JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

J. Agric. Food Chem. XXXX, XXX, 000–000 A
DOI:10.1021/jf904403u

Lipoic Acid: An Immunomodulator That Attenuates Glycinin-Induced Anaphylactic Reactions in a Rat Model

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The purpose of this study was to evaluate the attenuation effects of consuming a small dose of lipoic acid on soybean glycinin-induced hypersensitivity using a rat model. Sensitized by gavage with glycinin, weaned rats were orally supplemented with the diet containing 12.5 μg of lipoic acid/g (per diet). Results showed that untreated, sensitized rats demonstrated an increase in the level of serum IgE, the level of histamine release, and incidence of diarrhea and reduced growth performance compared with the controls ($P < 0.05$). Lipoic acid significantly ($P < 0.05$) improved growth performance while reducing mast cell numbers, the level of serum IgE, and the level of histamine release. Lipoic acid supplementation altered the balance of cytokines, attenuated the Th2-type immune response, and amended the ratio of $CD4^+$ to $CD8^+$ T cells ($P < 0.05$). Our results suggest that a small dose of lipoic acid has the potential to be an immunomodulator to prevent soybean-induced allergies.

KEYWORDS: α-Lipoic acid; glycinin; anaphylaxis; cytokines; T lymphocytes

INTRODUCTION

Food allergies are becoming more common and increasingly important in humans and food-producing animals. Today soybean proteins are widely used in foods and feeds, and their popularity can be attributed to several factors, including their widespread availability, their high protein, lysine, and energy content, and their palatability (*1*). However, glycinin, the main storage globulin in soybean protein, has long been recognized as a source of dietary allergens (*2*). Glycinin is a heterogeneous protein with a polymorphic subunit composition, which varies among different cultivars. Each of the glycinin subunits (58−69 kDa) can be dissociated under reducing conditions into acidic (A, 31−45 kDa) and basic (B, 18−20 kDa) polypeptide chains. At present, five major subunits have been characterized (*1*), namely, A1aB2 (G1), A1bB1b (G2), A2B1a (G3), A3B4 (G4), and A5A4B3 (G5) (*3*). The acidic chain of the G1 subunit has an immunoglobulin E-binding epitope (*4*), which has been shown to increase the level of serum glycinin-specific immunoglobulin E (IgE) antibody with induced anaphylaxis (*5*).

Recent research has focused on the mechanisms of food-induced allergy and sought methods for decreasing their immunoreactivity (*6*). Several strategies for preventing or treating food allergy are currently in development, but avoidance of the respective food from the diet is still the safest way to avoid food-induced allergic reactions. Approaches to preventing IgE-mediated food allergies such as the use of anti-IgE, probiotics, and food allergy vaccines are not yet suitable for long-term and widespread application (*7*).

It is well-known that vitamin C is an immunomodulator that functions as an electron donor to protect the body from radicals and pollutants (*8*). It is postulated that exogenous vitamin C can lower blood levels of histamine and thus help to reduce the severity of an allergic reaction (*9*); a previous study suggested that a megadose of vitamin C (1000 μg/g, per diet, Dry-Matter Basis, DM Basis) can be used to attenuate soybean allergies in pigs, but the mechanism is not known (*10*).

Lipoic acid, also called thioctic acid, exists in the cellular membranes and the cytosol in both plants and animals (*11*). Lipoic acid also functions as an electron donor to protect the body from radicals and pollutants (*12*) and is several-fold more powerful than vitamin C. Small doses of lipoic acid can attenuate oxidative damage in vivo (*13*) and therefore might protect humans from allergic asthma, diabetes, high blood pressure, and some other diseases with few adverse side effects; however, no further research has been performed, and the mechanism is still unknown (*14*−*16*).

The objective of this study was to investigate whether a small dose of lipoic acid could attenuate soybean glycinin-induced allergy and to explore its potential mechanism. We used an experimental rat model for glycinin anaphylaxis, and this model could eliminate interference from other potential allergic components contained in soybean.

MATERIALS AND METHODS

Purified Soybean Glycinin. Samples of purified glycinin were kindly provided by S. Guo of China Agricultural University. Sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) analysis confirmed that the samples contained more than 90% glycinin (data not shown).

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Table 1. Design of the Animal Test[a]

	group I (control)	group II (no additive)	group III (casein-based diet supplemented with 12.5 μg of lipoic acid/g)	group IV (casein-based diet supplemented with 1000 μg of vitamin C/g)
intragastric gavage	casein	glycinin	glycinin	glycinin
day 1	25 mg	25 mg	25 mg	25 mg
day 10	25 mg	25 mg	25 mg	25 mg
day 17	25 mg	25 mg	25 mg	25 mg
day 24	25 mg	25 mg	25 mg	25 mg
day 31	50 mg	50 mg	50 mg	50 mg

[a] On day 31, 3 h after intragastric gavage, samples of blood were collected. On day 32, all rats were sacrificed.

