

ORIGINAL ARTICLE

EXPERIMENTAL ALLERGY AND IMMUNOLOGY

Sensitization to acid-hydrolyzed wheat protein by transdermal administration to BALB/c mice, and comparison with gluten

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Keywords

acid-hydrolyzed wheat protein; active systemic anaphylaxis; food allergy; gluten; transdermal exposure.

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Abstract

Background: An increasing number of studies have shown that hydrolyzed wheat protein (HWP) can induce IgE-mediated hypersensitivity by skin contact and/or food ingestion. However, there has been no study of the sensitizing potential of HWP. In this study, the possibility of transdermal pathway for sensitization to acid-HWP (HWP1) was investigated using BALB/c mice, and compared with that of gluten.

Methods: HWP1 or gluten (500 µg/mouse) was transdermally administered using patches. After three or four cycles of sensitization for 3 days/week, active systemic anaphylaxis (ASA) was induced by intraperitoneal injection of the antigen, and rectal temperatures, scores of anaphylactic responses, and plasma histamine levels were determined. Because HWP1 was included in facial soap in Japan, the effect of detergent on the sensitizing potential was also investigated.

Results: Transdermal administration of HWP1 induced dose-dependent production of IgE and IgG1. After sensitization for 3 or 4 weeks, intraperitoneal injection of HWP1 caused ASA, leading to decreased rectal temperatures, increased anaphylaxis scores, and increased plasma histamine levels. In addition, splenocytes harvested after ASA produced IL-4, IL-5, and IL-10 by re-stimulation with HWP1. Transdermal exposure to gluten also induced IgE and IgG1 production, and intraperitoneal injection of gluten also induced ASA only in mice sensitized in the presence of sodium dodecyl sulfate.

Conclusions: Transdermal exposure to HWP1 is sufficient to activate key immune pathways necessary for sensitizing mice for immediate hypersensitivity reactions. This study shows that HWP has a sensitizing potential as well as gluten, whereas its allergenicity may be different from that of gluten.

Wheat protein is responsible for various allergic diseases. Wheat-dependent exercise-induced anaphylaxis (WDEIA) is one of the most important clinical phenotypes of adult wheat

allergy. Recently, immediate hypersensitivity to hydrolyzed wheat protein (HWP) was reported. Although it is generally believed that hydrolysis reduces the allergenicity of allergenic proteins, the number of studies indicating that HWP induces immediate hypersensitivity by skin contact and/or food ingestion has increased (1–8). Hydrolyzed wheat protein is a common food and nonfood ingredient. For example, it is used in cosmetics, shampoo, and hair conditioner. Laurière et al. (1) showed that immediate contact urticaria can be triggered by HWP present in cosmetics. Moreover, some patients with such allergies can also experience an immediate food allergy

Abbreviations

IgE, immunoglobulin E; PBS, phosphate-buffered saline, pH 7.2; EDTA-2Na, ethylenediamine-*N,N,N',N'*-tetraacetic acid, disodium salt; HRP, horseradish peroxidase; DTT, dithiothreitol; CBB, Coomassie brilliant blue; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; MW, molecular weight; Tris, tris(hydroxymethyl)aminomethane.

reaction by eating foods containing HWP. More recently, we reported five patients in Japan, who were primarily sensitized to HWP in facial soap and experienced WDEIA after ingesting normal wheat products (2). After publication of our report, as of June 10, 2012, more than 600 patients with such an allergy have been reported by various institutes around Japan. Importantly, most patients used the same facial soap (Cha no Shizuku®; Yuuka Co., Ltd., Fukuoka, Japan) containing acid-HWP.

Gluten is a complex of salt-insoluble proteins contained in wheat and has a strong viscoelastic property. Hydrolyzed wheat protein, mainly consisting of hydrolyzed gluten, is widely used in cosmetics. Hydrolyzed gluten is a mixture of variously sized polypeptides, and their sizes depend on the condition of hydrolysis. The commercial acid-HWP contained in the facial soap (Glupearl 19S®; Katayama Chemical Industries Co., Ltd., Osaka, Japan, described as HWP1 in this article) was produced from gluten by partial hydrolysis with hydrogen chloride.

