

Note

Percutaneous Sensitization to Soybean Proteins Is Attenuated by Oral Tolerance

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Summary Oral tolerance prevents allergic responses, but cutaneous exposure to food allergens predisposes individuals to food allergies. Soybean, a major allergenic food, is also an ingredient in various cosmetic products. However, it remains to be determined whether oral tolerance prevents percutaneous sensitization to soybean proteins in humans or animal models. In this study, BALB/c mice were divided into three groups; the SS group fed a soybean-containing diet, and the CS and control (C) groups fed a soybean-free diet. After being dorsally shaved, the CS and SS groups were epicutaneously exposed to a soybean extract while the control group was exposed to only the carrier. Specific IgE and IgG1 immunoglobulins secreted in response to the soybean proteins were measured using enzyme-linked immunosorbent assays. Exposure to the soybean extract elicited the secretion of IgE and IgG1 specific for Gly m 5 and Gly m 6, and trypsin inhibitor. Oral soybean consumption attenuated the secretion of all the soybean-specific IgEs and IgG1s, suggesting that percutaneous sensitization to soybean proteins is attenuated by oral tolerance.

Key Words oral tolerance, percutaneous sensitization, soybean allergen, food allergy

Food allergies disrupt the quality of life (QOL) and their prevalence appears to have increased. It is reported that they affect nearly 5% of adults and 8% of children in developed countries (1). Food allergy episodes are induced by Th2-type immune responses. Recent reports have shown that patients who use soaps containing wheat as an ingredient develop allergies to wheat (2), and that allergic episodes to peanuts and soybean may occur through the use of cosmetics containing these ingredients (3, 4). Cutaneous lesions and impaired skin-barrier function appear to be risk factors for food allergies (5).

It was previously largely accepted that elimination of highly allergenic foods such as peanuts, milk, or eggs from the diets of infants and their mothers is effective in the prevention of food allergies (6). However, in 2008, Du Toit et al. (7) suggested that peanut consumption during early childhood could prevent a peanut allergy from developing. Based on the association between oral tolerance and percutaneous sensitization, the dual-allergen-exposure hypothesis was proposed (8). According to this hypothesis, while oral food-ingestion induces immune tolerance and prevents food allergies, food allergens that enter through the skin may induce antigen-specific IgE and IgG1 production and predispose the immune milieu to Th2-type responses.

Food is digested and absorbed through the intestinal tract. In the small intestine, CD103⁺ dendritic cells take up potential food antigens in the lamina propria, which underlies the regular villus epithelium. Innocuous food antigens come from the intestinal lumen through enterocytes or CX3CR1^{high} macrophages that extend their dendrites through the epithelial layer and capture food antigens (9). Following antigen uptake, regulatory T-cells (Tregs) are activated and attenuate the antigen-specific Th2 immune cells; in other words, oral tolerance is executed. However, it remains to be elucidated why oral tolerance is disrupted in patients with food allergies.

Glycine max (L.), commonly referred to as soybean, is used in various foods, including in traditional Asian and Western cuisines, and is also used in some cosmetics. Soybean allergies not only develop at a high incidence in infants (class 1 food allergy), but are also common in adults with pollinosis (class 2 food allergy) (10). In 2004, Christensen et al. (11) reported that feeding mice a soybean-containing diet prevents sensitization resulting from intraperitoneal immunization with an extract from a mixture of soybean proteins. However, it remains to be determined whether oral tolerance prevents percutaneous sensitization to soybean protein. Therefore, we sought to clarify whether oral tolerance would prevent percutaneous soybean sensitization induced through the epidermal application of a crude soybean extract to the dorsal skin in a mouse model. We identified Gly m 5, Gly m 6, and trypsin inhibitor as antigens involved

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in percutaneous sensitization by soybean exposure, and demonstrated that oral consumption of soybean suppressed the IgE and IgG1 production specific for these soybean proteins.

Materials and Methods

Antibodies. Goat anti-mouse IgG1 antibody and horseradish peroxidase (HRP)-conjugated affinity-purified goat anti-mouse IgE and IgG1 antibodies were purchased from Bethyl Laboratories (Montgomery, TX). Mouse IgG1 isotype control was purchased from Southern Biotech (Birmingham, AL).