Animals, Diets, and Experimental Protocol. All animals used in this experiment were maintained according to the guidelines of the China Agricultural University Animal Care and Use Ethics Committee. Forty-eight, recently weaned, female Sprague-Dawley rats (21 days old and weighing an average of 36.2 ± 0.8 g) were individually housed in polycarbonate cages with soft wood granulate floors.

The rats were assigned to one of four treatments (N = 12) and fed a casein-based diet either unsupplemented (groups I and II) or supplemented with either 12.5 μg of α-lipoic acid/g (per diet, DM Basis, 98% pure as obtained from Healthjoy Chemical Co., Shanghai, China) (group III) or 1000 ppm of vitamin C (per diet, DM Basis) (group IV). The basal diet was formulated according to the recommendations of the American Institute of Nutrition. Water and feed were available ad libitum throughout the 31 day growth trial (**Table 1**). Rats and feeders were individually weighed at the start and end of the trial (days 0 and 31) to calculate weight gain, feed intake, and feed conversion.

As shown in **Table 1**, rats in groups II−IV were sensitized by means of intragastric gavage with 25 mg of purified glycinin [per rat, 0.5 mL of a solution containing 50 mg/mL glycinin dissolved in phosphate-buffered saline (pH 7.4)] on day 1, 10, 17, and 24. The control group (group I) was gavaged with 25 mg of casein (per rat, 0.5 mL of a solution containing 50 mg/mL casein dissolved in phosphate-buffered saline) using the same treatment schedule. On day 31, rats received a double dose of glycinin or casein on the basis of the recommendations of Fischer et al. (*17*).

Blood was obtained from the tail vein of each rat on day 1, 10, 17, 24, and 31 using a 2 mL Clot Activator tube (Greiner Bio-One GmbH, Kremsmunster, Austria) 3 h after intragastric gavage. The blood was left to stand for 2 h in a 37 °C incubator and then centrifuged at 4000 rpm in an Anke TGL-16B centrifuge (Shanghai Anting Scientific Instruments, Shanghai, China) for 10 min at 4 °C. The serum was immediately frozen and stored at −70 °C for later analysis.

On day 32, all rats were sacrificed. The blood and small intestine were quickly isolated, flushed with ice-cold saline to remove the digesta, and stored for analysis.

Passive Cutaneous Anaphylaxis Testing. Serum, obtained from the 48 rats in the four treatment groups on day 31, was split into two tubes and either heated at 56 °C for 3 h or remained untreated (1:5 dilution). An additional 84, three-week-old Sprague-Dawley rats were intradermally treated with 50 μL of heated or unheated serum in the abdomen. Twenty-four hours later, all rats were intravenously treated with 100 μL of 0.5% Evan's blue dye, followed immediately by an intradermal injection of 50 μL of glycinin [10 mg/mL in phosphate-buffered saline (pH 7.4)]. A reaction was scored as positive if the discoloration of the skin at the injection site was greater than 5 mm in diameter in any direction.

Morphology of the Small Intestine. The samples from the small intestine were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The photographs of the morphology of the small intestine were collected with an Axioskop-2 microscope (Olympus, Tokyo, Japan), a 1300 × 1030 pixel-cooled CCD camera (Princeton Instruments, Trenton, NJ), and an image processing system (Visitron Systems, Puchheim, Germany). The villus height and crypt depth were measured as described by Li et al. (*18*).

Histological Analysis of Intestinal Mast Cells. Paraffin sections from the small intestine of the rats fixed in 10% neutral-buffered formalin were dewaxed, rehydrated, and transferred to a potassium permanganate solution for 2 min. The sections were rinsed in distilled water and were transferred to a potassium metabisulfite solution for 1 min (or until the sections turned white). Then, they were washed in tap water for 3 min, rinsed in distilled water again, placed in an acidified toluidine blue solution (toluidine blue, T3266, Sigma, St. Louis, MO) for 5 min, rinsed in distilled water, dehydrated rapidly, cleared, and mounted. The photographs were collected as described above. The number of mast cells in the mucosa 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). Each sample was represented by three independent histological slides. The mast cell number was then calculated as the overall cell number divided by square millimeters.