Patients who had used the facial soap described above were presumed to be sensitized to HWP1 percutaneously or via the mucosal tissue of the palpebra. There have been several reports of percutaneous sensitization of mice to food allergens such as ovalbumin (OVA) (9) and hazelnut proteins (10). These reports show that transdermal exposure of food allergen proteins is sufficient to sensitize mice without adjuvants and induce a Th2-type response. In our study, the possibility of a transdermal pathway for sensitization to HWP1 was investigated using BALB/c mice, and the effect of HWP1 was compared with that of gluten. In addition, because HWP1 was included in the facial soap, the effect of detergent was also investigated.

Materials and methods

Reagents and HWP1

Rabbit anti-mouse IgE and IgG1 were purchased from Nordic Immunology (Tilburg, the Netherlands). An antibody against rabbit IgG (H+L)-conjugated to β -galactosidase was purchased from American Qualex Antibodies (San Clemente, CA, USA). 4-Methylumbelliferyl- β -D-galactopyranoside was purchased from Sigma-Aldrich (St. Louis, MO, USA). Glupearl 19S® (HWP1) was supplied by Katayama Chemical Industries Co., Ltd. (Osaka, Japan). It was produced from gluten by partial hydrolysis with hydrogen chloride at 95°C for 40 min. The sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) pattern of HWP1 showed that the molecular weight of the main band in HWP1 was 40–50 kDa, which was larger than the main band in gluten (Fig. S1). Gluten was purchased from Sigma-Aldrich (Cat. No. G5004).

Five-hundred milligrams of HWP1 and gluten were suspended in 5 ml of 1 M Tris solution (pH 11.4) and left to stand for 24 h at room temperature.

Transdermal sensitization

Seven-week-old female BALB/c mice were purchased from Japan SLC (Hamamatsu, Shizuoka, Japan), fed a commercial

Mouse Flat (MF) diet (Japan SLC), and maintained in a temperature- and light-controlled environment. All animal procedures were in accordance with the 'Ethical Guidelines of Animal Care, Handling and Termination' published by the National Institute of Health Sciences.

Mice were designated into five groups ($n = 8$): V group were patched with phosphate-buffered saline (PBS) containing 0.5% SDS (w/v), HS, and GS groups were sensitized with 500 μ g HWP1 and gluten containing 0.5% SDS (w/v), respectively, and H and G groups were sensitized with 500 μ g HWP1 and gluten without SDS, respectively. HS20, HS100, and HS500 groups ($n = 5$) were administered with 20, 100, and 500 μ g HWP1 containing 0.5% SDS (w/v), respectively. The suspensions of HWP1 and gluten were diluted with 1 $\times$ PBS and adjusted to pH 8 before applying.

On days 1, 8, 15, and 22, the stratum corneum of dorsal shaved mice was removed by tape-stripping repeated 10 times. A 50 μ l antigen solution was applied to the shaved area of each mouse with a patch (Patch tester Torii; Torii Pharmaceutical Co., Ltd., Tokyo, Japan), and then the patched area was covered with surgical tape. Mice were exposed to antigens for 3 days and then rested for the next 4 days after removing the patch. Transdermal antigen exposure was repeated for three or four cycles (days 1–3, 8–10, 15–17, and 22–24).