Soybean proteins. Soybeans were immersed in distilled water overnight, homogenized with a commercial food mixer, and filtered through four layers of gauze. The soybean Gly m 5 (β -conglycinin; 7S globulin) and Gly m 6 (glycinin; 11S globulin) were isolated as described previously (12). Recombinant Gly m 3 and Gly m 4 proteins were expressed in *Escherichia coli* from cDNAs cloned via polymerase chain reaction (PCR) from a soybean-leaf cDNA library. The protein concentrations were determined using the Bradford method.

Oral tolerance and percutaneous sensitization. All animal experiments were approved by the Kindai University Animal Care and Use Committee (Approval No. KAAG-26-004). Six-wk-old female BALB/c mice were purchased from Japan SLC, Inc. (Shizuoka, Japan). The mice were randomly divided into three groups, CS (casein feeding and soybean application), SS (soybean feeding and soybean application), and C (control). Mice in the CS and C groups were fed a diet excluding all soybean ingredients, including casein as a protein (Supplemental Online Material, Table S1), from 1 wk prior to the start of the experimental protocol until its completion. Mice in the SS group were fed the same diet as the C and CS mice except beginning at 2 d prior to the start of the protocol until its completion, they were fed a diet including soy protein (SPI: soy protein isolate).

For the percutaneous sensitization experimental protocol, after the dorsal hair was shaved with an electric shaver and stripped with adhesive tape, 50 mg/mL of crude soybean extract containing 0.5% sodium dodecyl sulfate (SDS) was applied to the skin of each mouse in groups CS and SS using a paint brush (Supplemental Online Material, Fig. S1A). Animals in the control group C received a treatment of 0.5% SDS in distilled water. Each week, the skin was shaved and stripped 20 times using adhesive tape under anesthetic conditions using midazolam (Astellas Pharma, Tokyo, Japan), butorphanol (Meiji Seika Pharma, Tokyo, Japan), and medetomidine (Nippon Zenyaku Kogyo, Fukushima, Japan). The crude soybean extract or control was epidermally applied three times a week for 8 wk.

Detection of IgE and IgG1. The binding of mouse serum IgE and IgG1 to the soybean antigens was detected by enzyme-linked immunosorbent assay (ELISA). On ELISA plates, 50 μ L of crude soybean extract, or each individual purified soybean protein at 20 μ g/mL in phosphate buffered saline (PBS) was added and incubated overnight at 4°C. After blocking with 1%

BSA in 10 mM PBS containing 0.1% Tween 20 (PBST) for 1 h and washing with PBST three times, the mouse sera diluted with PBS were added to the wells and incubated for 1 h. After the wells were washed with PBST five times, 1 : 50,000 dilutions of the HRP-conjugated goat anti-mouse IgE antibody and HRP-conjugated goat anti-mouse IgG1 antibody were added to the wells and incubated for 1 h. After the wells had been washed with PBST five times, the bound secondary antibodies were detected through reaction with 50 μ L of 3,3',5,5'-tetramethylbenzidine (TMB) as a peroxidase substrate. The reaction was stopped by addition of 50 μ L of 1 M phosphoric acid. The absorbance of the plate wells was measured at 450 nm using a Wallac ARVO SX 1420 multi-label counter (PerkinElmer, Waltham, MA). The allergen-specific IgG1 concentrations in the sera were determined by sandwich ELISA. A standard curve was generated using the anti-mouse IgG1 antibody as the capture antibody, and the mouse IgG1 isotype control as an IgG1 standard control.

Statistical analysis. Statistical differences were determined by the Tukey-Kramer test for feed intake and by the Fisher's protected least significant difference (PLSD) test for the others. A p -value < 0.05 was considered significant. Statistical analyses were performed using StatView 5.0 software (SAS Institute, Cary, NC).