Determination of Serum IgE Antibody Levels. The total level of serum IgE antibody was determined using a rat IgE enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's instructions (RapidBio Laboratory, Calabasas, CA). Briefly, the antibody-coated plate was washed three times with washing buffer; 50 μL of the standard IgE solution was placed in the wells for the standard curve, and 50 μL of the diluted sample solution (diluted 1:100 with dilution buffer) was placed in sample wells. The plate was kept at room temperature (20−25 °C) for 2 h. After the reaction, the plate was washed three times with washing buffer, and 50 μL of the biotin-conjugated antibody solution was added to each well. The plate was kept for 2 h at room temperature. Then the plate was washed three times with washing buffer, and 50 μL of the HRP−avidin solution was added to each well. The plate was kept for 1 h at room temperature. After the plate had been washed, 50 μL of the chromogenic substrate (TMB) reagent was pipetted into each well, and the plate was kept for 20 min at room temperature. Then 50 μL of the reaction stopper was added to each well to stop further color development, and the absorbance of each well at 450 nm was measured. The standard curve was prepared by plotting absorbances obtained from standard wells versus IgE standard concentrations, and IgE concentrations of the diluted samples were obtained from their absorbance. The IgE concentrations of the original samples were obtained by multiplying the IgE concentrations by dilution factors of the samples (in our assay procedure, the dilution factor is 100).

The glycinin-specific IgE level was determined using an indirect ELISA as described by Sun et al. (*5*) with slight modifications. Briefly, 96-well microtiter plates (Canda Jet Bio-Chemicals International, Guangzhou, China) were coated with 5 μg/mL glycinin in carbonate buffer [$NaHCO_3$/Na_2CO_3 (pH 9.6)]. Then, appropriately diluted rat serum was added and the mixture incubated for 1 h. Biotinylated anti-rat IgE and streptavidin-conjugated horseradish peroxidase (RapidBio Laboratory) were added, and the mixture was incubated for 1 h following each addition. *O*-Phenylenediamine in substrate buffer was then added and the mixture incubated at 37 °C for 15 min in the dark. Prior to each addition, the plates were washed five times with PBST [0.05% Tween 20 in 0.01 mol/L phosphate-buffered saline (pH 7.4)]. After the addition of 50 μL of stop buffer (2 mol/L H_2SO_4) to each well, the absorbance was measured at 492 nm and the data were expressed in optical density units.

Measurement of Histamine Levels and Histamine Release Ratios. Samples of small intestine, ∼10 cm in length, were weighed, finely minced by syringe, lysed in lysis buffer [10 mM HEPES (pH 7.4), 0.15 M NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 0.5% NP-40, and 0.05% SDS with proteinase inhibitor cocktail freshly added], and diluted to 1 mg/mL (per gram of tissue). The histamine contents of the isolated supernatant and the serum were measured using a commercially available rat ELISA kit according to the instructions of the manufacturer (Adlitteram Diagnostic Laboratories, San Diego, CA). Briefly, 50 μL of

Table 2. Effect of Lipoic Acid on Growth Performance in Glycinin-Sensitized Rats[a]

		glycinin sensitization		
	control	no additive	12.5 μg of lipoic acid/g (per diet, DM basis)	1000 μg of vitamin C/g (per diet, DM basis)
ADG (g/day)[b,c]	4.76 ± 0.49 a	2.98 ± 0.57 b	4.45 ± 0.31 a	3.67 ± 0.33 b
ADFI (g/day)[c,d]	18.64 ± 0.69	17.52 ± 0.81	17.83 ± 0.57	18.69 ± 0.71
feed efficiency (F/G)[c,e]	3.92 ± 0.30 a	5.88 ± 0.41 b	4.01 ± 0.34 a	5.10 ± 0.44 b
diarrhea incidence (%)[f]	0 a	41.7 b	8.3 a	33.3 b

[a] Data are representative of three separate experiments. [b] Average daily gain. [c] Values are means ± standard deviations ($N = 12$). Values within the same row not sharing common letters are significantly different ($P < 0.05$) from each other, as analyzed by analysis of variance (ANOVA), followed by Duncan's test. [d] Average daily feed intake. [e] ADFI divided by ADG. [f] Significantly different as determined by a χ^2 contingency test.

Table 3. Passive Cutaneous Anaphylaxis Reactions after Injection of Heated or Unheated Serum Obtained from Glycinin-Sensitized Rats

	dietary treatment	heat treatment (56 °C for 3 h)	diameter (mm)[a]	positive reaction	
				N/total	%
glycinin sensitization	control	unheated	1.12 ± 0.28	0/12	0
	no additive	unheated	10.15 ± 1.32[b]	12/12	100
		heated	1.52 ± 0.22	0/12	0
	12.5 μg of lipoic acid/g (per diet, DM basis)	unheated	2.26 ± 0.43	1/12	8.3
		heated	1.34 ± 0.33	0/12	0
	1000 μg of vitamin C/g (per diet, DM basis)	unheated	7.44 ± 2.56[b]	8/12	66.7
		heated	1.38 ± 0.62	0/12	0

[a] Values are means ± standard deviations. Within the same group, data between the heated and unheated serum injection were analyzed by an unpaired Student's *t* test. [b] Significant difference between the heated and unheated serum ($P < 0.05$).

standards or samples (optimum dilution) was added to the appropriate wells in duplicate; 50 μL of the ready-to-use enzyme conjugate was added to each well and mixed when the plate was gently shaken. The plate was covered with plastic film and incubated at room temperature (20−25 °C) for 45 min. After the incubation of the conjugate, the contents of the plate were dumped out and tapped out thoroughly on a clean lint-free towel. Each well was filled with 300 μL of diluted wash buffer, and then the contents were dumped out three times; 150 μL of substrate was added to each well, and the plate was incubated at room temperature for 30 min. The plate was read in a microplate reader using a 650 nm filter. The standard curve was plotted, and the concentrations of the samples were estimated from the curve.