Measurement of IgE and IgG1 production

Antigen-specific IgE and IgG1 production was detected based on the method in our previous report (11). Production of both antibodies has been reported to be induced by Th2-dominant helper T-cell activation (12). Briefly, 1 μ g of antigen was coated on a 96-well plate overnight at 4°C. After washing, the wells were blocked with 0.1% casein in PBS for 1 h at room temperature and then incubated with mouse serum (diluted with 0.1% casein/PBS at 5×10^{-1} for IgE and 5×10^{-2} for IgG1) for 20 h at 4°C. Plates were then incubated with rabbit anti-mouse IgE/IgG1, followed by a goat anti-rabbit IgG (H+L)-conjugated β -galactosidase solution. Then, 0.1 mM 4-methylumbelliferyl- β -galactopyranoside was added to each well, and plates were incubated for 1 h at 37°C. The substrate reaction was stopped with 25 mM sodium carbonate, and the fluorescence intensity of liberated 4-methylumbelliferone was monitored at 355 and 460 nm for excitation and emission, respectively, using a Fluoroskan Ascent (Thermo Fisher Scientific Inc., Waltham, MA, USA).

Systemic anaphylaxis induction

Transdermal-sensitized mice were intraperitoneally challenged with 1 mg of antigen (100 μ l) on day 18 or 25. Rectal temperatures were measured with a rectal thermometer for mice (Shibaura Electronics Co., Ltd., Saitama, Japan) during the 30 min after intraperitoneal challenge. Concurrently, anaphylactic response to the antigen challenge was evaluated by scoring symptoms (10, 13, 14). Scores were as follows: 0, no symptoms; 1, scratching and rubbing around the snout and head; 2, puffiness around the eyes and snout, pilar erection,

reduced activity or standing still with an increased respiratory rate; 3, periods of motionless for more than 1 min, lying prone on stomach, transient convulsion, wheezing, labored respiration; 4, no response to whisker stimuli, slight or no activity after prodding, tremor, convulsion, loss of consciousness; 5, death.

Measurement of plasma histamine and cytokines secreted from splenocytes

At 30 min after challenge, blood was collected in a tube containing 1% ethylenediamine-*N,N,N',N'*-tetraacetic acid, disodium salt (EDTA-2Na), and then mice were killed. Plasma histamine levels were measured with a Histamine EIA Kit (Bertin Pharma, Montigny le Bretonneux, France) according to the manufacturer's instructions. Splenocytes were collected from mice, and 5×10^6 cells/ml were re-stimulated with antigen at a 100 $\mu\text{g}/\text{ml}$ final concentration in a 24-well culture plate at 37°C for 72 h. Cytokine secretion from splenocytes was measured with a Bio-Plex Pro Mouse Cytokine Th1/Th2 Panel (Bio-Rad Laboratories Inc., Hercules, CA, USA) according to the manufacturer's instructions.

Statistics

The statistical significance of difference between experimental groups was determined using one-way analysis of variance

followed by Dunnett's test vs the V group. Differences were regarded as significant at $P < 0.05$. For anaphylaxis scores, statistical differences were analyzed using Kruskal–Wallis test with Bonferroni/Dunn's test (15).

Results

Sensitization of BALB/c mice to HWP1 and gluten by transdermal administration

BALB/c mice were sensitized to HWP1 or gluten by transdermal administration as described in Materials and methods. Transdermal administration was repeated for three or four cycles, depending on the increase in the levels of specific antibodies in mouse sera. The content of HWP1-specific IgE in sera on Day 23 is shown in Fig. 1A. Group names were as follows ($n = 8$): V, vehicle (PBS with SDS); HS, HWP1 with SDS; H, HWP1; GS, gluten with SDS; G, gluten. The IgE contents of HS, H, and GS groups were significantly increased compared with that of the vehicle group, while the content in G group was barely increased. The IgG1 contents of HS, H, and GS groups were also significantly higher than that of the vehicle group (Fig. 1B). For both IgE and IgG1, antibodies against HWP1 were markedly raised in HS and H groups. These results indicated that HWP1 possessed remarkable antigenicity.