Results

No significant differences in body-mass increase or feed intake were observed among the three groups of mice (Supplemental Online Material, Fig. S1B and Table S2). We sought to examine whether the mouse serum IgEs and IgG1s would bind to soybean proteins using ELISA in which crude soybean extract was added as capture antigen to the solid phase. Significant increases in soybean protein-specific IgEs and IgG1s were observed in the CS group, compared with the control group C (Fig. 1A–D). However, no significant difference was observed between the control and SS groups.

Next, we conducted solid-phase ELISAs using soybean allergens Gly m 5, Gly m 6, trypsin inhibitor, Gly m 3, and Gly m 4. We found that the serum IgE and IgG1 levels for Gly m 3 and Gly m 4 did not differ significantly between the C and CS groups; however, the levels of IgEs and IgG1s specific for Gly m 5 and Gly m 6, and trypsin inhibitor were significantly higher in the CS group compared with the control group C (Fig. 1E and F). No significant differences were observed between the C and SS groups, suggesting that oral soybean consumption prevented percutaneous sensitization to each of the soybean proteins.

Discussion

In the present study, we investigated the effect of soybean oral consumption on soybean percutaneous sensitization. We found that oral consumption of soybean attenuated the secretion of IgE and IgG1 immunoglobulins specific for soybean proteins.

The skin has attracted attention as an alternative sensitization route to the intestine, and damage to the skin's

