

Soybean isoflavones regulate dendritic cell function and suppress allergic sensitization to peanut

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Background: Although peanut and soybean proteins share extensive amino acid sequence homology, the incidence and severity of allergic reactions to soy are much less than those to peanut. Soybeans are rich in anti-inflammatory isoflavones and are the most common source of isoflavones in the human food supply. **Objective:** We hypothesized that the active isoflavones in the gut milieu are capable of modulating immune responses to dietary antigens by regulating dendritic cell (DC) function.

Methods: We tested this hypothesis in a murine model of peanut allergy and in human monocyte-derived dendritic cells (MDDCs). C3H/HeJ mice were fed a diet containing genistein and daidzein. The mice were sensitized and challenged with peanut, and the anaphylactic symptoms were compared with those of mice fed a soy-free diet. Human MDDCs were activated with cholera toxin in the presence of isoflavones. The surface expression of DC activation markers and DC-mediated effector functions were analyzed by means of flow cytometry.

Results: Dietary isoflavones significantly reduced the anaphylactic symptoms and mast cell degranulation *in vivo* after peanut challenge. Serum peanut-specific antibodies were markedly reduced in mice fed the isoflavone diet. Isoflavones inhibited cholera toxin-induced DC maturation in the mesenteric lymph nodes and human MDDCs and subsequent DC-mediated CD4⁺ T-cell function *in vitro*.

Conclusions: These data suggest that dietary isoflavones suppress allergic sensitization and protect against peanut allergy *in vivo*. Dietary supplementation of soybean isoflavones could be a novel strategy to prevent the development of allergic reactions to food. (J Allergy Clin Immunol 2011;■■■:■■■-■■■.)

Key words: Peanut allergy, soybean, isoflavones, genistein, daidzein, dendritic cells, T cells

Peanuts and tree nuts are among the most allergenic foods in children and adults. Allergic reactions to these foods can be severe and sometimes fatal and affect about 3% of children and

Abbreviations used

CT: Cholera toxin
DC: Dendritic cell
MDDC: Immature monocyte-derived dendritic cell
MLN: Mesenteric lymph node
MMCP-1: Mouse mast cell protease 1

1% to 2% of adults in Western countries.¹ Once established, peanut and tree nut allergies are rarely outgrown. Because there are no therapies currently available, avoiding the offending allergen in the diet is the primary treatment for food allergy.^{2,3}

Several allergenic proteins in peanut share significant amino acid sequence homology to soy. For example, the major peanut allergen Ara h 1 (7S globulin fraction) shares approximately 70% homology to Gly mBd 30K/P34, the major allergen in soy.^{4,5} Similarly, Ara h 2 and Ara h 3 are 2S albumin and 11S globulin family members, respectively, with about 40% to 50% homology to their counterparts in soy.^{6,7} Despite the high similarity between peanut and soy allergens, the incidence and severity of soy allergy is much lower than that of peanut and is usually resolved very early in life.^{8,9} Soy is reported to be the least frequent cause of food allergy among all the allergenic food types.⁹ We hypothesize that these observations could be attributed to the abundance of phytonutrients known as isoflavones in soybean.

Isoflavones belong to a class of molecules related to flavonoids (Fig 1). Soybeans are the most common source of isoflavones in the human diet,¹⁰ whereas peanuts contain negligible/undetectable amounts. Isoflavones, such as genistein, daidzein, and glycitein, have been shown to have anti-inflammatory and antioxidant properties.¹¹ The immune-regulatory effects of isoflavones, specifically genistein, have been extensively investigated.¹² Genistein has been widely used as an inhibitor of protein tyrosine kinases.^{13,14} Isoflavones have been shown to inhibit a number of cellular processes^{13,14} and influence immune responses,^{12,15} including humoral,¹⁶ cellular,¹⁷ and natural killer cell¹⁸⁻²⁰ responses. Most of these studies used pharmacologically high doses of isoflavones *in vitro*, injection of active aglycone forms of isoflavones into animals, or both, conditions that might not reflect the true effects of isoflavones *in vivo*. Nevertheless, these studies provide insights into the possible cellular mechanisms that could be regulated by isoflavones.

Several studies in human subjects and animals have shown that dietary soy intake has beneficial effects against cancer,²¹ obesity,²² cardiovascular conditions,²³ and menopausal symptoms.^{12,16,17} Some epidemiologic studies have linked soy intake with beneficial effects in patients with allergic diseases: the high intake of soy-containing foods and isoflavones is associated

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with reduced prevalence of allergic rhinitis²⁴ and better lung function in asthmatic patients.²⁵ Dietary soy supplementation reduced antigen-induced eosinophilia in the lungs in a guinea pig model of asthma²⁶ and reduced eosinophil leukotriene C₄ synthesis and eosinophilic airway inflammation in asthmatic patients.²⁷ A koji-probiotic fermentation product that also contains soy isoflavones has been shown to reduce peanut allergy symptoms in a murine model.²⁸ However, in spite of these favorable outcomes, the role of isoflavones in the pathogenesis of food allergy has not been investigated in detail, and the mechanisms of action of isoflavones are not yet clear.

We tested the hypothesis that soy isoflavones could have beneficial effects on the onset and development of food allergy. We show that dietary isoflavones suppress allergic sensitization to peanut and protect against peanut allergy *in vivo*. Using human dendritic cells (DCs) *in vitro*, we show that isoflavones inhibit DC maturation.

METHODS

Induction of peanut allergy and assessment of anaphylaxis

Peanut allergy was induced in mice (6- to 8-week-old C3H/HeJ mice, 5 mice per group) fed with a soy-free diet (TestDiet; Purina Feed LLC, Richmond, Ind) or a diet containing 1500 ppm each of genistein and daidzein (LC Laboratories, Woburn, Mass). The animals were housed in pathogen-free conditions according to standard institutional guidelines. The mice were maintained on their respective diets for at least 2 to 3 weeks before start of a sensitization protocol. Mice were sensitized weekly with 10 mg of freshly ground crude peanut solution plus 20 μ g of cholera toxin (CT; List Biological Laboratories, Campbell, Calif) as adjuvant for 5 weeks, boosted with 50 mg of peanut plus 20 μ g of CT for 2 weeks, and finally challenged twice in 30-minute intervals with 200 mg of peanut at week 10. Feeding was performed by means of intragastric gavage by using a ball-ended mouse-feeding needle with a final volume of 500 μ L of 1.5% sodium bicarbonate in PBS.