An additional sample of jejunum was obtained from the control rats for histamine release analysis. Intestinal mast cells were isolated, and histamine release testing was conducted as previously described by Sun et al. (*5*). Briefly, purified mast cells were resuspended in complete Hank's Balanced Salt Solution (1×10^6 cells/mL) and incubated in medium that was supplemented with serum obtained from the four treatment groups for 2 h. Then, glycinin was added to the suspension (final concentration of 100 μg/mL) and cultured for an additional 1 h. The histamine contents of both the cell pellet and the supernatant were measured. The histamine release ratio (%) was calculated as [(supernatant histamine)/(supernatant histamine + cell pellet histamine)] × 100.

Flow Cytometry Analysis of CD4$^+$ and CD8$^+$ Lymphocyte Subsets. The lymphocytes of the mesenteric lymph nodes (MLNs) were prepared and cultured as previously described (*19*). As for flow cytometry analysis, the lymphocytes were suspended with a cell density of 2×10^6 cells/mL, and CD4$^+$ and CD8$^+$ subset phenotypes were analyzed by flow cytometry.

Determination of Cytokines by an ELISA. The lymphocytes of MLNs were prepared and suspended as described above. The cell suspension was incubated with 50 μg/mL glycinin for 24 h at 37 °C with 5% CO_2. The cell culture supernatant was harvested. The concentrations of interleukin-2, interleukin-4, interleukin-10, and interferon-γ were determined using commercially available rat ELISA kits according to the instructions of the manufacturer (Biosource, Camarillo, CA).

Statistical Analysis. Differences in the occurrence of diarrhea among the four treatment groups were tested by χ^2 contingency test. All other data were analyzed using the analysis of variance (ANOVA) procedure of SAS (version 8.2, SAS Institute, Inc., Cary, NC). A P value of < 0.05 was considered statistically significant.

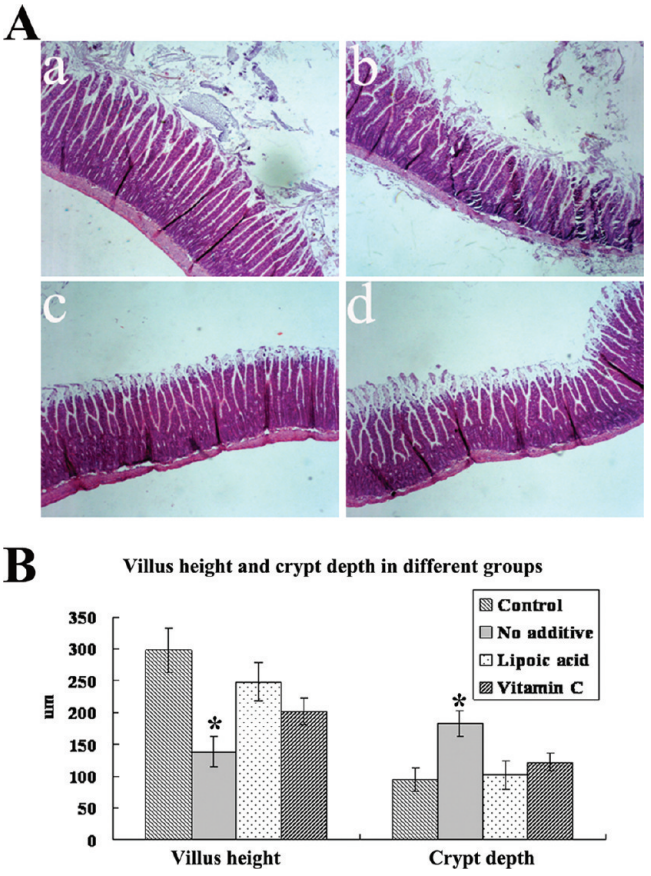

Figure 1. Effects of lipoic acid on the morphological structure of jejunum in glycinin-sensitized rats. (**A**) Morphological observation of different groups (the stained sections were photographed at 10× magnification): (**a**) control group, (**b**) sensitized group, (**c**) lipoic acid group, and (**d**) vitamin C group. (**B**) Villus height and crypt depth were measured. Values are means ± standard deviations ($N = 12$). Asterisks indicate $P < 0.05$ for lipoic acid (group III) vs sham (group II).

