 shows that the intestinal IgA levels in the sensitised mice were increased ($p < 0.01$) compared with those of the control group (0.27 μ g/mg and 0.37 μ g/mg, respectively).

3.5. Differential cytokine expression in the jejunum

To investigate cytokine changes in the intestinal mucosa of glycinin- and β -conglycinin-sensitised mice, IL-4, IL-10, IFN- γ and TGF- β levels were measured (Figure 4). mRNA

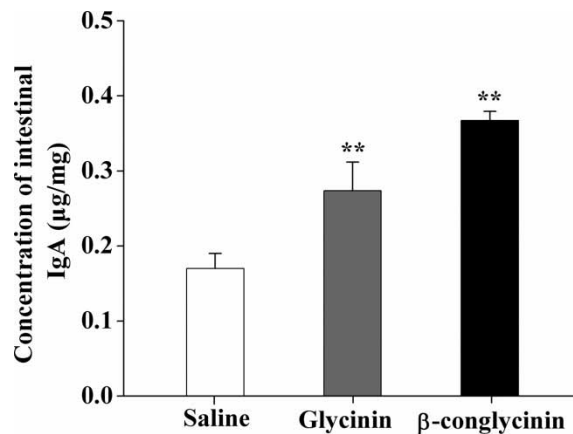


Figure 3. Effect of soybean glycinin or β -conglycinin on mucosal IgA in mice. The data are presented as mean $\pm$ SEM of 10 mice per group. Results are expressed as the amount of IgA (μ g) in 1 mg of total mucosal protein (mg). ** represents $p < 0.01$ versus saline-treated group.

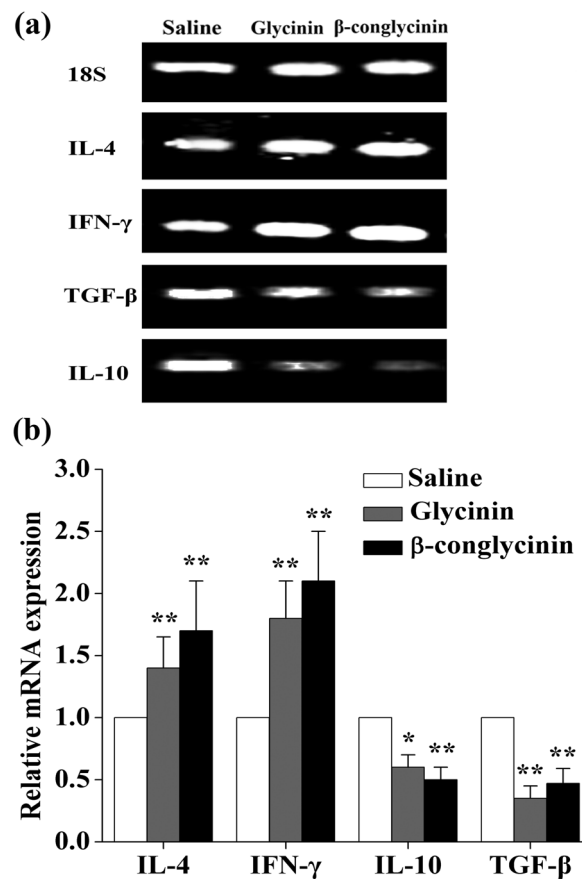


Figure 4. Expression of IL-4, IL-10, IFN- β and TGF- β mRNA in the jejunum tissue of Balb/c mice sensitised with soybean glycinin or β -conglycinin. (a) The profile of cytokine gene expression (IL-4, IL-10, IFN- β and TGF- β) in jejunum from control and immunised mice was evaluated by real-time PCR; 18S expression was shown as RNA control. (b) Relative cytokine mRNA expression. All the results were normalised against 18S gene expression and are presented as mean $\pm$ SEM of 10 mice per group. * represents $p < 0.05$ and ** represents $p < 0.01$ versus saline-treated group.

levels of IL-4 and IFN- γ were increased ($p < 0.05$) in the mice sensitised with glycinin and β -conglycinin, whereas levels of IL-10 and TGF- β mRNA expression were decreased ($p < 0.01$).

4. Discussion

Numerous immune cells and molecules scatter throughout the intestinal mucosa to provide efficient protection against pathogens and macromolecules. Abrogation of this barrier might be associated with intestinal inflammation and may promote food allergy (Chehade & Mayer, 2005). Animal models provide an effective method to evaluate allergens *in vivo*. We previously reported IgE-mediated anaphylactic reactions and intestinal damages induced by purified glycinin and β -conglycinin in

a Balb/c mice model (Liu et al., 2008). In this study, the same Balb/c mice model was used to characterise the intestinal immune responses by intragastric gavage with glycinin and β -conglycinin. We also analysed the cytokine and lymphocyte subsets in intestinal mucosa to explore its potential mechanisms.