The mice were scored for the development of allergic symptoms after 30 minutes of second challenge by using a previously published scoring method²⁹: (1) scratching and rubbing around the snout and head; (2) puffiness around the eyes and snout, diarrhea, pilar erecti, and reduced activity and/or decreased activity with increased respiratory rate; (3) wheezing, labored respiration, and cyanosis around the mouth and tail; (4) no activity after prodding, tremor, and convulsions; and (5) death. The scoring was done in a blinded manner by 2 investigators unaware of the identities of the groups.

Rectal temperatures were measured with a thermal probe (Harvard Apparatus, Holliston, Mass). Blood collection by means of submandibular or retro-orbital bleeding was performed on weeks 3, 6, and 8. Serum was analyzed for peanut-specific IgE, IgG₁, and IgG_{2a} by means of ELISA, as described previously.²⁹ Paraffin-embedded sections of ear specimens obtained from mice sacrificed within 60 minutes of challenge were stained with toluidine blue, and visually examined for mast cell degranulation. Plasma mouse mast cell protease 1 (MMCP-1) levels were analyzed from blood collected 1 hour after the final challenge by means of ELISA (Moredun Scientific, Scotland, United Kingdom), as described elsewhere.³⁰

Cells and reagents

Immature monocyte-derived dendritic cells (MDDCs) were generated by culturing CD14⁺ monocytes from human buffy coat PBMCs for 4 to 5 days with 10 ng/mL GM-CSF and 10 ng/mL IL-4 in RPMI medium (reconstituted with 10% FCS, 2 mmol/L glutamine, 100 U/mL penicillin, and 100 μ g/mL streptomycin). Naive CD4⁺ T cells were isolated from PBMCs by means of negative selection (StemCell Technologies, Vancouver, British Columbia, Canada).

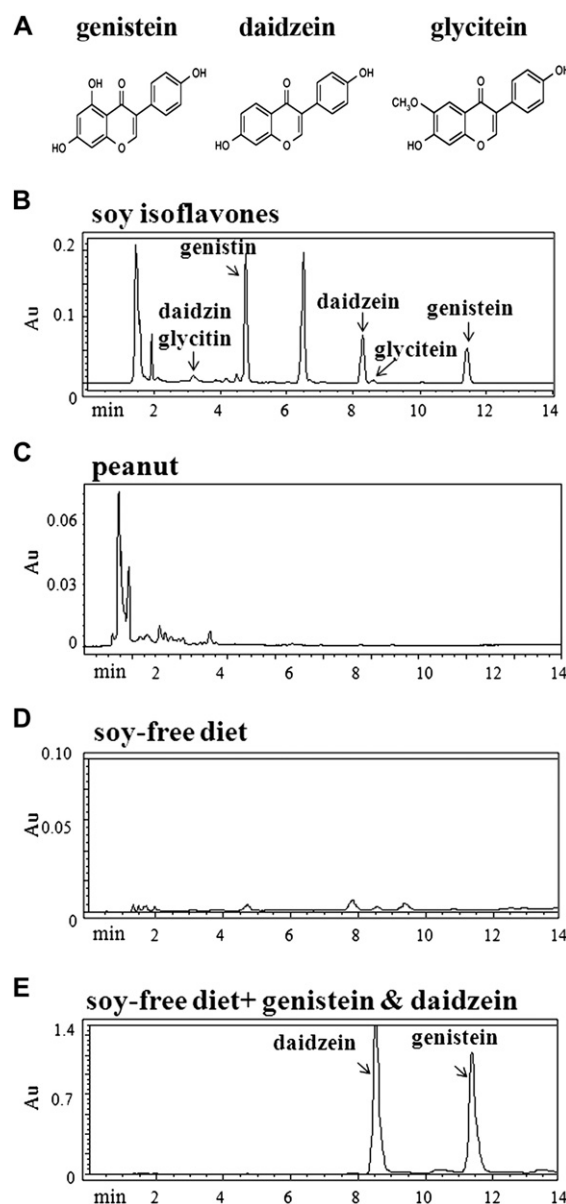


FIG 1. A, Chemical structure of the soybean isoflavones genistein, daidzein, and glycitein. B-E, Isoflavone profile of soybean and animal diets: powdered defatted soybeans (Fig 1, B), peanut (Fig 1, C), soy-free diet (Fig 1, D), and isoflavone diet (Fig 1, E) were extracted with acetonitrile, and HPLC was performed with a C-18 column. Peanut and the soy-free diet were completely devoid of isoflavones (Fig 1, C and D). Two distinct peaks representative of genistein and daidzein were seen in the isoflavone diet (Fig 1, E).

HPLC

The soy-free and isoflavone diets were lyophilized and directly extracted with acetonitrile at room temperature for 16 to 18 hours. Defatted protein extracts were prepared from soybean and peanuts and dissolved in PBS before extracting with an equal volume of acetonitrile. HPLC was performed with the Waters Alliance 2695 HPLC system (Waters Corp, Milford, Mass). The separation was performed on a ZORBAX SB-C18 column (Agilent Technologies, Santa Clara, Calif). Individual isoflavones are distinguished by their absorption peaks at 254 nm. The peaks were identified by using pure compounds as controls and determination of molecular weights by means of mass spectrometry.

DC activation, isoflavone treatment, and flow cytometry

The immature MDDCs were activated for 18 hours with 1 $\mu\text{g/mL}$ CT in the presence/absence of genistein, daidzein, or glycitein (100 $\mu\text{mol/L}$). Cells were stained with fluorescent antibodies against CD83, CD80, and CD86 and analyzed by means of flow cytometry (BD-LSR2 [BD Biosciences, San Jose, Calif] and FlowJo Software [TreeStar, Inc, Ashland, Ore]).

