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Effects of glycinin on IgE-mediated increase of mast cell numbers and histamine release in the small intestine

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Abstract

Soybean allergy represents a significant health threat to individuals with food allergies. Glycinin, the main storage protein in soybean, has been identified as a major food allergen. The present study was conducted to investigate the mechanism of glycinin-induced hypersensitivity in a swine model. The relationship between glycinin dose and the severity of hypersensitive reactions was also explored. Twenty-four piglets weaned at 18 days were gastric sensitized and subjected to repeated oral challenges with diets containing 0%, 2%, 4% and 8% glycinin. The results showed that dietary supplementation of glycinin reduced piglet performance ($P<.01$) while increasing occurrence of diarrhea ($P<.05$) and erythema area ($P=.01$) in response to an intradermal injection of glycinin. Intestinal mast cell numbers ($P<.05$) and immunoglobulin E (IgE) levels ($P<.05$) were increased linearly, whereas the histamine content in intestinal specimens (except in the duodenum) was decreased ($P<.01$), indicating that more histamine had been released in glycinin-fed piglets than in control. Serum concentrations of total IgE, glycinin-specific IgG1 and interleukin (IL)-4 and IL-10 were also greater ($P<.05$) in the pigs treated with glycinin. In this study, we found that glycinin-induced hypersensitivity is a predominantly Th2-type immune response, mediated by IgE and associated with increases in intestinal mast cell numbers and histamine release as well as IL-4 and IL-10 concentrations in the serum of sensitized piglets, resulting in diarrhea and reduced performance. The severity of the hypersensitive reactions depends on the dose of glycinin. Higher dose may cause more severe anaphylactic symptoms.

Keywords: Glycinin; Piglets; IgE; Mast cells; Histamine

1. Introduction

Soybean is a source of high-quality protein due to its relatively well-balanced composition of amino acids [1,2]. However, soybean is also a dietary allergic source for humans [3], which threatens individuals with soybean-sensitive food allergies. Glycinin (Mr≈360,000), also called 11S protein, accounts for more than 40% of the total soybean protein. It is composed of five subunits, each containing an acidic and a basic polypeptide linked by a disulfide bond [4,5]. Available evidence suggests that glycinin has an immunoglobulin E (IgE)-binding region in the acidic polypeptide chain [6]. Results of clinical studies indicate an increase in concentrations of glycinin-specific IgE antibody in sera of soybean-allergic subjects [7–9] and, thus, glycinin allergenicity [10]. Although previous studies have shown that glycinin contributes to a series of allergic reactions, damage to intestinal morphology, disorder of immune function, growth depression and diarrhea in human infants [11] and piglets [12–15], the underlying mechanisms causing these symptoms are largely unknown. Because the piglet is widely used as an animal model for studying sensitization of infants to soy proteins [13–18], it was used in the present study to define mechanisms whereby glycinin increases hypersensitivity in response to consumption of soybean products.

Mast cells are located in the gut mucosa and submucosa to function in intestinal peristalsis, inflammatory process and related immune responses [19–21]. Many studies involving food allergies suggest that mast cells are prominent in the development of IgE-mediated hypersensitivity through an excessive release of large amounts of inflammatory factors,

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including histamine and some cytokines after activation and degranulation [22]. Actually, food-allergic patients often have an increase in the number of mast cells in the intestinal mucosa [23]. Histamine, a major mediator released by mast cells, contributes to increasing vascular permeability of the gastrointestinal mucosa and plays an important role in the IgE-dependent hypersensitivity [24]. Studies on rodents showed that mice sensitized by ovalbumin experienced mucosal histamine depletion [25]. Collectively, these results indicate that mast cells and histamine are effective in IgE-mediated hypersensitive reactions to food allergens.

To date, there is insufficient evidence linking soybean allergy with the alteration in mast cell numbers and histamine release in the small intestine as well as cytokine production. Therefore, we hypothesized that allergic reactions after ingestion of soybean glycinin might result from hypersensitivity mediated by IgE produced by T-helper 2 (Th2) lymphocytes in response to stimulation by cytokines, such as interleukin (IL)-4 and IL-10. We used an experimental piglet model fed purified glycinin to eliminate the interference from other components in soybean meal or whole soybean protein extract. We also determined the effects of various dosages of glycinin on IgE levels, mast cell numbers and histamine content in the small intestine, which are essential criteria to explore the mechanism of glycinin-induced hypersensitivity in human and animals.

2. Materials and methods

2.1. Characterization of purified soybean glycinin

Purified glycinin suspension was kindly donated by Professor Shuntang Guo from the Food Institute of China Agricultural University (Patent No. 200410029589.4, China). After lyophilization, protein content was determined by the Kjeldahl method and glycinin purity was analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) followed by Coomassie Brilliant Blue (CBB) R-250 staining [26]. The gel was scanned by Bio Imaging System with Gene Genius (Syngene, USA), and the purity of glycinin was measured by GeneTools Analysis Software, Version 3.03.03 [27].