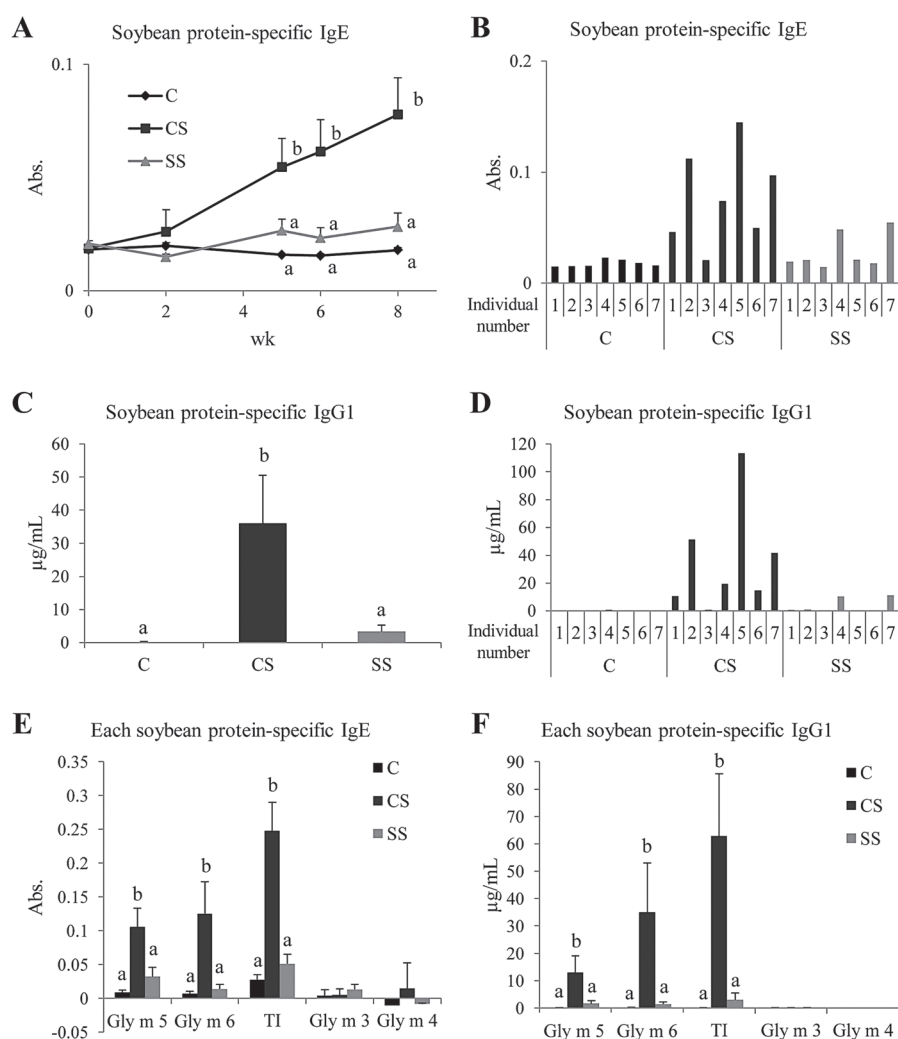


Fig. 1. (A) Mean level changes at each week (wk) of the protocol period and (B) individual levels at week eight. Soybean protein-specific IgEs were detected by ELISA. (C) Mean and (D) individual levels of soybean protein-specific IgG1 at week eight were detected by ELISA. Mean levels of IgE (panel E) and IgG1 (panel F) specific for Gly m 5, Gly m 6, trypsin inhibitor, Gly m 3, and Gly m 4 at week eight of the experimental protocol were detected by ELISA. TI, trypsin inhibitor. C ($n=7$), CS ($n=7$), SS ($n=7$). Data are presented as means $\pm$ standard errors. $p < 0.05$ (a vs. b).

epithelial barrier has been suggested as a risk factor for percutaneous sensitization (5). In the present study, the skin of mice was disrupted using adhesive tape and by the addition of SDS to the soybean-extract solution since surfactants likely promote disruption of the epithelial barrier. Accordingly, following barrier disruption, application of soybean extract to the dorsal skin elicited the production of soybean-protein-specific IgE and IgG1, both of which were detectable in the sera. Mice of the SS group had been fed a diet including isolated soybean protein resulting in prevention of the elicitation of secreted IgE and IgG1 immunoglobulins specific for soybean protein. Both the secreted IgEs and IgG1s bound to soybean proteins Gly m 5, Gly m 6, and trypsin inhibitor. Oral consumption of soybean attenuated the IgE and IgG1 secretion specific for all these proteins, compared with serum levels in mice fed a soybean-restricted diet. Our results suggest that oral consumption of food containing soybean proteins prevented percutaneous sensitization, which supports the dual-allergen-expo-

sure hypothesis that has recently attracted attention (8).

In regard to Gly m 3 and Gly m 4, these proteins might not sensitize percutaneously and thus, the effect of oral tolerance was not able to be observed. The investigation of oral tolerance will be needed with animal models specifically allergic to Gly m 3 or 4 in the future. Gly m 5, Gly m 6, and TI are known as class 1 food allergens but Gly m 3 and Gly m 4 are known as class 2 food allergens. It is popularly believed that whereas class 1 food allergens are resistant to heat and digestion, class 2 food allergens do not have these properties (10). In addition, the content of Gly m 3 and Gly m 4 are less than Gly m 5, Gly m 6, and TI. These factors are hypothesized to contribute to differences in allergenicity through the epidermal application.

Soybean proteins reportedly contain several food allergens such as Gly m Bd 28k, Gly m Bd 30k, and Gly m 8 (2S albumin), which were not investigated in the present study (13). In 2018, Lu et al. (14) reported high incidence of TI in addition to Gly m 5 and Gly m 6 in

soybean allergic patients. Although it remains unclear why TI more likely induced the secretion of specific IgEs than Gly m 5 and Gly m 6 in this model, this characteristic may be related to the three-dimensional structure including S-S bondages. Therefore, more detailed studies are required for the assessment of percutaneous sensitization antigens and oral tolerance in future.

Large individual differences were found in the CS group, which may have been attributable to differences in oral tolerance, since rodent feed normally contains soybean ingredients. We noted that the mice may have consumed such a diet prior to our oral tolerance and percutaneous sensitization protocol. In addition, not all of the mice from the SS group were prevented from secreting IgE and IgG1, suggesting that skin exposure could elicit sensitization over oral tolerance. Factors determining superiority of sensitization or tolerance were not determined in the current study and need to be investigated in future experiments. Despite this, our results are consistent with oral consumption of soybean inducing preventive-effects on percutaneous sensitization in this animal model.

In conclusion, we have demonstrated that oral consumption of soybean may potentially attenuate the secretion of IgE and IgG1 antibodies specific for soybean proteins, including Gly m 5, Gly m 6, and trypsin inhibitor. This is consistent with the dual-allergen-exposure hypothesis.

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Supporting Information

Supplemental online material is available on J-STAGE.

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