Cytokine secretion

MDDCs were activated with CT with or without isoflavones for 18 hours. The cells and the supernatants were separated by means of centrifugation. IL-6 and IL-8 levels were estimated in DC supernatants by using the cytometric bead array (BD Biosciences). The washed DCs were cultured with freshly isolated allogeneic naive CD4⁺ T cells (1:4 ratio) for 5 days. IL-13, IL-5, and IL-9 levels were estimated in the coculture supernatants by using the cytometric bead array.

Statistical analysis

Statistical analyses were performed with Prism software (GraphPad Software, Inc, La Jolla, Calif). The 2-tailed *t* test, 1-way ANOVA with the Bonferroni posttest, or repeated-measures ANOVA with 95% CIs was performed as appropriate to assess statistical significance.

RESULTS

Dietary isoflavones suppress peanut-induced anaphylactic reactions *in vivo*

The major active isoflavones (aglycones) present in soybean are genistein, daidzein, and glycitein (Fig 1, A), and their respective inactive glycosides are genistin, daidzin, and glycitin. These isoflavones can be identified by their characteristic retention times in HPLC (Fig 1, B). In contrast, peanuts have negligible quantities of isoflavones (Fig 1, C).

We have used the C3H/HeJ murine model to investigate the effect of isoflavones on the onset and pathogenesis of peanut allergy. This murine strain has been previously shown to be susceptible to peanut allergy by means of oral sensitization.²⁹ We maintained mice on a soy-free rodent diet (<1 ppm isoflavones; Fig 1, D) or a diet containing genistein and daidzein (1500 ppm each; Fig 1, E) for at least 2 weeks before starting the sensitization protocol (Fig 2, A) until the end of the experiment. Mice were sensitized and boosted with crude peanut solution and CT as adjuvant. The mice were finally challenged with peanut, and allergic/anaphylactic reactions were recorded and compared with each other (Fig 2, B). Mice fed a soy-free diet showed severe symptoms of anaphylaxis (average score of 3 and a decrease in body temperature [up to 2°C–3°C], which is a hallmark of anaphylaxis; Fig 2, C). In contrast, 35% to 40% of the mice in the isoflavone diet group showed no symptoms at all or significantly less severe symptoms (average score of 1; $P < .001$; Fig 2, B) and no significant decrease in body temperature ($P < .05$) compared with mice in the soy-free diet group (Fig 2, C). Naive unsensitized mice showed none of the above allergic symptoms after peanut challenge. These results demonstrate that dietary isoflavones suppressed the development of allergic reactions to peanut in this model of peanut allergy.

Suppression of mast cell degranulation by isoflavones *in vivo*

One of the major factors contributing to anaphylaxis is the degranulation and release of inflammatory mediators from mast

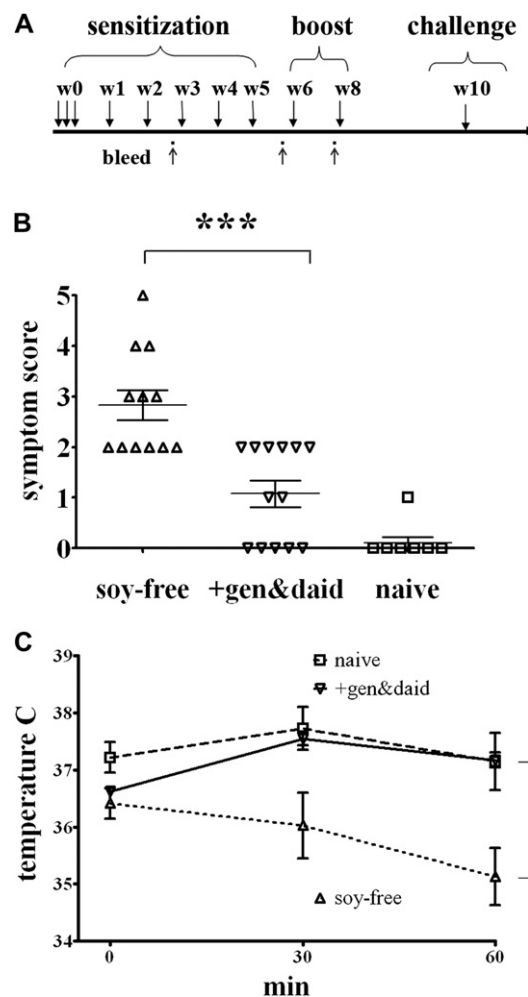


FIG 2. A, Murine sensitization protocol (see the Methods section). B, Mice fed with either the soy-free diet ($n = 12$) or the diet containing genistein and daidzein (*gen&daid*; 1500 ppm each; $n = 12$) for at least 2 weeks before the experiment were sensitized and challenged with peanut extract as shown in the Methods section and compared with naive unsensitized mice ($n = 9$). The clinical symptoms of anaphylaxis were given a score of between 0 and 5. Data from 3 independent experiments are shown ($***P < .001$, 1-way ANOVA). C, Rectal temperatures of mice receiving the soy-free diet ($n = 8$) or the genistein and daidzein diet ($n = 4$) were measured at 30 minutes after 2 successive challenges ($*P < .05$, *t* test). Data from 2 independent experiments are shown.

cells and basophils. We assessed the skin mast cell degranulation by means of histology in murine ears obtained 1 hour after challenging the mice with peanut (Fig 3, A). Mice fed the soy-free diet had numerous degranulated mast cells comprising about 50% to 65% of total cells counted (mean, 57%; Fig 3, A). In mice fed the isoflavone diet, the percentage of degranulated mast cells was significantly reduced to about 25% to 30% (mean, 29%; $P < .001$). The mean percentage of degranulated mast cells in naive mice was 18%. The suppression of mast cell degranulation in ear specimens indicates a systemic tolerance toward peanut sensitization.