2.2. Animals and diets

Twenty-four crossbred (Large White×Landrace) barrows, weaned at 14 days of age, were used in this trial. All the piglets were individually housed in an environmentally controlled room with the temperature at 26–28°C. Animals were maintained according to the rules of China Agricultural University Animal Care and Use Committee. After 4 days of adaptation, all the piglets, with an average initial body weight of 4.98±0.67 kg, were randomly allotted to four treatments. Each treatment had six replicates with one piglet per pen. Each pen was equipped with a feeder and a waterer to allow the pigs to have unlimited access to water and feed at all times. All the pigs were weighed on Days 0, 7 and 10 of the trial in order to calculate the weight gain. Incidents of piglets with diarrhea were recorded every day throughout the whole experiment. Pig feces was classified at four levels: 0, normal; 1, pasty; 2, semiliquid; and 3, liquid [28,29]. The occurrence of diarrhea was defined as maintenance of feces at Level 2 or Level 3 for two continuous days. Diarrhea incidence (%) = diarrhea piglets in each treatment×diarrhea days/6/32×100%.

The control diet, devoid of soybean protein, contained skimmed milk powder and casein as the protein source. Different doses of purified glycinin (2%, 4% and 8%) were substituted for the skimmed milk powder and casein in the corresponding experimental diets (Table 1). All the piglets were fed the control diet, except for the experimental piglets during the sensitization and challenge periods.

2.3. Experimental design

Experimental piglets were gastric sensitized and orally challenged with the diets containing 2% 4%, and 8% glycinin at Days 0 to 10 and Days 16 to 18, respectively. Thereafter, piglets were challenged either by skin test or by intragastric challenge at 1-week intervals. The control pigs, which were fed the soy-free diet throughout the trial, also received skin prick tests.

Table 1
Ingredient composition and nutrient levels of the diets (as-fed basis)

	Dietary treatment			
	0% glycinin	2% glycinin	4% glycinin	8% glycinin
Ingredient composition (%)				
Corn	60.2	61.0	60.9	60.8
Skimmed milk powder	9.5	8.4	8.6	8.6
Whey powder	10.0	10.0	10.0	10.0
Casein	11.6	9.9	7.7	3.5
Glycinin	0.0	2.0	4.0	8.0
Spray dried porcine plasma	3.0	3.0	3.0	3.0
Fish meal	3.0	3.0	3.0	3.0
Limestone	0.5	0.5	0.5	0.5
Calcium phosphate	1.0	1.0	1.0	1.0
Salt	0.2	0.2	0.2	0.2
Premix [a]	1.0	1.0	1.0	1.0
L-Lys	0.0	0.0	0.05	0.2
DL-Met	0.0	0.0	0.05	0.2
Chemical analysis [b]				
Crude protein (%)	22.98	22.96	23.02	23.08
Lysine (%)	1.65	1.58	1.57	1.57
Calcium (%)	0.92	0.89	0.88	0.86
Phosphorus (%)	0.75	0.72	0.71	0.66
Calculated DE (MJ/kg)	14.20	14.19	14.21	14.22

[a] Premix provided per kilogram of complete diet: vitamin A, 10,000 IU; vitamin D_3, 1500 IU; vitamin E, 30 IU; vitamin K_3, 2.5 mg; vitamin B_1, 1.5 mg; vitamin B_2, 10 mg; vitamin B_6, 10 mg; vitamin B_{12}, 0.05 mg; folic acid, 1 mg; biotin, 0.5 mg; niacin, 30 mg; pantothenic acid, 20 mg; Cu, 20 mg; Fe, 100 mg; Zn, 110 mg; Mn, 40 mg; Se, 0.3 mg; I, 0.5 mg.

[b] Analyzed value.

2.4. Sample collection

Three hours after the final oral challenge (Day 32), all the piglets were slaughtered with an intracardial injection of sodium pentobarbital (50 mg/kg body weight) and jugular exsanguination. Blood (7 ml) was collected from the precaval vein before the slaughter, and the serum was separated and stored at −20°C for IgE and glycinin-specific IgG1 antibody analysis. Intestinal segments were immediately removed from the duodenum (5 cm from the pylorus), the jejunum (the middle part of the whole jejunum) and the ileum (5 cm anterior to the ileocecal valve) and then flushed by physiological saline to remove gut contents. One sample of each intestinal tissue, about 2 cm in length, was fixed in Carnoy's fluid. Another part of a 5-cm section was frozen in liquid nitrogen and stored at −80°C until analysis.