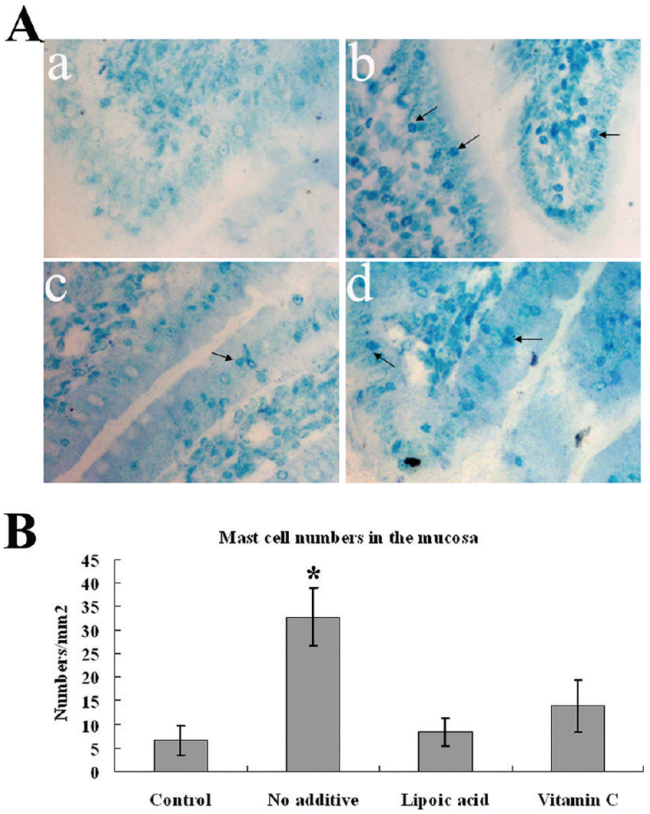

Figure 2. Effect of lipoic acid on mast cells of jejunum in glycinin-sensitized rats. (**A**) Morphological observation of mast cells of jejunum in different groups (the stained sections were photographed at 40× magnification): (**a**) control group, (**b**) sensitized group, (**c**) α-lipoic acid group, and (**d**) vitamin C group. (**B**) The mast cell numbers in the mucosa were measured (Materials and Methods). Values are means ± standard deviations (N = 12). The asterisk indicates P < 0.05 for lipoic acid (group III) vs sham (group II).

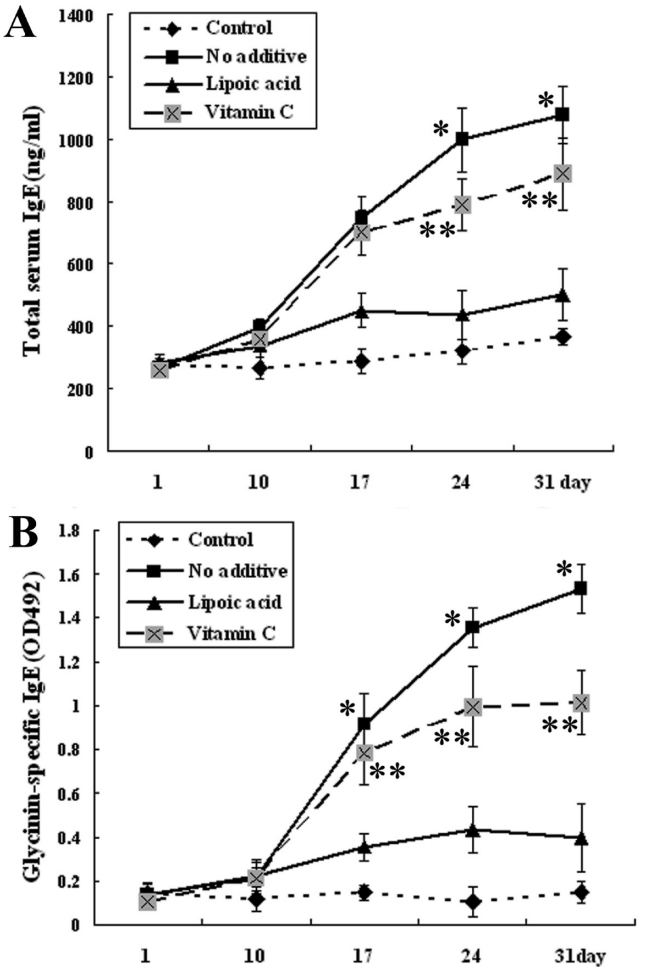

Figure 3. Total serum IgE (**A**) and glycinin-specific IgE (**B**) levels in the rats on days 1, 10, 17, 24, and 31 after weaning. Values are means ± standard deviations (N = 12). Experiments were performed in triplicate with similar results obtained each time, and data from one experiment are presented. Single asterisks indicate P < 0.05 for lipoic acid (group III) vs sham (group II); double asterisks indicate P < 0.05 for lipoic acid (group III) vs vitamin C (group IV).