MMCP-1, a β -chymase, is predominantly expressed in intestinal mucosal mast cells and is released during mast cell degranulation.^{31,32} We tested for plasma MMCP-1 levels in blood obtained 30–60 minutes after the final peanut challenge (Fig 3, B). MMCP-1 levels were significantly increased in mice fed the soy-free diet (mean, 120 ng/mL) compared with levels in mice

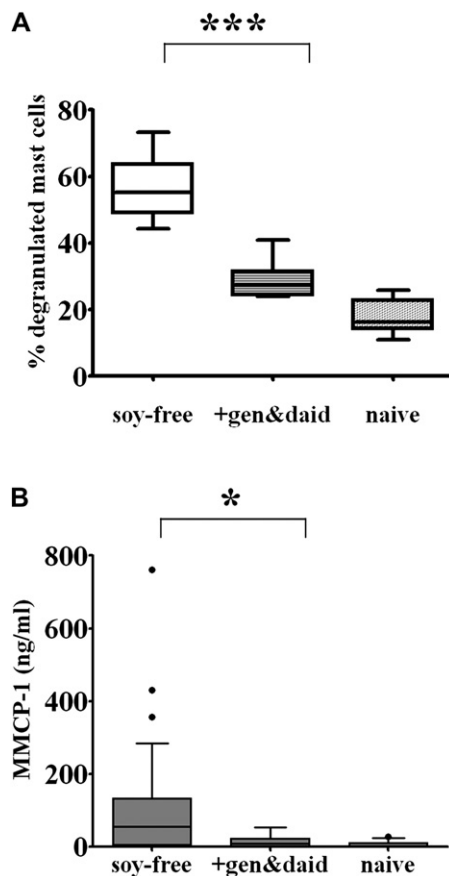


FIG 3. **A**, The percentage of mast cells in ear sections from mice receiving the soy-free diet ($n = 12$) or the genistein and daidzein (*gen&daid*) diet ($n = 12$) that are degranulated in each group and compared with naive unsensitized mice ($n = 9$). Data from 3 independent experiments are shown as box-and-whisker Tukey plots ($***P < .001$, 1-way ANOVA). **B**, Plasma MMCP-1 levels were tested in blood obtained within 60 minutes of challenge by means of ELISA. Data from 3 independent experiments are shown as box-and-whisker Tukey plots ($*P < .05$, 1-way ANOVA).

fed the isoflavone diet (mean, 15 ng/mL; $P < .05$), which are comparable with those in naive mice, indicating a reduction of mast cell degranulation in the mice fed the isoflavone diet. These results correlate very well with the decrease seen in mast cell degranulation (Fig 3, A) and indicate that dietary isoflavones suppress allergic reactions to peanut (Fig 2).

Peanut-induced antibody levels in mice

Because the presence of allergen-specific antibodies in blood is a good marker for immune response against allergens, we evaluated peanut-specific antibody levels in peanut-sensitized mice. Mice were bled on weeks 3, 6, and 8 during the sensitization protocol and tested for peanut-specific IgE, IgG_{2a}, and IgG₁. Peanut-specific IgE levels were markedly reduced in mice fed the isoflavone diet compared with those seen in mice fed the soy-free diet at all time points tested ($P < .01$; Fig 4, A). Similarly, peanut-specific IgG_{2a} levels were also lower in mice fed the isoflavone diet ($P < .05$; Fig 4, B). IgG₁ levels were similar in both mice fed the soy-free diet and those fed the isoflavone diet (Fig 4, C). These results indicate that the dietary isoflavones regulate the humoral immune response to peanut by suppressing allergen-specific IgE and IgG_{2a} production.

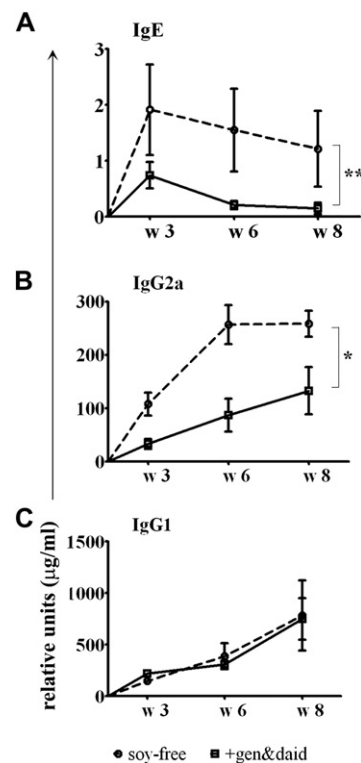


FIG 4. Peanut-specific serum IgE (**A**), IgG_{2a} (**B**), and IgG₁ (**C**) levels were estimated by means of ELISA from murine blood collected on weeks 3, 6, and 8 and plotted as line graphs. Data from 5 to 10 mice from each group from 2 independent experiments are shown ($**P < .01$, and $*P < .05$, t test). *gen&daid*, Genistein and daidzein.

Isoflavones inhibit DC maturation

Oral administration of CT is known to induce the activation and migration of DCs from the lamina propria to the mesenteric lymph nodes (MLNs).³³ To investigate the mechanism of isoflavone-mediated immune suppression, we fed CT (20 μ g/250 μ L of PBS) to mice that were maintained on either a soy-free or isoflavone diet and tested the activation status of MLN DCs after 18 hours. Single-cell populations from MLNs were stained for DC markers, such as CD86, CD80, and CD83, followed by flow cytometry (Fig 5). The expression of these molecules was analyzed in the CD103^{hi}CD11c⁺MHC-II^{hi} DC population. Oral administration of CT resulted in upregulation of these molecules in this MLN DC population in mice fed soy-free diets, which is indicative of DC maturation *in vivo*. In contrast, in MLNs from mice fed isoflavone diets, there was no such upregulation of these molecules ($P < .05$), suggesting a suppression of DC maturation by isoflavones.