2.5. Cutaneous skin testing

The skin testing was conducted as previously described with modifications [12,30]. Briefly, 0.5 mg of glycinin dissolved in physiological saline solution was intradermally injected to skin-shaved flank regions of piglets. The physiological saline solution was injected as the negative control. The allergic reaction was evaluated by diameters of red flares at 30 min after the injection.

2.6. Histological analysis of intestinal mast cells

Mast cells were counted by analysis of histology of the small intestinal segments. Briefly, three intestinal segments, the duodenum, the jejunum and the ileum, were fixed in Carnoy's solution for 24 h [31], followed by routine dehydration, paraffin embedment and 6-μm histological slide cutting. Mast cells were visible by toluidine blue (Gurr, Poole, UK) staining under a microscope (BX 50, Olympus Co., Japan). The number of mast cells in the mucosa and submucosa was quantified by numbering mast cells in eight defined 1-mm^2 areas of one segment histological slide with a Microcheck Grid (Beijing KeYi Optical Ltd., Beijing, China) containing 400 microchecks (1 mm^2) under a ×20 power. Each sample was represented by three independent histological slides. The mast cell number was then calculated by overall cell number divided by square millimeters.

2.7. Determination of intestinal histamine levels with high-performance liquid chromatography

The determination of histamine levels in the duodenum, the jejunum and the ileum was performed with high-performance liquid chromatography (Waters, MA, USA), as described by Liu et al. [31]. Briefly, 0.5 g of intestinal tissue was dissolved in 0.6 mol/L perchloric acid. After homogenization and extraction, the sample was loaded into a reversed-phase column (Symmetry, C_{18}, 5 μm, 4.6×150 mm, Waters Ireland, Dublin), which was eluted using a gradient elution program at a flow rate of 1 ml/min. Histamine then reacted with *o*-phthaldialdehyde at 40°C, and fluorescence was detected at 340 nm excitation and 450 nm emission. A standard sample of histamine was supplied by Sigma, USA. A data integrator (Waters TM 600, Waters Ireland) was used to analyze the data.

2.8. Analysis of intestinal and serum antibodies by ELISA

Approximately 0.5 g intestinal segments were resolved in PBS buffer containing 1 mM phenylmethanesulfonyl fluoride. After homogenization, samples were centrifuged at 10,000×*g* at 4°C for 10 min, and the supernatant was obtained. The measurement of IgE levels in the intestine was performed using swine IgE enzyme-linked immunosorbent assay (ELISA) Kit (RapidBio Lab, Calabasas, CA, USA), following the instructions of the manufacturer. Protein was quantified with CBB Protein Assay Kit (Jiancheng Institute of Biological Technology, Nanjing, China).

Total IgE antibodies were also determined using the swine IgE ELISA Kit (RapidBio Lab). Since specific antiserum to swine IgE is not commercially available, glycinin-specific IgG1 in the serum was measured by indirect ELISA as described by Helm et al. [30], and some modifications were made. Briefly, 96-well microtiter plates (BioFil, Canada) were coated with 3.5 μg/ml glycinin in 100 μl carbonate buffer ($NaHCO_3/Na_2CO_3$, pH 9.6). Then, 100 μl of 20-fold diluted swine serum was added to each well and incubated. After being washed five times, goat antipig serum horseradish peroxidase conjugated (Sigma) and 0.4 mg/ml *O*-phenylenediamine-dihydrochloride (Sigma) buffer containing 0.015% H_2O_2 were added to measure the absorbance at 492 nm. The data were expressed as optical density (OD) units.

2.9. Quantification of cytokines in the serum

Levels of IL-4 and IL-10 in the serum were determined using the swine ELISA kit (Biosource, Camarillo, CA, USA) according to the manufacturer's instructions.

2.10. Statistical analysis

Differences in occurrence of diarrhea among treatment groups were determined by chi-square contingency test. All other data were analyzed using the General Linear Model procedures of SAS system (version 8.2, SAS Institute, Inc., Cary, NC, USA). Linear and quadratic polynomial contrasts were used to determine the dose-dependent effect of soybean glycinin. P values ≤.05 were considered statistically significant.

3. Results

3.1. Quality check of glycinin

The soybean glycinin sample contained more than 85% glycinin determined by the Kjeldahl method and SDS-PAGE.

3.2. Performance during the sensitization period and diarrhea throughout the trial

The performance data and occurrence of diarrhea are presented in Table 2. Generally, average daily gain declined linearly (P=.002) whereas the feed-to-gain ratio increased linearly (P=.004) with the increasing of glycinin in the diets. Piglets fed 4% and 8% glycinin had lower average daily gain (P=.002) compared to those in the control and the 2% glycinin group either at 1 week after weaning (237 and 222 g/day vs. 283 and 264 g/day) or during the 10 days of sensitization (320 and 291 g/day vs. 372 and 339 g/day), although average daily feed intake was not influenced (P=.305).