Diarrhoea is a common symptom of food allergies in infants and adults, which is also taken to evaluate the degree of sensitisation (Sun et al., 2008b). Many studies have showed reduced performance and increased occurrence of diarrhoea in soybean protein-sensitised piglet models (Dreau et al., 1994; Huang et al., 2010; Sun et al., 2008b). Similarly, our result showed increased diarrhoea in Balb/c mice after glycinin and β -conglycinin ingestion.

Our result demonstrated villous atrophy and crypt hypertrophy in jejunum of the mice sensitised with soybean proteins. Histological change of the intestine is a common phenomenon in animals fed soybean allergens (Dreau et al., 1994; Hao et al., 2009; Liu et al., 2008). The precise reason for such phenomenon is still not known. Liu et al. (2008) indicated that IgE and IgG1 were involved in the allergic reactions of Balb/c mice. These antibodies activate mast cells, resulting in an excessive release of histamine, which may contribute to the intestinal damage and diarrhoea (Brandt et al., 2003; Guo et al., 2008). Additionally, substantial amounts of glycinin polypeptides escape digestion in the small intestine (Perez, Mills, Lambert, Johnson, & Morgan, 2000). The undigested remaining may damage the intestinal mucosa, disturb the absorption of nutrients and lead to diarrhoea (Dunsford, Knabe, & Haensly, 1989).

The intestinal damage allows antigens to pass rapidly into the systemic circulation and induces specific mediator release (Li, Schofield, Huang, Kleiner, & Sampson, 1999). Several studies have reported a significantly increased histamine level resulting from mast cell degranulation after antigen challenge in the sensitised animals (Guo et al., 2008; Liu et al., 2008; Sun et al., 2008b). Consistent with these reports, we found an increased histamine release in mice sensitised with soybean allergens. Histamine influences immune reactions through the activation of different T-cell cytokine profiles (Jutel et al., 2001), which drives the CD4⁺ T cells towards either Th1 or Th2 subset. Therefore, we further examined the differentiation of intestinal lymphocyte subsets.

One important finding is that the numbers of CD4⁺ T lymphocyte subpopulations were markedly increased in the jejunal lamina propria of mice gavaged with glycinin or β -conglycinin. This may partly agree with Sun et al. (2008a), who demonstrated an increased number of peripheral blood CD4⁺ lymphocytes in glycinin-sensitised piglets. However, differentiation of peripheral blood T lymphocytes mainly presents systemic immune responses. The measurement of lymphocytes in the intestinal mucosa provides a more precise picture of the local immune reactions. Since CD4⁺ T cells are helper T cells which secrete cytokines to control the immune response, the increased number of these cells may affect the intestinal inflammation. Interestingly, our study also demonstrated that the number of IgA⁺ plasma cells elevated in the jejunum after oral sensitisation. IgA⁺ cells in the intestinal lamina propria are a major source of IgA precursor cells for IgA production (Xu, Ayala, Monfils, Cioffi, & Chaudry, 1997). The development of IgA⁺ B cells is dependent on the activation of T cells (Wittig & Zeitz, 2003). Therefore, the significantly increased proportions of CD4⁺ T cells in jejunal lamina propria could, by itself, explain the increased density of IgA-producing cells.

IgA is well known as the major class of antibody present in the mucosal secretions. At least 80% plasma cells located in the intestinal lamina propria secrete IgA, which defends against ingested food antigens (Brandtzaeg, 2001). Our result showed an increase of intestinal IgA level in the jejunal mucosal sample, which is in agreement with the results obtained in pig models (Hao et al., 2009; Sun et al., 2008a).

IL-4 belongs to Th2 cytokines, which enhances humoral immunity while IFN- γ is Th1 cytokine, promoting cell-mediated immunity (Wittig & Zeitz, 2003). Our data showed that mRNA expression of IL-4 and IFN- γ were significantly increased in mice sensitised with soybean proteins compared with the saline-treated group. A previous study has revealed that serum IgE level and mRNA expression of IL-4 are correspondingly depended, suggesting that IgE-mediated hypersensitive responses partly depend on the production of IL-4 (Romagnani, 1990). Therefore, the increased expression of IL-4 may provide evidence for the elevated IgE level observed in our previous study (Liu et al., 2008). IL-4 and IFN- γ produced in spleen cells may play important roles in systemic immune responses to glycinin and β -conglycinin in sensitised mice (data not shown). Results of this study indicate that the production of Th2-type cytokines is also related to intestinal mucosal regulation mode (Huang et al., 2010; van Wijk & Knippels, 2007). In addition, although food allergy is recognised as Th2-type immune reactions, a mixed Th1/Th2 regulation mode in animal models has recently been described (Cardoso et al., 2008; Hao et al., 2009). Our data also suggest that a Th1/Th2 combined intestinal hypersensitive reaction was developed after oral administration of soybean proteins. Since the effects of Th1 or Th2 cytokines are counter-regulatory (Wittig & Zeitz, 2003), the results may indicate that continuous exposure to soybean allergens induced tolerance, which prevents persistent tissue injury (Cardoso et al., 2008). Future studies will be needed to identify the specific T cells activated by these cytokines.