We then tested the effects of isoflavones on human DC activation. We chose to use monocyte-derived dendritic cells (MDDCs) because of the relative ease in generation of large numbers of DCs *in vitro*. CT stimulation of MDDCs has been shown to induce T_H2 responses *in vitro*.³⁴ The half maximal inhibitory concentration (IC₅₀) values of various related flavonoids in suppressing *in vitro* degranulation of IgE cross-linked RBL-2H3 cells ranged from 1 to greater than 100 μ mol/L.³⁵ Using titrated concentrations, we found that the effect of isoflavones on DC maturation was dose dependent (see Fig E1 in this article's Online Repository at www.jacionline.org). Isoflavones did not

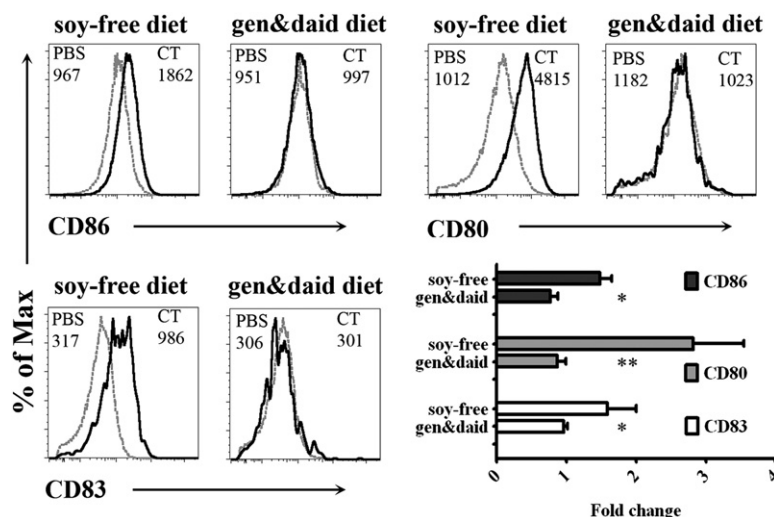


FIG 5. Mice were maintained on either a soy-free or isoflavone diet for at least 2 weeks and administered 20 μ g of CT/100 μ L of PBS by means of intragastric gavage. Single-cell preparations of the MLNs isolated after 18 hours of CT feeding were stained with the following fluorescent-conjugated antibodies followed by flow cytometry: CD11c, MHC-II (A/E), CD103, CD80, CD83, and CD86. The histograms for CD86, CD80, and CD83 stainings of MLN DCs falling in CD103⁺CD11c⁺MHC-II^{hi} gates are shown. The histograms shown are from a representative mouse from each group from 3 independent experiments. PBS- and CT-treated mice are indicated as dotted gray and solid black lines, respectively (* $P < .05$, t test). *gen&daid*, Genistein and daidzein.

affect cell viability at the concentrations used (100 μ mol/L, data not shown). Because it is not possible to measure exactly the concentration of active isoflavones to which DCs are exposed in the gut, we chose to use the maximum concentration of isoflavones that had a minimal effect on cell viability in our titration experiment (see Fig E1), hoping to mimic the effect of a high soy-containing meal *in vitro* at this concentration. MDDCs were activated with CT (1 μ g/mL) in the presence or absence of isoflavones (100 μ mol/L) for 18 hours. The surface expressions of CD83, CD80, and CD86 were compared with those of unstimulated controls (No CT, taken as 100%; Fig 6). CT treatment induced CD83 and CD86 upregulation on DC surfaces, which is suggestive of DC maturation *in vitro*. CD80 upregulation was only marginal during CT treatment. The presence of isoflavones during CT treatment inhibited the activation-induced upregulation of these molecules to varying extents when compared with CT or vehicle control (dimethyl sulfoxide). Both genistein and daidzein significantly suppressed CT-induced CD83 and CD80 expression (Fig 6, A and B). No significant changes were seen in CT-induced CD86 expression by these isoflavones (Fig 6, C). The observed inhibition of activation-induced DC marker expression suggests a potential role of isoflavones in DC maturation. Moreover, the effect of isoflavones in DC maturation is specific to the expression of CD80 and CD83 but not CD86 and other activation-induced DC markers (data not shown).

Isoflavones inhibit cytokine secretion from CT-activated DCs

DC maturation is associated with secretion of various cytokines required for the development of appropriate immune responses. Therefore we tested the cell-culture supernatants of CT-activated MDDCs with or without isoflavones for IL-6, IL-8, IL-10, IL-12, and TNF- α . As in previous reports,^{34,36} we could not detect T_H1 cytokines, such as IL-12, TNF- α , and IL-10, in DC supernatants

stimulated with CT (data not shown). However, these cytokines were readily detected with LPS stimulation of DCs (data not shown). CT-induced DCs secreted detectable amounts of the proinflammatory cytokines IL-6 (mean, 36 pg/mL; Fig 7, A) and IL-8 (mean, 832 pg/mL; Fig 7, B). When DCs were stimulated with CT in the presence of isoflavones, the CT-induced IL-6 and IL-8 secretion was significantly suppressed by genistein (mean IL-6, 18 pg/mL [$P < .05$]; mean IL-8, 159 pg/mL [$P < .05$]). We observed a nonsignificant trend toward suppression of IL-6 and IL-8 secretion by daidzein (mean IL-6, 31 pg/mL [nonsignificant]; mean IL-8, 130 pg/mL [nonsignificant]). These data demonstrate the anti-inflammatory properties of genistein during DC maturation.

Isoflavone-treated activated DCs inhibit T_H2 cytokine secretion from CD4⁺ T cells

To further explore the functional consequences of isoflavone-induced phenotypic changes in DCs, we investigated the effect of isoflavone-treated DCs in a mixed lymphocyte reaction. CT-activated DCs with or without isoflavones were washed and cultured with allogeneic naive CD4⁺ T cells for 5 days. IL-13 (Fig 7, C), IL-5 (Fig 7, D), and IL-9 (Fig 7, E) levels were estimated in culture supernatants on day 5. CT-activated DCs induced differentiation of allogeneic CD4⁺ T cells to secrete these T_H2 cytokines (mean IL-13, 1852 pg/mL; mean IL-5, 168 pg/mL; mean IL-9, 41 pg/mL). The presence of genistein during DC activation significantly inhibited the ability of DCs to induce the production of IL-13 and IL-9 from CD4⁺ T cells *in vitro* (mean IL-13, 858 pg/mL [$P < .05$]; mean IL-9, 12 pg/mL [$P < .05$]). We observed a nonsignificant trend toward suppression of IL-13 and IL-9 from CD4⁺ T cells by daidzein (mean IL-13, 1500 pg/mL [nonsignificant]; mean IL-9, 23 pg/mL [nonsignificant]). In addition, there was a nonsignificant trend toward suppression of IL-5 from CD4⁺ T cells by genistein but not daidzein (mean