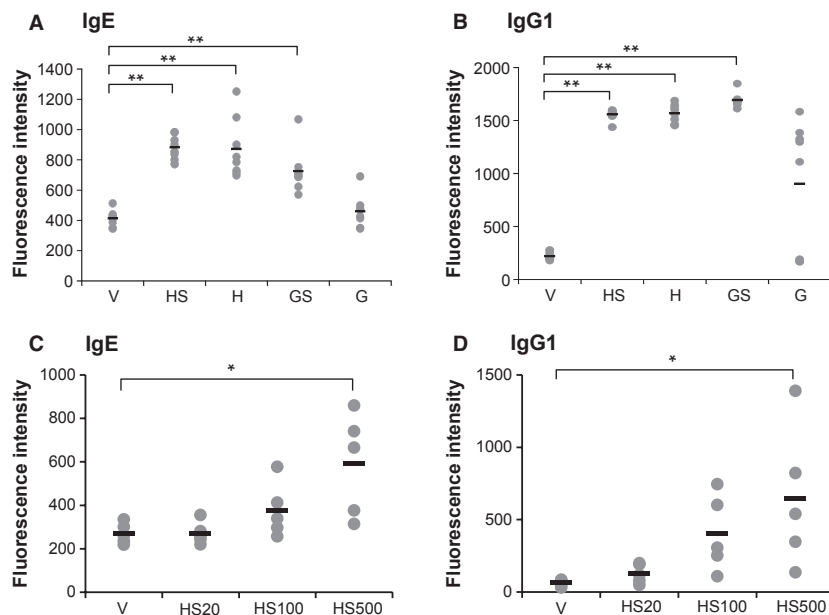


Figure 1 HWP1-specific IgE/IgG1 in the sera of mice transdermally sensitized to HWP1/gluten. HWP1-specific IgE (A and C) and IgG1 (B and D) in the sera of mice transdermally sensitized to HWP1/gluten were determined by ELISA. Fluorescence intensities in diluted sera (50-fold diluted for IgE and 500-fold for IgG1) are shown. Materials administered to each group were as follows: V (vehicle), phosphate-buffered saline + sodium dodecyl sulfate

(SDS); HS, HWP1 500 μg + SDS; H, HWP1 500 μg ; GS, gluten 500 μg + SDS; G, gluten 500 μg ; HS20, HWP1 20 μg + SDS; HS100, HWP1 100 μg + SDS; HS500, HWP1 500 μg + SDS. Transdermal antigen exposure was repeated for three (C and D, $n = 5$) or four (A and B, $n = 8$) cycles. Bars represent the means of each group. * $P < 0.05$, ** $P < 0.01$ by Dunnett's test vs the V group.

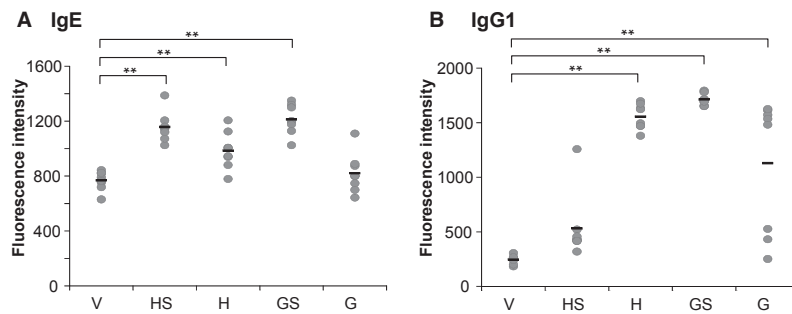


Figure 2 Gluten-specific IgE/IgG1 in the sera of the mice transdermally sensitized to HWP1/gluten. Gluten-specific IgE (A) and IgG1 (B) in the sera of mice transdermally sensitized to HWP1/gluten were determined by ELISA. Groups are the same as described in

Fig. 1. Transdermal antigen exposure was repeated for four cycles. Bars represent the means of each group ($n = 8$). * $P < 0.05$ by Dunnett's test vs the V group.