TGF- β and IL-10 play a central role in mucosal immunity. TGF- β inhibits the proliferation of naive T cells, whereas IL-10 blocks antigen presentation and T cell activation; thus, they both function as immunosuppressive cytokines (Weiner, 2001). In the present study, we found a significant decrease of TGF- β and IL-10 mRNA expression in mice gavaged with soybean proteins. The result is in accordance with Cardoso et al. (2008), who reported a reduced expression of both TGF- β and IL-10 mRNA in gut mucosal inflammatory induced by peanuts. The result suggests that the modulation of gut immune response and induction of inflammation by soybean proteins could be, at least, a consequence of the loss of regulatory mechanisms as demonstrated by the low expression of TGF- β and IL-10 (Hori, Haury, Coutinho, & Demengeot, 2002).

Ma, He, Sun, and Han (2010) suggested that the cytokine production by lymphocytes from the mesenteric lymph nodes (MLN) played an important role in regulating the gut immunity of the glycinin-sensitised rats. However, considering the increased numbers of IgA+ plasma cells and CD4+ T cells, we determined the expression profile of cytokines in intestinal lamina propria rather than in the MLN. Similar studies have showed that cytokines secreted by T lymphocytes in the mucosa sections of the small intestine may control the immune response in humoral immunity (Bjersing, Teleme, Dahlgren, & Hanson, 2002; Liu et al., 2009). Moreover, Hao et al. (2009) demonstrated that both IFN- γ and IL-4 were unregulated in jejunum tissues of piglets after oral β -conglycinin challenge. Additionally,

CD4⁺ effector T cells in the intestinal mucosa are mainly developed from the naive CD4⁺ T cells in the MLN, which were activated by antigen-presenting cells via the afferent lymph (van Wijk & Knippels, 2007). Therefore, the soybean protein-induced local immune responses could be associated with cytokine production and lymphocyte differentiation in the MLN, which requires further investigation.

Generally, allergic reactions are difficult to induce by allergens without an adjuvant. However, the use of adjuvant may undermine the ability to discriminate between proteins concerning allergenic potential, resulting in false positives (Penninks & Knippels, 2001). Therefore, no adjuvant was used in the present trial. Recent studies have showed an increased level of allergen-specific IgE and histamine after administration of soybean allergens without the addition of an adjuvant in pig and rat models (Hao et al., 2009; Ma et al., 2010; Sun et al., 2008a, 2008b). Since the purities of glycinin or β -conglycinin used in these studies were 85–95%, we cannot rule out that the remaining part of soy impurities has adjuvant functions, which needs further research. It also seems difficult to induce allergic reactions without challenge after oral sensitisation (Guo et al., 2008; Ma et al., 2010). However, Akiyama et al. (2001) reported typical allergic responses in both mice and rat models induced by daily ovalbumin administration. Our previous study also demonstrated allergy syndrome and anaphylactic reactions in BALB/c mice caused by long-term gavage with soybean proteins (Liu et al., 2007). Our result confirms that an animal model of allergy could be built after oral administration with soybean proteins.

The lack of data in other parts of intestine (duodenum, ileum and colon) may be acknowledged as a limitation in this study. However, Capobianco et al. (2008) suggested that jejunum might be the main gut site in which allergen sensitisation occurs. Several studies have used jejunum to investigate the immune responses after exposure to allergenic food antigens (Hao et al., 2009; Sun et al., 2008a). The effects of soybean proteins on other intestinal segments would be assessed in future studies.

Taking together, our study demonstrates that oral sensitisation with soybean glycinin or β -conglycinin has induced intestinal allergic responses in the mice model, including histological changes of the intestine, increased levels of histamine and IgA antibodies, and an increase in IgA⁺ and CD4⁺ lymphocyte number in jejunal lamina propria. The results also suggest that the mixed Th1/Th2 immune response along with suppression of IL-10 and TGF- β production may play an important role in the development of intestinal hypersensitive reactions in soybean protein-sensitised mice.

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