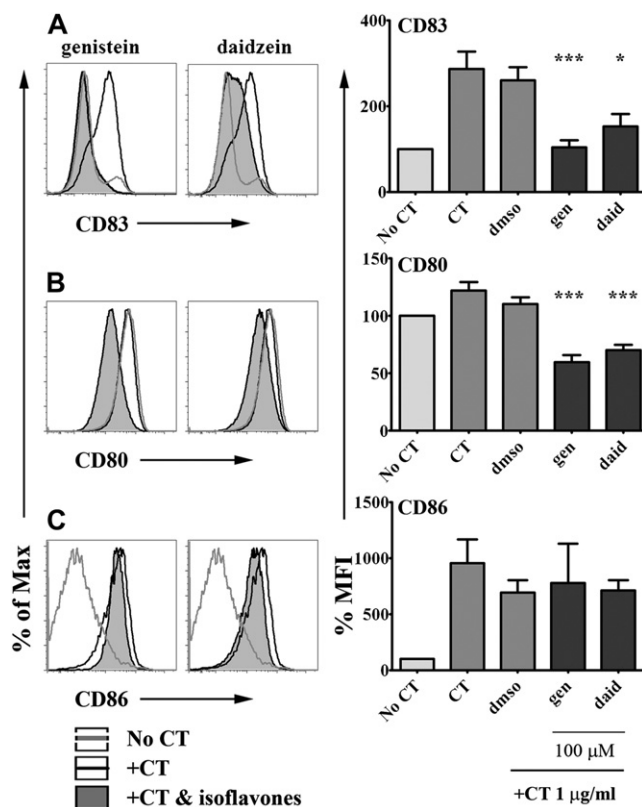


FIG 6. Human MDDCs were activated with 1 μ g/mL CT with or without 100 μ M isoflavones or dimethyl sulfoxide (*dms*; vehicle control) for 18 hours and stained with CD83 (A), CD80 (B), and CD86 (C). Unstimulated controls (No CT) or CT- or CT & isoflavone-treated cells were shown in solid gray, solid black, and shaded histograms, respectively. The percentage surface expression levels were calculated from the geometric mean fluorescent intensities, in which unstimulated controls (No CT) were taken as 100%, and shown as bar graphs. Data from a total of at least 4 experiments are shown (* P < .05 and *** P < .001, 1-way ANOVA). *daid*, Daidzein; *gen*, genistein.

IL-5, 87 and 179 pg/mL, respectively). These data demonstrate that genistein suppressed DC-mediated CD4⁺ T-cell activation and cytokine secretion.

DISCUSSION

The majority of the dietary isoflavones in soybean are present as inactive glycosides (genistin, daidzin, and glycitin). The bacterial microbiome in the digestive tract and epithelial membrane-bound lactase phlorizin hydrolase converts these glycosides to active aglycone forms (genistein, daidzein, and glycitein, respectively).³⁷⁻⁴⁰ The active aglycones are taken up by the gut cells and immediately conjugated with glucuronides before entering the circulation.¹⁰ Therefore the maximum bioavailability of active isoflavones is only in the gastrointestinal tract. It is possible to achieve very high concentrations of active aglycones in the gut local environment after a soy meal. Nevertheless, only a fraction of the active aglycone forms can enter the circulation and be available to all other cells of the body. Consequently, the direct effect of isoflavones on cells and tissues other than the gut, if any, is negligible. The monocyte-derived lamina propria DCs have direct access to dietary antigens through the protrusion of their dendrites into the gut lumen⁴¹ and are therefore poised to take up isoflavones directly from the lumen.

DCs have to internalize, process, and present antigen to CD4⁺ T cells, which in turn regulate B-cell responses depending on its polarization toward the T_H1 or T_H2 phenotype, to generate an effective immune response. We hypothesized that the active isoflavones in the gut milieu are capable of modulating the immune responses to dietary antigens through their action on DCs. We have addressed this hypothesis in an *in vivo* food allergy model in which we show that dietary isoflavones significantly reduced the allergic hypersensitivity reactions to peanut (Figs 2, 3, and 4). As previously published,²⁹ we sensitized C3H/HeJ mice orally with peanut and CT as an adjuvant, which induces a robust T_H2 immune response in this species. As with all murine models of allergy and immunologic disorders, there is a question of how well these findings will translate to human subjects. In this model robust IgE-mediated reactions are dependent on the use of CT because the C3H/HeJ murine strain is unresponsive to LPS as a result of a lack of functional TLR-4.⁴² Nevertheless, this model does mimic clinical symptoms and most of the immunologic parameters identified in subjects with food allergy in spite of the potential side effects of CT (eg, increased intestinal permeability and diarrhea), particularly during the early stages of sensitization.⁴³ Dietary isoflavones did not affect the immune responses to peanut allergen when sensitized and challenged through the intraperitoneal route with alum as adjuvant (data not shown), suggesting that the gut-derived active aglycone isoflavone concentrations (if any) in blood and tissue spaces is not sufficient to suppress activation of DCs located in the draining lymph nodes of the peritoneum. Moreover, isoflavones did not inhibit absorption of peanut protein from the gut because there was no difference in the levels of peanut peptides detected in the sera from mice fed with a soy-free or genistein plus daidzein-containing diet (data not shown).

CD83 is a DC maturation marker, and CD80 and CD86 belong to the B7 group of costimulatory molecules expressed on the DC surface that are essential for T-cell activation. These molecules are upregulated during DC maturation and serve as definite markers of maturation.^{34,44} We propose that an altered/partial maturation of DCs exposed to isoflavones could account for this observed suppression of allergic reactions, which is demonstrated by reduced expression of these molecules in CD103^{hi}CD11c⁺MHC-II^{hi} MLN DCs (Fig 5). The expression of CD103 molecules differentiated the migrating DC population from the lamina propria in MLNs.^{33,41} We have extended the investigation to human DCs by establishing an *in vitro* cell-culture system with MDDCs and found a similar suppression of CT-induced CD83 and CD80 expression by isoflavones (Fig 6). This suppression of DC maturation functionally translates into suppressed cytokine secretion from DCs and altered regulation of CD4⁺ T-cell cytokine secretion *in vitro* (Fig 7).