As shown in Table 2, the occurrence of diarrhea differed (P<.05) among piglets fed 2%, 4% and 8% of glycinin. When glycinin supplementation increased from 2% to 4%, the incidence of diarrhea increased from 14.5% to 15.6% (P<.05), but it did not further increase when glycinin supplementation increased further to 8%.

3.3. Cutaneous sensitization

Skin prick tests were performed following glycinin-induced hypersensitivity. Results of the erythema diameters and areas are presented in Table 2. Skin prick test with glycinin was positive in glycinin-sensitized pigs, and the diameters of the red flare were more than 5 mm, which differed from the control group (P<.05). There were no differences among piglets fed the 2%, 4% and 8% glycinin diets.

3.4. Mast cell numbers in the intestine

The results of mast cell numbers in the intestine of pigs are shown in Table 2. There was a dose-dependent influence

Table 2
Effects of different doses of glycinin on performance during the sensitization period, diarrhea incidence throughout the trial, skin prick test, the number of mast cells and histamine content in the small intestine, intestinal and serum total IgE titers, glycinin-specific IgG1 levels and cytokine concentrations in the serum of piglets

Item	Glycinin level				S.E.M.	P value	
	0%	2%	4%	8%		Linear	Quadratic
Performance							
0–7 days							
ADG (g/day)	283^{a}	264^{ab}	237^{bc}	222^{c}	12.8	.002	.877
ADFI (g/day)	327	325	319	304	15.7	.305	.675
F/G	1.16^{a}	1.23^{ab}	1.34^{ab}	1.41^{b}	0.06	.004	.971
0–10 days							
ADG (g/day)	372^{a}	339^{ab}	320^{b}	291^{b}	16.1	.002	.891
ADFI (g/day)	423	412	396	381	23.7	.200	.940
F/G	1.14^{a}	1.22^{ab}	1.24^{ab}	1.31^{b}	0.04	.004	.898
Diarrhea incidence (%)	0	14.5	15.6	15.6	–	<.05 *	–
Erythema diameter (mm) and area (mm^2) by intradermal injection of glycinin							
Diameter	1.9^{b}	5.8^{a}	6.2^{a}	6.7^{a}	0.72	.002	.043
Area	2.8^{b}	27.5^{a}	31.1^{a}	36.1^{a}	6.84	.010	.190
Mast cell numbers in the mucosa (number/mm^2)							
Duodenum	12.4^{b}	19.6^{ab}	22.0^{a}	27.4^{a}	2.83	.003	.747
Jejunum	20.8^{b}	28.7^{a}	32.8^{a}	33.9^{a}	2.65	.002	.221
Ileum	20.0^{c}	31.0^{b}	39.1^{ab}	47.4^{a}	3.17	<.001	.672
Mast cell numbers in the submucosa (number/mm^2)							
Duodenum	29.2	33.1	38.1	39.3	2.85	.014	.638
Jejunum	33.8^{c}	39.3^{c}	49.3^{b}	62.3^{a}	2.97	<.001	.223
Ileum	36.9^{b}	51.2^{a}	59.4^{a}	65.3^{a}	4.75	<.001	.385
Histamine content in the intestine (μg/g)							
Duodenum	23.03	21.09	20.15	17.17	2.22	.087	.819
Jejunum	23.43^{a}	20.10^{ab}	17.76^{b}	17.08^{b}	1.35	.004	.346
Ileum	20.25^{a}	19.97^{a}	14.54^{b}	10.89^{b}	1.53	<.001	<.001
IgE concentration in the intestine (μg/g)							
Duodenum	21.8^{b}	24.3^{ab}	30.3^{a}	31.5^{a}	2.45	.006	.784
Jejunum	19.6^{b}	21.1^{b}	24.8^{b}	39.5^{a}	4.67	.008	.175
Ileum	10.5	16.7	22.8	21.7	3.89	.037	.365
Total serum IgE (ng/ml)	722.56^{b}	916.16^{ab}	1090.29^{a}	1235.19^{a}	106.95	.004	.824
Glycinin-specific IgG1 (OD units)	0.34^{b}	0.63^{ab}	0.78^{a}	0.89^{a}	0.10	.001	.377
Cytokine concentrations in the serum							
IL-4 (pg/ml)	26.89^{c}	37.63^{bc}	50.40^{ab}	61.45^{a}	4.63	<.001	.973
IL-10 (pg/ml)	9.05^{c}	13.70^{bc}	19.14^{b}	31.64^{a}	1.76	<.001	.056

Each value is the mean of data from six piglets (average initial body weight of 4.98±0.67 kg) per group.
Mean values with different superscripts are different at P<.05.
* Significantly different by chi-square contingency test.

of glycinin supplementation on mast cell numbers ($P<.05$). Compared to the control, mast cell numbers were steadily increased as glycinin supplementation increased. In the case of the 8% glycinin group, the number of mast cells in the mucosa and submucosa was increased by 121.0% (27.4 vs. 12.4) and 34.6% (39.3 vs. 29.2) in the duodenum, by 63.0% (33.9 vs. 20.8) and 84.3% (62.3 vs. 33.8) in the jejunum and by 137.0% (47.4 vs. 20.0) and 77.0% (65.3 vs. 36.9) in the ileum, respectively. In addition, the mast cell numbers were greater in the distal intestine (in the ileum) than those in the proximal intestine (in the duodenum and jejunum).