RESULTS

Growth Performance and Diarrhea. As shown in **Table 2**, untreated rats sensitized with glycinin had significantly (P < 0.05) lower average daily weight gain and poorer feed conversion compared with the control. Supplementation with lipoic acid significantly (P < 0.05) improved the weight gain and feed conversion of rats sensitized with glycinin. In contrast, vitamin C was not effective in overcoming the depression in performance when rats were sensitized with glycinin. Daily feed intake was not affected by any treatment (P > 0.05). The occurrence of diarrhea was significantly (P < 0.05) decreased in the rats from the lipoic acid group compared with those from the unsupplemented challenged rats. Vitamin C-supplemented rats had diarrhea incidence similar to that of the unsupplemented challenged rats.

Passive Cutaneous Anaphylaxis Reactions. The development of a passive cutaneous anaphylaxis reaction was used to follow the induction of glycinin hypersensitivity. When unheated serum was injected, all rats in the unsupplemented challenged group exhibited positive reactions compared with none of the controls (**Table 3**). Only 8.3% of the sensitized rats supplemented with lipoic acid exhibited positive reactions compared with 66.7% of the rats treated with vitamin C. All reactions were eliminated when the serum was heated at 56 °C for 3 h (**Table 3**).

Morphology Structure of the Small Intestine and Mast Cells. To investigate the effect of α-lipoic acid on the glycinin-induced damage to the small intestine, the morphological structure of the jejunum was observed. **Figure 1** shows that the intestines were damaged with decreased villus height and increased crypt depth in the glycinin-challenged group compared to the control group (P < 0.05). Supplementation with either α-lipoic acid or vitamin C can improve the morphology of the small intestine injured by glycinin (P < 0.05). In particular, α-lipoic acid was more protective than vitamin C.

Increased degranulation of mast cells was clearly observed in the rats sensitized with soybean glycinin (**Figure 2**, P < 0.05). The mast cell numbers in the mucosa were measured, and the results showed that supplementation with either α-lipoic acid or vitamin C can restrain degranulation of mast cells and that α-lipoic acid was more protective (**Figure 2**, P < 0.05).

Total Serum IgE and Glycinin-Specific IgE Levels. To evaluate glycinin-induced anaphylactic reactions and explore the possible effects of lipoic acid and vitamin C on the development of allergy in sensitized rats, total IgE and glycinin-specific IgE levels in rat serum were determined four times after initial sensitization. As shown in **Figure 3**, the total serum IgE and glycinin-specific IgE concentrations were modestly increased on day 17 and significantly elevated (P < 0.05) on days 24 and 31 in the unsupplemented glycinin-treated group and the vitamin C-supplemented group compared with the control group and the lipoic acid-supplemented group.

Histamine Content and Histamine Release Ratio. The histamine content in the duodenum and jejunum of the unsupplemented glycinin-treated rats was significantly lower than the control (**Table 4**). However, treatment with lipoic acid significantly ($P < 0.05$) increased the histamine content compared with that of the untreated glycinin-sensitized group. Values for the vitamin C-supplemented group were intermediate between the untreated glycinin-sensitized group and the lipoic acid-supplemented group. The levels of histamine in the ileum were unaffected by treatment ($P > 0.05$).

In contrast to the effects of glycinin in the intestine, the histamine content of the serum in untreated sensitized rats was significantly ($P < 0.05$) higher than the control. This increase was amended to a large degree by supplementation with lipoic acid ($P < 0.05$).

Compared with the untreated glycinin-sensitized group, supplementation with lipoic acid suppressed ($P < 0.05$) the release of histamine from mast cells as indicated by a lower release ratio (**Table 4**). The histamine release ratio for the vitamin C-supplemented group did not differ from that of the untreated glycinin-sensitized group ($P > 0.05$).

$CD4^+$ and $CD8^+$ Lymphocyte Subset. As shown in **Table 5**, intragastric gavage with purified glycinin increased the percentage of the $CD4^+$ lymphocyte subset in the mesenteric lymph nodes (MLNs) compared with the control ($P < 0.05$), while the percentage of the $CD8^+$ lymphocyte subset was unaffected by treatment ($P > 0.05$). This resulted in an increase in the ratio of $CD4^+$ to $CD8^+$ in the untreated glycinin-sensitized group compared with the control ($P < 0.05$). The percentage of the $CD4^+$ lymphocyte subset and the ratio of $CD4^+$ to $CD8^+$ were decreased in the lipoic acid-treated group compared with those of the untreated sensitized group.