At HWP1 doses of 20–500 $\mu\text{g}/\text{mouse}$, both IgE and IgG1 contents were clearly increased dose dependently (Fig. 1C,D, $n = 5$).

The content of gluten-specific IgE in sera on Day 23 is shown in Fig. 2A. The IgE contents of GS, HS, and H groups were significantly increased compared with that of the vehicle group, while the content in G group was barely increased. The IgG1 contents of GS, G, and H groups were significantly increased, although there were some individual differences in G group (Fig. 2B).

The difference in antibody content between GS and G groups showed that SDS played an important role in sensitization by transdermal administration.

As shown in Fig. 1A,B, antibodies against gluten in the mouse sera of GS group reacted with HWP1. And antibodies against HWP1 in the mouse sera of HS and H groups also reacted with gluten (Fig. 2). These results showed that there was some cross-reactivity between HWP1 and gluten.

Elicitation of immediate hypersensitivity against HWP1

After three or four transdermal administrations of HWP1 or gluten, on Day 18 or 25, immediate hypersensitivity reactions

were elicited by intraperitoneal injection of the antigens used for sensitization (1 mg HWP1 or gluten/mouse). Figure 3 shows the change in rectal temperatures of mice during the 30 min after injection. Among the four groups tested ($n = 8$), mice in HS, H, and GS groups immediately reacted to their antigens. After 30 min, average rectal temperatures of mice in HS, H, and GS groups decreased to approximately 3.5, 2.6, and 2.1°C, respectively, which were lower than that of the vehicle group (Fig. 3A). The results of varied HWP1 doses for sensitization are shown in Fig. 3B. All mice in the three groups ($n = 5$) were injected intraperitoneally with 1 mg/mouse HWP1 to elicit immediate hypersensitivity. The average rectal temperature of HS20 group was the same level as that of the vehicle group, and the temperature of HS100 group tended to decrease compared with that of the vehicle group. The average temperature of HS500 group was significantly lower than that of the vehicle group by approximately 1.2°C. These results demonstrated the clear dependency of the extent of immediate hypersensitivity reactions on the dose of HWP1 used for sensitization by transdermal administration.

At the same time as the rectal temperature was measured, anaphylactic responses of mice were also observed. Manifestations of systemic anaphylaxis after the challenge were

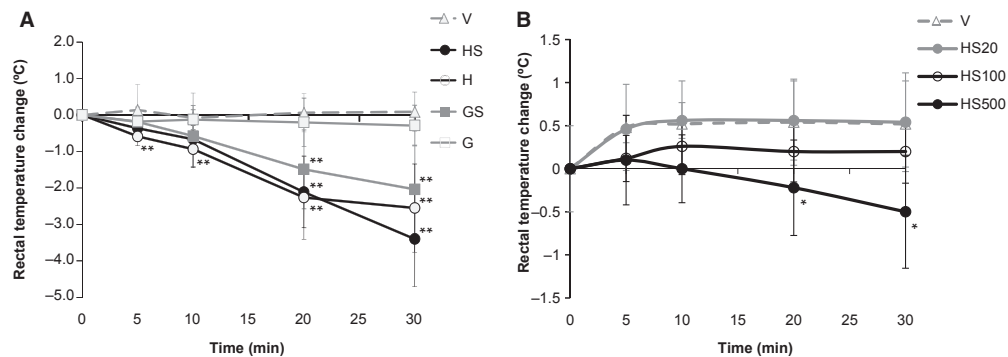


Figure 3 Changes in the rectal temperatures of mice transdermally sensitized to HWP1/gluten after intraperitoneal challenge. A, HWP1/gluten-sensitized mice ($n = 8$); B, HWP1-sensitized mice.

Groups are the same as described in Fig. 1. Data are the mean $\pm$ standard deviation (SD) of each group. * $P < 0.05$, Dunnett's test vs the V group.