3.5. Histamine content in the small intestine

The effects of different doses of glycinin on histamine levels in the small intestine are summarized in Table 2. In contrast to the trend of mast cell numbers, a linear decrease was observed in the jejunum and ileum of piglets as supplementation of glycinin increased ($P<.01$). The histamine contents in the jejunum and ileum of piglets treated with 4% and 8% glycinin supplementation were decreased ($P<.01$) compared with control, which suggested that more histamine had been released and involved in metabolism of the small intestine.

3.6. Antibody levels in the intestine and serum

To investigate the effects of glycinin on anaphylactic reactions in sensitized pigs, we determined the levels of IgE antibody in the small intestine, total IgE and glycinin-specific IgG1 levels in the serum. The antibody levels in both the intestine and the serum exhibited a trend similar to that for the mast cell numbers, but there was an inverse trend with the histamine content. As shown in Table 2, the IgE levels in pigs fed increasing doses of glycinin were linearly elevated in the intestine ($P<.05$). Total IgE and glycinin-specific IgG1 in the serum were also increased ($P<.01$) in the groups treated with glycinin. The 4% and 8% glycinin treatments elevated the total IgE levels as well as glycinin-specific IgG1 levels compared with the control and the 2% glycinin group ($P<.01$).

3.7. Cytokine concentrations in the serum

Concentrations of IL-4 and IL-10 in the serum increased linearly ($P<.01$) with increasing glycinin supplementation (Table 2). The results are consistent with a Th2-type immune response in glycinin-induced hypersensitivity.

4. Discussion

The present study utilized an experimental pig model to elucidate the mechanism of soybean glycinin-induced food allergy, which is an important concern related to both animal nutrition and human health. Soybean allergy represents a significant health problem, which occurs more frequently early in life [10]. Infants consuming soybean-based formulae tend to suffer from hypersensitivity [32]. With the popularized application of soybean in the food and feed industries, the incidence of soybean allergy tends to rise in children and adults [33] as well as in young animals [12–15].

The major soybean storage globulin 11S glycinin has been identified as an allergen [8,9], and its allergenicity is known to contribute to hypersensitivity in human, whose clinical symptoms range from gastrointestinal distress to life-threatening asthma and death [3]. To date, there have been many studies using young pigs as an animal model to investigate the effects of soybean proteins (including glycinin) [13,18], which showed that soybean protein could reduce performance with frequent diarrhea. Clinically, diarrhea is a concomitant apparent symptom for soybean or other food allergies in infants and adults [34–36], which is one of the criteria to evaluate the degree of sensitization [30]. Consistent with those reports, our study demonstrated reduced weight gain and lowered feed efficiency in glycinin-fed piglets during the sensitization period. All the diets were formulated with similar levels of protein and energy to minimize the effect of the replacement of high-quality skimmed milk powder and casein with a plant protein. Furthermore, the performance of piglets was dose-dependently related to the supplementation of soybean glycinin. In addition, the frequency of diarrhea increased in pigs fed glycinin after the initial oral challenge (data not shown), indicating that a successful animal model has been established.

Allergenicity of a dietary allergen is attributed to a variety of factors including the structure of the protein and the stability resistant to processing as well as enzyme digestion. Glycinin, especially its acid polypeptide, is heat resistant and can escape small intestinal digestion. Lallès et al. [37] detected immunoreactive glycinin and acid polypeptides of glycinin in the digesta of the duodenum and ileum in preruminant calves, respectively. Perez et al. [38] found immunoreactive glycinin in tissue homogenates in rats that received intragastric doses of soya isolate as well. The undigested residues may damage the gastrointestinal mucosa [39], affect the absorption of nutrients and result in diarrhea.