Cytokine Levels in the Cultured MLN Lymphocyte Supernatant. In the cultured MLN lymphocyte supernatant, both interferon-γ and interleukin-2 levels of rats sensitized with glycinin were significantly ($P < 0.05$) lower while interleukin-4 and interleukin-10 levels were dramatically higher ($P < 0.05$) compared with the control group. Supplementation with lipoic acid increased interferon-γ and interleukin-2 levels but depressed interleukin-4 and interleukin-10 levels compared with those of the untreated sensitized group (**Table 5**).

Table 4. Effects of Lipoic Acid on the Histamine Contents and Release Ratios of Glycinin-Sensitized Rats[a]

		glycinin sensitization		
	control	no additive	12.5 μg of lipoic acid/g (per diet, DM basis)	1000 μg of vitamin C/g (per diet, DM basis)
Histamine Content in the Small Intestine (μg/g, per tissue)				
duodenum	27.2 a	10.4 b	22.3 a	16.8 c
jejunum	25.0 a	13.5 b	21.9 a	17.9 a,b
ileum	20.7	16.3	21.0	18.9
Histamine Content in the Serum (ng/mL)				
	8.8 a	25.4 b	12.7 a	19.3 c
Histamine Release Ratio (%)				
	4.9 a	37.8 b	11.2 c	29.6 b

[a] Data are representative of three separate experiments. Values are means ± standard deviations ($N = 12$). Values within the same row not sharing common letters are significantly different ($P < 0.05$) from each other, as analyzed by analysis of variance (ANOVA), followed by Duncan's test.

DISCUSSION

Glycinin, the main storage globulin in soybean protein, increases the level of serum glycinin-specific immunoglobulin E (IgE) antibody in soybean allergic subjects (*2*, *5*, *20*). In this study, the Sprague-Dawley rat was selected to establish a model for the anaphylactic reactions induced by soybean glycinin, and the attenuation effects of consuming a small dose of lipoic acid were evaluated.

Our results showed that glycinin-sensitized rats had reduced growth performance and transient diarrhea. Moreover, total serum IgE and glycinin-specific IgE levels were significantly increased after sensitization, which was associated with a high rate of histamine release in the rats from the sensitized group. These findings indicate that a successful animal model for glycinin sensitivity via oral administration was established with the Sprague-Dawley rat (*17*, *21*). In general, it is very difficult to induce allergen-specific IgE by oral administration without an adjuvant. Because 90% pure glycinin was used in our animal sensitivity model, there is some possibility that the remaining 10% of soy impurities has adjuvant effects, which requires further research. Since previous research has suggested the Sprague-Dawley rats are comparatively allergy-resistant (*22*), we could hypothesize that some other more allergy-susceptible strains (such as Brown Norway rats) might exhibit more allergen-specific IgE production by glycinin. Further research will be performed to assess the responses of other rat strains.

Our results also showed that feeding a low dose of lipoic acid to glycinin-sensitized rats significantly reduced mast cell numbers in the mucosa, the level of histamine release, and total serum IgE and glycinin-induced IgE levels, which suggested that the

Table 5. Effects of Lipoic Acid on Lymphocyte Subset and Cytokine Levels of MLNs in Glycinin-Sensitized Rats[a]

		glycinin sensitization		
	control	no additive	12.5 μg of lipoic acid/g (per diet, DM basis)	1000 μg of vitamin C/g (per diet, DM basis)
Lymphocyte Subset of MLNs				
$CD4^+$ subset	37.4 ± 3.2 a	59.2 ± 4.1 b	40.3 ± 2.7 a	43.1 ± 2.9 a
$CD8^+$ subset	22.5 ± 0.5	19.4 ± 1.0	21.4 ± 0.9	18.9 ± 1.7
ratio of $CD4^+$ to $CD8^+$	1.66 ± 0.12 a	3.05 ± 0.27 b	1.88 ± 0.24 a	2.28 ± 0.35 a
Cytokine Concentrations in the Cultured Lymphocyte Supernatant of MLNs (pg/mL)				
interleukin-2	175.5 ± 15.4 a	42.5 ± 19.1 b	155.8 ± 18.4 a	132.6 ± 22.9 a
interleukin-4	100.8 ± 17.8 a	259.4 ± 20.4 b	144.5 ± 34.5 a	220.4 ± 21.0 b
interleukin-10	67.2 ± 6.4 a	210.1 ± 15.2 b	77.5 ± 23.3 a	80.2 ± 24.1 a
interferon-γ	552.3 ± 40.9 a	189.5 ± 33.6 b	497.1 ± 34.8 a	300.2 ± 49.0 c

[a] Data are representative of three separate experiments. Values are means ± standard deviations ($N = 12$). Values within the same row not sharing common letters are significantly different ($P < 0.05$) from each other, as analyzed by analysis of variance (ANOVA), followed by Duncan's test.

supplementation of the diet with lipoic acid exerts its inhibiting effect on the sensitization and not on the anaphylactic response (*23*). In a previous study using pigs, it was reported that supplementation of a megadose of vitamin C attenuated glycinin-induced anaphylactic reactions to a certain degree (*10*). The results of our study demonstrate that a small dose of lipoic acid was much more effective than a megadose of vitamin C, at least in the weaned rat model. In addition to its ability to attenuate glycinin-induced allergy, recent studies have shown that lipoic acid can intervene in anaphylaxis (especially allergic asthma), dermatitis, and the development of early pediatric allergies in infants and children (*16*, *24*−*26*), as well as decelerate the progression of some kinds of autoimmune diseases such as cirrhosis and collagen-induced arthritis (*27*). However, the pathways and mechanisms of lipoic acid action that lead to these phenotypic features have not been fully elucidated.