Allergic immune responses are strongly associated with T_H2 immunity. Whereas DCs are known to secrete cytokines, such as IL-12p70, TNF- α , and IL-10, which induce T_H1 polarization of naive CD4⁺ T cells, a specific DC-derived T_H2 signal is yet to be discovered.⁴⁵ In both human⁴⁶ and murine⁴⁷ DCs, CT suppressed IL-12p70 and TNF- α production but not IL-8 production.³⁴ In addition, CT is also shown to promote IL-6 production from murine DCs.⁴⁷ We could detect the proinflammatory cytokines IL-6 and IL-8 from CT-induced human MDDCs (Fig 6), although at lesser quantities compared with those seen after LPS stimulation (data not shown), and showed that CT-induced IL-6 and IL-8 secretion are inhibited by isoflavone

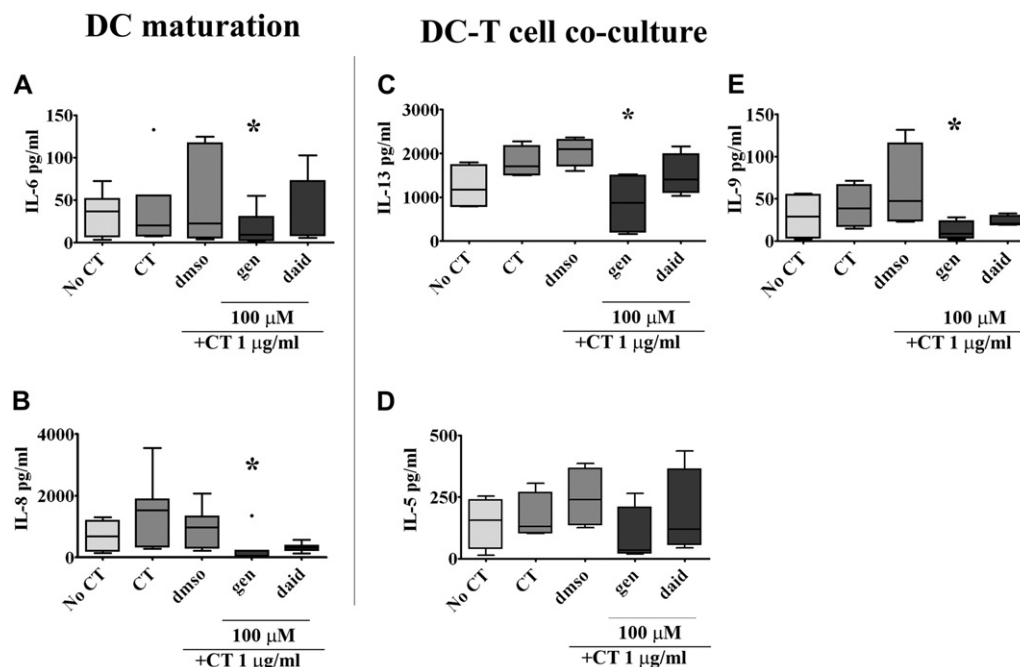


FIG 7. Human MDDCs were activated with 1 μ g/mL CT with or without isoflavones (100 μ mol/L) or dimethyl sulfoxide (*dms*; vehicle control) for 18 hours. IL-6 (**A**) and IL-8 (**B**) levels were measured in DC supernatants by using the cytometric bead array. The data from 7 individual experiments are shown as box-and-whisker Tukey plots. Activated DCs were washed and incubated with allogeneic CD4⁺ naive T cells for 5 days. IL-13 (**C**), IL-5 (**D**), and IL-9 (**E**) levels were measured by using the cytometric bead array in supernatants of DC-T-cell cultures. Data from 4 individual experiments are shown as box-and-whisker Tukey plots (* P < .05, 1-way ANOVA).

treatment. IL-6 is also secreted by T cells and macrophages and promotes T_H2 responses by directing CD4⁺ T cells to secrete IL-4⁴⁸ and also by inhibiting T_H1 immune responses.⁴⁹ IL-8 is an important mediator of inflammatory and chemotactic responses.⁵⁰ IL-8 is secreted by monocytes, fibroblasts, and T cells in addition to DCs. IL-8 plays a major role in chemotaxis of neutrophils, eosinophils, T cells, and several other cell types⁵¹⁻⁵³ by inhibiting leukocyte adhesion to endothelial monolayers.^{54,55} Both IL-6 and IL-8 are shown to be involved in Der p 1-induced allergic asthma and allergic rhinitis.^{56,57} Increased levels of IL-8 were found in airway secretions of patients with food allergy, with an associated increase in neutrophil numbers in airway mucosa.⁵⁸ Based on our observation that isoflavones suppress activation-induced IL-8 and IL-6 secretion from MDDCs (Fig 7, A), it is tempting to speculate that harnessing this mechanism could be useful for the treatment of allergic inflammation.

The major cytokines secreted by T_H2 cells are IL-4, IL-5, IL-9, and IL-13, which are required for the production of antigen-specific IgE by B cells, as well as eosinophilic responses,^{2,59,60} and can also augment anaphylaxis.⁶¹⁻⁶³ Allergen-stimulated T-cell clones from subjects with peanut allergy secrete T_H2 cytokines.⁶⁴ Among the T_H2 cytokines, IL-13 seems to play a critical role because targeted deletion of IL-13 protects mice against allergic airway hyperreactivity in a murine model of asthma.⁶⁵ In addition, IL-13-overexpressing transgenic mice have been shown to be predisposed to fatal antigen-induced anaphylaxis.⁶¹ IL-5, on the other hand is required for the proliferation, differentiation, and activation of eosinophils.⁶⁶ IL-9 is a pleiotropic T_H2 cytokine produced from various cell types, such as lymphocytes, eosinophils, mast cells, and neutrophils.⁶² Recently, it was shown

that IL-9/IL-9 receptor signaling is critical for oral antigen-induced systemic anaphylaxis.⁶³ DCs isolated from MLNs of mice fed with CT induced IL-13 secretion from antigen-specific CD4⁺ T cells.³³ We show that naive human CD4⁺ T cells cultured with genistein-treated and activated DCs secrete significantly less IL-13 and IL-9 (Fig 7). These observations are in line with the finding that peanut-specific IgE levels are decreased in isoflavone-fed mice. These data are indicative of protective responses to allergic sensitization caused by the suppression of the T_H2 response by isoflavones. However, we cannot rule out the contribution of other cytokines or cell types, if any, in the observed isoflavone-mediated effects, warranting further investigation.