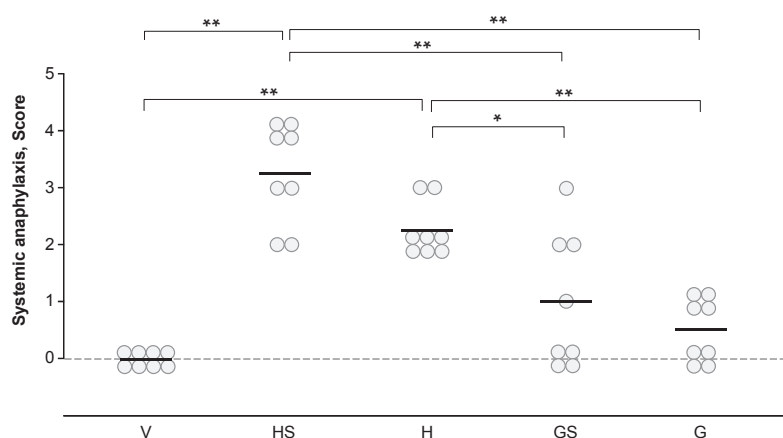


Figure 4 Anaphylactic responses to intraperitoneal challenge in mice transdermally sensitized to HWP1/gluten. Anaphylactic symptoms within 30 min after the challenge were scored as described in

scored as shown in Fig. 4. There was little or no anaphylactic response in all mice in vehicle and G groups. In GS group, half of the mice showed mild to slightly severe responses (score 1–3), while the other mice showed no responses. All the mice in H group exhibited mild to slightly severe symptoms (score 2–3). In HS group, half of the mice experienced severe symptoms (score 4). These results indicated a relationship between the decrease in rectal temperatures and systemic anaphylaxis scores after elicitation of immediate hypersensitivity reactions.

Histamine content in the plasma of mice after 30 min of immediate hypersensitivity elicitation was determined as shown in Fig. 5. The mean value of histamine content in H group was significantly higher than that of the vehicle group (Fig. 5A). In HS group, a significant increase in histamine content in the sera was not observed, while the decrease in rectal temperature and manifestations of systemic anaphylaxis were most remarkable as shown in Figs 3A and 4. In GS group, an increase in histamine content was observed; however, the difference between GS and vehicle groups was

Materials and methods. Each symbol shows the greatest score of symptoms in each mouse. Bars represent the average score for each group ($n = 8$). * $P < 0.05$, ** $P < 0.01$ by Bonferroni/Dunn's test.

not significant. The results of various HWP1 doses for sensitization are shown in Fig. 5B. Larger amounts of HWP1 used for sensitization resulted in a higher histamine content in mouse plasma.

Cytokine secretion from the splenocytes of mice sensitized to HWP1

After elicitation of immediate hypersensitivity reactions, the splenocytes of mice were collected and cultured for 72 h with HWP1 for re-stimulation. The content of several cytokines in the culture medium was determined after 72 h as shown in Fig. 6. In HS100 and HS500 groups, the content of IL-4, IL-5, and IL-10 in the culture medium of splenocytes was increased (Fig. 6A–C). On the other hand, the content of IL-2 was significantly decreased in HS500 group. In HS20, HS100, and HS500 groups, interferon (IFN)- γ content was also significantly decreased (Fig. 6D,E). These results demonstrated that transdermal sensitization to HWP1 resulted in a state of Th2-dominant helper T-cell activation.