To explore potential mechanisms, analysis of cytokine and T lymphocyte subsets in the mesenteric lymph nodes (MLNs) was performed. The results showed that both interferon-γ and interleukin-2 levels of rats supplemented with a small dose of lipoic acid were significantly higher, while interleukin-4 and interleukin-10 levels were drastically lower than the sham group sensitized with purified glycinin or the megadose vitamin C group. In addition, the results of the $CD4^+/CD8^+$ lymphocyte subset analysis showed that supplementation with a small dose of lipoic acid altered the percentage of the $CD4^+$ lymphocyte subset and decreased the ratio of $CD4^+$ to $CD8^+$, which was disordered by glycinin-induced allergy.

T lymphocytes can be classified as $CD4^+$ or $CD8^+$ subsets according to their surface markers (*28*). $CD4^+$ lymphocytes can be further divided into T-helper 1 (Th1) cells and T-helper 2 (Th2) cells according to their pattern of secretion of cytokines (*29*). Th1 lymphocytes secrete interleukin-2, tumor necrosis factor, and interferon to initiate cell-mediated immune responses, while Th2 lymphocytes are mainly involved in humoral immune responses via the production of cytokines such as interleukin-4 and interleukin-10 that play important roles in allergic responses (*29*−*31*). In this study, the $CD4^+$ lymphocytes were significantly increased in percentage due to the oral administration of glycinin, which in turn increased the ratio of $CD4^+$ to $CD8^+$ lymphocytes.

It has been well documented that food-induced hypersensitivity is a predominantly Th2-type immune response (*32*), in which the Th2-type representative cytokine interleukin-4 plays a key role in allergic inflammation by favoring the production of total IgE and antigen-specific IgE antibodies (*31*). Our study showed that levels of interleukin-4 and interleukin-10 were suppressed by lipoic acid which subsequently inhibited the Th2-type immune response. At the same time, lipoic acid promoted the production of interferon-γ, which functioned reciprocally on the induction of IgE responses and histamine release, thus alleviating the inflammation in vivo (*33*). Similar results were reported by Lee et al. (*24*), who found that lipoic acid inhibited tumor necrosis factor-α-induced NF-κB activation. These results suggest that the antihistamine effect of lipoic acid might be mediated by the attenuation of the Th2-type immune response in the allergic subjects in response to glycinin-induced anaphylaxis.

Besides, our findings are in agreement with previous results from an animal allergic asthma model showing that either oral *N*-acetylcysteine or lipoic acid, which acted as a free radical scavenger, could suppress NF-κB DNA binding activity and reduce intracellular reactive oxygen species (ROS) levels (*16*, *34*, *35*). Taken together, the findings of our study suggest that the functional mechanism underlying the therapeutic efficacy of lipoic acid as an antioxidant in glycinin-induced allergy may be related to inhibition of NF-κB activation in T lymphocytes through decreased oxidative stress, which led to the decrease in the level of activated $CD4^+$ lymphocytes and subsequent reduction in the level of proinflammatory chemical mediators. However, because lipoic acid may act by other mechanisms, such as a direct intervention in intracellular inflammatory signaling pathways without reduction of ROS (*36*, *37*), lipoic acid may induce antiallergic effects through several different anti-inflammatory mechanisms, which needs to be further elucidated.

In conclusion, our study demonstrates that lipoic acid altered the balance of cytokines, attenuated the Th2-type immune response, and amended the ratio of $CD4^+$ to $CD8^+$ T cells. A small dose of lipoic acid has considerable potential to be used as an immunomodulator to prevent soybean-induced allergies. Adding a small dose of lipoic acid might have potential application in attenuating other food protein-induced allergies.

ACKNOWLEDGMENT

We thank Dr. Phil Thacker (University of Saskatchewan, Saskatoon, SK) for excellent assistance in editing the manuscript.

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Received for review December 13, 2009. Revised manuscript received February 2, 2010. Accepted March 12, 2010. The investigation was supported by the National Natural Science Foundation of P. R. China (Grant 30800790), the Doctoral Program Foundation of Institutions of Higher Education of China (Grant 200800191018), and the National Key Laboratory Foundation of Animal Nutrition (Grant 2004DA125184-0806-05).