Although there are a few reports on the effect of isoflavones on allergic responses,^{26,67} studies on the direct effect of isoflavones in the development of food allergy are lacking. Dietary isoflavones (2 g of isoflavone glycosides with 35% purity/kg diet) have been shown to reduce ovalbumin-induced eosinophilia in the lungs in a guinea pig model of asthma,²⁶ whereas intraperitoneal injection of genistein (15 mg/kg body weight) inhibited ovalbumin-induced bronchoconstriction.⁶⁷ We have used 1500 ppm each of genistein and daidzein in the diet in our murine model. This concentration of isoflavones in the murine diet has been shown to result in plasma isoflavone levels equivalent to 50% of those in plasma of human infants fed with soy milk.^{68,69} The European Society for Paediatric Gastroenterology, Hepatology and Nutrition Committee on Nutrition⁷⁰ and various other reports^{16,68} have advocated against the use of soy infant formula partly because of its isoflavone content and partly because of the inferior quality of soy protein compared with cow's milk protein. Isoflavones mimic the structure of estrogens and are weak

agonists for the estrogen receptor.^{12,71} The potency of isoflavone is about 1,000 to 10,000 times less than that of endogenous estrogens.⁷² Therefore it is possible that high consumption of soy products (as in soy infant formula) might increase the serum isoflavone levels enough to induce estrogenic activity. However, it has been shown that dietary isoflavones from soy formula do not adversely affect human growth and reproductive development.^{73,74} Moreover, soybeans have comprised an important part of the diet in Asian cultures for centuries and are reported to be beneficial in various disorders,^{12,21-23} including allergy.^{24,25} Yet no large-scale human studies have been performed with isoflavones, warranting further research in this regard.

In conclusion, we have clearly demonstrated *in vivo* the protective effects of dietary soybean isoflavones in allergic sensitization to food in a murine model of peanut-induced anaphylaxis (Fig 2). The beneficial effect of isoflavones in food allergy is a result of immune suppression, during which DCs and CD4⁺ T-cell responses are inhibited. Further research will be directed toward testing the beneficial effects of isoflavones in food allergy in human subjects. Development of new therapeutic approaches for food allergy is of significant importance in terms of public health. In this light our results could have wide implications in future treatment strategies of food allergies.

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Key messages

- Dietary isoflavones protect mice against peanut sensitization.
- Isoflavones suppress DC maturation and subsequent DC-mediated regulation of T-cell function *in vitro*.

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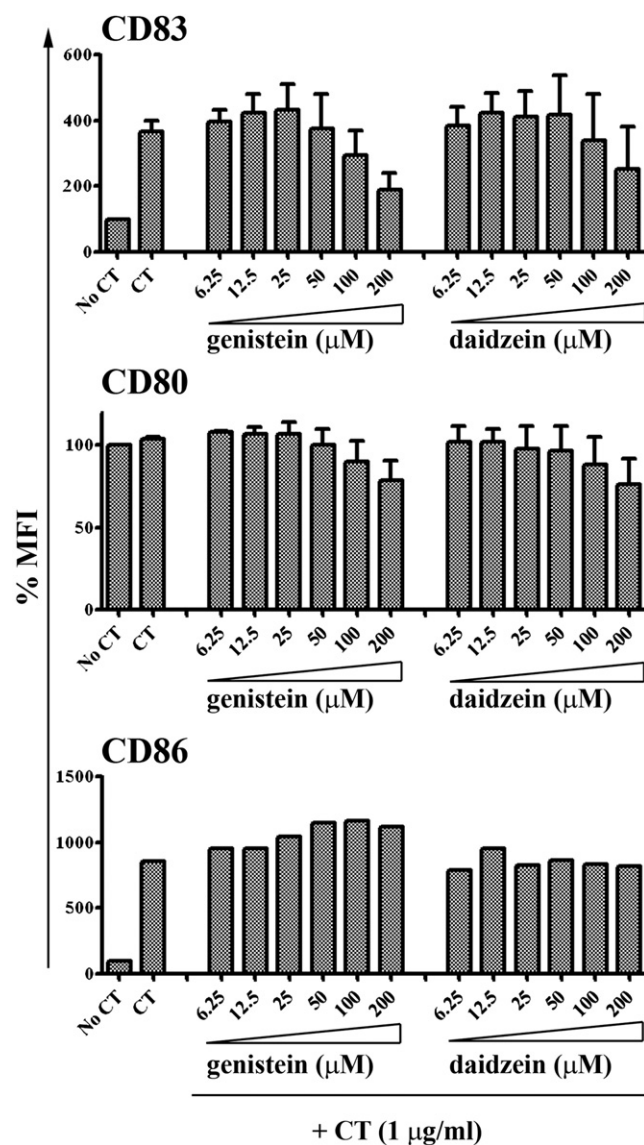


FIG E1. Isoflavone titration. Human MDDCs were activated with 1 µg/mL CT in the presence or absence of titrated amounts of genistein or daidzein. The concentration of isoflavones ranged from 6.25 to 200 µmol/L. The percentage surface expression levels of CD83 (*top panel*), CD80 (*middle panel*), and CD86 (*bottom panel*) were calculated from the geometric mean fluorescent intensities, in which the unstimulated control (*No CT*) was taken as 100%, and shown as bar graphs.