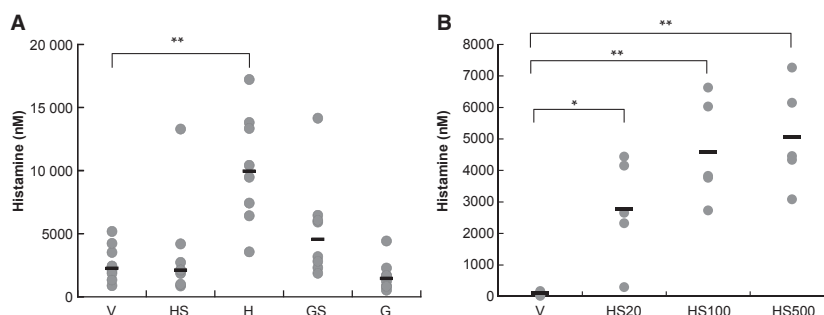


Figure 5 Plasma histamine levels of mice transdermally sensitized to HWP1/gluten after intraperitoneal challenge. A, HWP1/gluten-sensitized mice ($n = 8$); B, HWP1-sensitized mice ($n = 5$). Groups

are the same as described in Fig. 1. Bars represent the means of each group. * $P < 0.05$, ** $P < 0.01$ by Dunnett's test vs the V group.

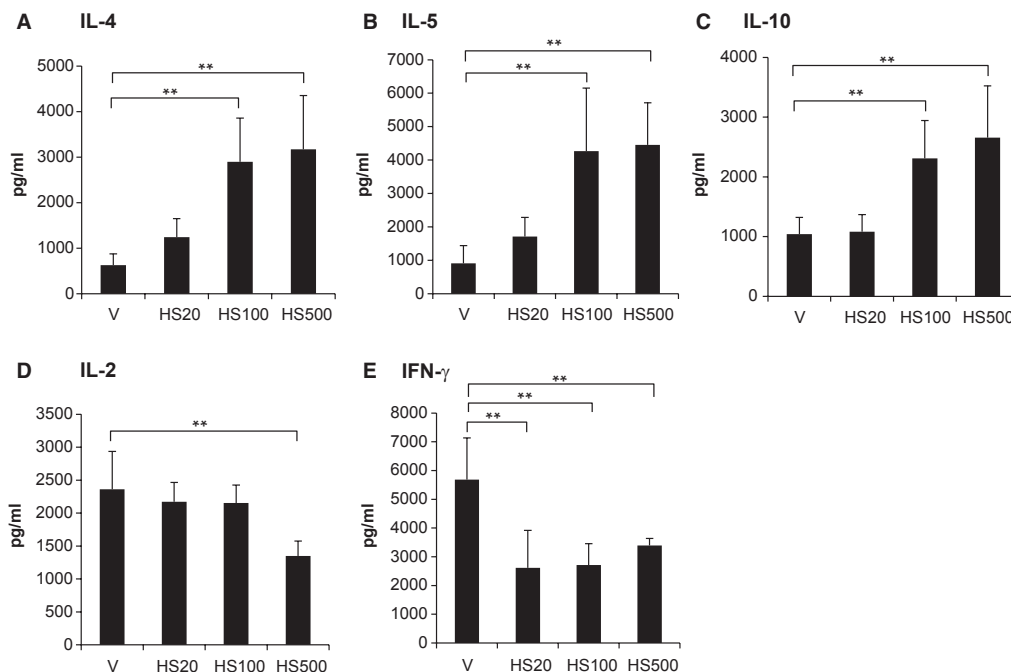


Figure 6 Cytokine secretion by the splenocytes of mice transdermally sensitized to HWP1/gluten. Splenocytes were harvested from HWP1-sensitized mice and re-stimulated with 100 µg/ml HWP1 in 24-well plates. After re-stimulation for 72 h, cytokines in

medium supernatants were determined with a Bio-Plex system. Groups are the same as described in Fig. 1. Data are the mean ± SD of each group ($n = 5$). ** $P < 0.01$ by Dunnett's test vs the V group.

Discussion

Hydrolyzed wheat protein has been recently reported to induce immediate hypersensitivity via skin contact and/or food ingestion (1–8). In this study, we chose BALB/c mice as model animals and compared the sensitizing potential of HWP1 with that of gluten by transdermal exposure.