Gastrointestinal food allergy is a food-stimulated IgE-mediated event. Soybean-allergic patients often have increased IgE antibodies in the sera [8,9]. In the current study, we also found a significant increase in IgE levels both in the intestine and in the serum of glycinin-treated pigs. The results of skin prick tests in this experiment were also positive in the pigs fed glycinin, which is consistent with the results obtained by Dréau et al. [12]. IgE plays an important role in most food-induced allergies, which are IgE mediated and characterized by Type I allergic reactions [40]. Furthermore, IgE levels can reflect the activation of mast cells in the small intestine. The large quantity of IgE antibodies generated in the allergic individuals immediately bind to the corresponding receptor FcεRI on the surface of the mast cells in the gastrointestinal tract and other tissues, which provoke the mast cells to degranulate and release active mediators, such as histamine, 5-hydroxytryptamine

and some cytokines, leading to a typical hypersensitivity [41]. Consistent with these reports, serum IL-4 and IL-10 levels increased as IgE did, and the IgE production was dependent on IL-4 and IL-10. It is well documented that IL-4 and IL-10 are produced by Th2 cells [25], which bring about strong antibody-producing responses (including antigen-specific IgG1 and IgE). Previous studies have shown that IgE and IgG1 are involved in the immediate allergic reaction in murine [33,42,43] and other animals [30,44]. Indeed, an increase in IgE and IgG antibodies specific for the soy extract has been detected in the serum of children with atopic dermatitis and positive soy challenges [8]. In the current study, we observed elevated levels of glycinin-specific IgG1 and IgE in the serum of sensitized piglets, indicating that glycinin-induced hypersensitivity was a predominantly Th2-oriented immune response.

An important finding of the current study is that the number of mast cells in the mucosa and submucosa in the small intestine of pigs fed glycinin was greater ($P<.05$) than that of the control group, which demonstrated that as a food allergen, glycinin could elevate the intestinal mast cell numbers in accordance with the previous results [45,46]. These mast cells can be activated by soybean antigen-specific IgE, resulting in an excessive release of histamine, which may contribute to the change of intestinal motility [47] and malabsorption of nutrients, supporting the results of the reduced performance and diarrhea of pigs in previous studies [13,36,39] as well as in our study.

As described above, histamine is the major mediator released by activated mast cells. Since mast cells are regarded as the major source of histamine in the gut and other tissues [47], the assay of histamine has been considered a good indicator of the activation and number of the mast cells [48]. Mast cells proliferated to release more histamine, as reported by other investigators [45]. Furthermore, histamine release can be a criterion in the diagnosis to test food allergy [49], as patients with food allergy manifest increased release of histamine from the gastrointestinal mucosa after being sensitized by food allergen [50]. Cellular histamine content may be a sensitive indicator of histamine release because secreted histamine can be instantly terminated after an interaction with corresponding cellular targets [47], which means that less cellular histamine and proliferated mast cells indicate more histamine release from mast cells to the hollow canal of the gastrointestinal tract. We found a linear depletion of histamine levels in the small intestine in glycinin-sensitized pigs ($P<.05$), indicating that more histamine had been released. The histamine concentrations in different parts of the gut were correlated with the number of mast cells in the corresponding tissues. As the number of mast cells increased, the analyzed histamine content decreased, which is in accordance with those reported by other investigators [25,51]. The excessive histamine would promote the dilation and permeability of local microvessels, leading to the absorption of undigested glycinin, inducing the impairment of the gut and elevating the serum IgG, as shown in our research and as described by other investigators [13]. In addition, there is compelling evidence that mast cells and histamine contribute to allergen-induced diarrhea [34], which was also found in our study.

In conclusion, this study has established a swine model to elucidate a mechanism responsible for glycinin-induced hypersensitivity in response to consumption of soybean products. The immunological abnormality is a predominantly Th2-type immune response, mediated by IgE and associated with the increase of mast cell numbers and histamine release as well as a concomitant increase of IL-4 and IL-10 production, which may affect the digestion and absorption of nutrients, leading to poor performance and occurrence of diarrhea in piglets. These findings may have important implications for preventing and treating glycinin-related allergic disease in human, especially in children and infants.

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References

[1] Friedman M, Brandon DL. Nutritional and health benefits of soy proteins. J Agric Food Chem 2001;49:1069–77.
[2] Hancock JD, Cao H, Kim IH, Li DF. Effects of processing technologies and genetic modifications on nutritional value of full-fat soybeans in pigs. Asian–Aust J Anim Sci 2000;13(Special Issue):356–65.
[3] Foucard T, Yman IM. A study on severe food reactions in Sweden — is soy protein an underestimated cause of food anaphylaxis? Allergy 1999;54:261–5.
[4] Golubovic M, van Hateren SH, Ottens M, Witkamp GJ, van der Wielen LAM. Novel method for the production of pure glycinin from soybeans. J Agric Food Chem 2005;53:5265–9.
[5] Hou DH, Chang SKC. Structural characteristics of purified glycinin from soybeans stored under various conditions. J Agric Food Chem 2004;52:3792–800.
[6] Zeece MG, Beardslee TA, Markwell JP, Sarath G. Identification of an IgE-binding region in soybean acidic glycinin G1. Food Agric Immunol 1999;11:83–90.
[7] Awazuhara H, Kawai H, Maruchi N. Major allergens in soybean and clinical significance of IgG4 antibodies investigated by IgE- and IgG4-immunoblotting with sera from soybean-sensitive patients. Clin Exp Allergy 1997;27:325–32.
[8] Burks AW, Brooks JR, Sampson HA. Allergenicity of major component proteins of soybean determined by enzyme linked immunosorbent assay (ELISA) and immunoblotting in children with atopic dermatitis and positive soy challenges. J Allergy Clin Immunol 1988;81:1135–42.
[9] Shibasaki M, Suzuki S, Tajima S, Nemoto H, Kuroume T. Allergenicity of major component proteins of soybeans. Int Arch Allergy Appl Immunol 1980;61:441–8.
[10] Herian AM, Taylor SL, Bush RK. Allergenic reactivity of various soybean products as determined by RAST inhibition. J Food Sci 1993; 58:385–8.
[11] Duke WW. Soybean as a possible important source of allergy. J Allergy 1934;5:300–2.

[12] Dréau D, Lallès JP, Philouze-Romé V, Toullec R, Salmon H. Local and systemic immune responses to soybean protein ingestion in early-weaned pigs. J Anim Sci 1994;72:2090–8.
[13] Li DF, Nelssen JL, Reddy PG, Blecha F, Hancock JD, Allee GL, et al. Transient hypersensitivity to soybean meal in the early-weaned pig. J Anim Sci 1990;68:1790–9.
[14] Li DF, Nelssen JL, Reddy PG, Blecha F, Klemm RD, Goodband RD. Interrelationship between hypersensitivity to soybean protein and growth performance in early-weaned pigs. J Anim Sci 1991; 69: 4062–9.
[15] Stokes CR, Miller BG, Bailey M, Wilson AD, Bourne FJ. The immune response to dietary antigens and its influence on disease susceptibility in farm animals. Vet Immunol Immunopathol 1987;17:413–23.
[16] Helm RM. Food allergy animal models: an overview. Ann N Y Acad Sci 2002;964:139–50.
[17] Kimber I, Dearman RJ, Penninks AH, Knipples LMJ, Buchanan RB, Hammerberg B, et al. Assessment of protein allergenicity on the basis of immune reactivity: animal models. Environ Health Perspect 2003; 111:1125–30.
[18] Hankins CC, Noland PR, Burks AW, Connaughton JC, Cockrell G, Metz CL. Effect of soy protein ingestion on total and specific immunoglobulin G concentrations in neonatal porcine serum measured by enzyme-linked immunosorbent assay. J Anim Sci 1992; 70: 3096–101.
[19] de Jonge F, Nassauw LV, Meik FV, Miller HRP, Marck EV, Timmermans JP. Temporal distribution of distinct mast cell phenotypes during intestinal schistosomiasis in mice. Parasite Immunol 2002;24: 225–31.
[20] Golkar L, Bernhard JD. Mastocytosis. Lancet 1997;349:1379–85.
[21] Schneider E, Rolli-Derkinderen M, Arock M, Dy M. Trends in histamine research: new functions during immune responses and hematopoiesis. Trends Immunol 2002;23:255–62.
[22] van Wijk F, Knippels L. Initiating mechanisms of food allergy: oral tolerance versus allergic sensitization. Biomed Pharmacother 2007;61: 8–20.
[23] Bischoff SC. Mucosal allergy: role of mast cells and eosinophil granulocytes in the gut. Baillieres Clin Gastroenterol 1996;10:443–59.
[24] Wershil BK, Furuta GT, Wang ZS, Galli SJ. Mast cell-dependent neutrophil and mononuclear cell recruitment in immunoglobulin E-induced gastric reactions in mice. Gastroenterology 1996;110:1482–90.
[25] van Halteren AGS, van der Cammen MJF, Biewenga J, Savelkoul HFJ, Kraal G. IgE and mast cell responses on intestinal allergen exposure: a murine model to study the onset of food allergy. J Allergy Clin Immunol 1997;99:94–9.
[26] Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970;227:680–5.
[27] Guo PF, Piao XS, Ou DY, Li DF, Hao Y. Characterization of the antigenic specificity of soybean protein β-conglycinin and its effects on growth and immune function in rats. Arch Anim Nutr 2007;61: 210–21.
[28] Azevedo MSP, Yuan L, Pouly S, Gonzales AM, Jeong KI, Nguyen TV, et al. Cytokine responses in gnotobiotic pigs after infection with virulent or attenuated human rotavirus. J Virol 2006;80:372–82.
[29] Yuan L, Ward LA, Rosen BI, To TL, Saif LJ. Systemic and intestinal antibody-secreting cell responses and correlates of protective immunity to human rotavirus in a gnotobiotic pig model of disease. J Virol 1996; 70:3075–83.
[30] Helm RM, Furuta GT, Stanley JS, Ye JH, Cockrell G, Connaughton C, et al. A neonatal swine model for peanut allergy. J Allergy Clin Immunol 2002;109:136–42.
[31] Liu YH, Piao XS, Ou DY, Cao YH, Huang DS, Li DF. Effects of particle size and physical form of diets on mast cell numbers, histamine, and stem cell factor concentration in the small intestine of broiler chickens. Poult Sci 2006;85:2149–55.
[32] Halpern SR, Sellars WA, Johnson RB, Anderson DW, Saperstein S, Reisch JS. Development of childhood allergy in infants fed breast, soy, or cow milk. J Allergy Clin Immunol 1973;51:139–51.
[33] Gizzarelli F, Corinti S, Barletta B, Iacovacci P, Brunetto B, Butteroni C, et al. Evaluation of allergenicity of genetically modified soybean protein extract in a murine model of oral allergen-specific sensitization. Clin Exp Allergy 2006;36:238–48.
[34] Brandt EB, Strait RT, Hershko D, Wang Q, Muntel EE, Scribner TA, et al. Mast cells are required for experimental oral allergen-induced diarrhea. J Clin Invest 2003;112:1666–77.
[35] Herman E. Soybean allergenicity and suppression of the immunodominant allergen. Crop Sci 2005;45:462–7.
[36] Miller BG, Newby TJ, Stokes CR, Hampson DJ, Brown PJ, Bourne FJ. The importance of dietary antigen in the cause of postweaning diarrhea in pigs. Am J Vet Res 1984;45:1730–3.
[37] Lallès JP, Tukur HM, Salgado P, Mills ENC, Morgan MRA, Quillien L, et al. Immunochemical studies on gastric and intestinal digestion of soybean glycinin and β-conglycinin in vivo. J Agric Food Chem 1999; 47:2797–806.
[38] Perez MD, Mills ENC, Lambert N, Johnson IT, Morgan MRA. The use of anti-soya globulin antisera in investigating soya digestion in vivo. J Sci Food Agric 2000;80:513–21.
[39] Dunsford BR, Knabe DA, Haensly WE. Effect of dietary soybean meal on the microscopic anatomy of the small intestine in the early-weaned pig. J Anim Sci 1989;67:1855–63.
[40] Ladics GS, Holsapple MP, Astwood JD, Kimber I, Knippels LMJ, Helm RM, et al. Workshop overview: approaches to the assessment of the allergenic potential of food from genetically modified crops. Toxicol Sci 2003;73:8–16.
[41] Metcalfe DD, Baram D, Mekori YA. Mast cells. Physiol Rev 1997;77: 1033–79.
[42] Faquim-Mauro EL, Coffman RL, Abrahamsohn IA, Macedo MS. Cutting edge: mouse IgG1 antibodies comprise two functionally distinct types that are differentially regulated by IL-4 and IL-12. J Immunol 1999;163:3572–6.
[43] Knippels LMJ, Penninks AH, Spanhaak S, Houben GF. Oral sensitization to food proteins: a Brown Norway rat model. Clin Exp Allergy 1998;28:368–75.
[44] Foster AP, Knowles TG, Moore AH, Cousins PDG, Day MJ, Hall EJ. Serum IgE and IgG responses to food antigens in normal and atopic dogs, and dogs with gastrointestinal disease. Vet Immunol Immunopathol 2003;92:113–24.
[45] Ashida Y, Denda M. Dry environment increases mast cell number and histamine content in dermis in hairless mice. Br J Dermatol 2003;149: 240–7.
[46] Mathan MM, Mathan VI. Ultrastructural pathology of the rectal mucosa in *Shigella* dysentery. Am J Pathol 1986;123:25–38.
[47] Rangachari PK. Histamine: mercurial messenger in the gut. Am J Physiol 1992;262:G1–G13.
[48] Li P, Yin YL, Li DF, Kim SW, Wu G. Amino acids and immune function. Br J Nutr 2007;98:237–52.
[49] Crockard AD, Ennis M. Basophil histamine release tests in the diagnosis of allergy and asthma. Clin Exp Allergy 2001;31:345–50.
[50] He HH, Hua X, Zhang X. Inhibition of histamine release from human mast cells by natural chymase inhibitors. Acta Pharmacol Sin 2004;25: 822–6.
[51] Ou DY, Li DF, Cao YH, Li XL, Yin JD, Qiao SY, et al. Dietary supplementation with zinc oxide decreases expression of the stem cell factor in the small intestine of weanling pigs. J Nutr Biochem 2007;18: 820–6.