There are three important and novel findings from this study. First, repeated transdermal exposure of BALB/c mice to HWP1 is sufficient to sensitize mice and induces active systemic anaphylaxis (ASA) by intraperitoneal injection in a dose-dependent manner. Second, the mechanism underlying the transdermal sensitization involves the activation of type-2 cytokine responses (IL-4, IL-5, and IL-10). Third, repeated transdermal exposure to gluten sensitizes mice in the presence of 0.5% SDS and induces ASA.

In this study, the BALB/c strain was chosen to develop a HWP allergy model because this strain has been recently used for transdermal sensitization models using peanuts (12, 16), hazelnuts (10), and OVA (9, 17) as antigens. We are not aware of previous animal studies that examine the immune consequences of transdermal exposure to wheat proteins and HWP.

There are some reports using mouse models of dermatitis, which indicate effective transdermal sensitization in the presence of 0.5% Tween 20 (18, 19). In our study, by comparing GS and G groups, we showed that the presence of SDS evidently increased the efficiency of sensitization (Figs 1 and 2). It is possible that the solubility (20) or

transdermal permeability of gluten was increased by SDS. In addition, the effect of SDS may possibly depend on its adjuvant activity by activating cytokine secretion (such as IL-1α) from epidermal cells including keratinocytes (21), or an irritant activity (22). In the case of patients sensitized to HWP1, detergents contained in facial soap might facilitate such sensitization.

For transdermal sensitization, the effectiveness of an additional intraperitoneal injection has been reported (9). Therefore, in our study, the effect of an intraperitoneal injection of HWP1 or gluten (10 µg/mouse) at 1 week prior to the start of transdermal administration was investigated. However, no effect of the intraperitoneal injection on IgE and IgG1 production, rectal temperature, or histamine content in the plasma after elicitation of immediate hypersensitivity was observed (data not shown).

As shown in Figs 1 and 2, antibodies against gluten in the mouse sera of GS group reacted with HWP1, and antibodies against HWP1 in the mouse sera of HS and H groups also reacted with gluten. These results show that there is some cross-reactivity between HWP1 and gluten. However, some of the epitopes in gluten could be cleaved by hydrolysis, or some new epitopes could appear in HWP1 owing to destruction of the gluten conformation and/or deamidation of glutamine residues, which are often included in gluten, by hydrolysis. In addition, as shown in Fig. 3, the decrease in rectal temperature in HS group was larger than that of GS group. Furthermore, as shown in Fig. 4, the scores of systemic anaphylactic responses in HS group were significantly

higher than those of GS group. These results show that the capabilities of HWP1 and gluten to induce ASA appear to be different. The molecular aspect of HWP1 antigenicity and/or allergenicity may be an important point that should be examined further.

In H group, almost the same levels of IgE and IgG1 specific for HWP1 were produced as that in HS group (Fig. 1), whereas the levels of IgE and IgG1 specific for gluten in the sera of G group were lower than that of GS group (Fig. 2). In addition, the rectal temperature after immediate hypersensitivity elicitation was decreased markedly in H group as well as in HS group. However, the change in rectal temperature in G group was much smaller than that of GS group and almost the same level as that of the vehicle group (Fig. 3). The comparison between H and G groups suggests that a certain degree of hydrolysis of proteins increases their solubility and transdermal permeability and, as a result, the ability to induce the production of specific antibodies and cause immediate hypersensitivity reactions.

As shown in Fig. 5A, histamine content in the plasma of HS group mice after 30 min of immediate hypersensitivity elicitation was not very high compared with that of H group, whereas both the decrease in rectal temperature and scores of systemic anaphylactic responses were most remarkable among the groups of mice in this study. The reason for this result is unclear, but it is possible that the transdermal sensitization of mice with HWP1 in HS group was successful enough, and the latter part of sensitization (fourth sensitization) played a role of transdermal challenge that induced partial release of histamine from mast cells. Because of such a condition, the histamine content in the plasma after intraperitoneal challenge might not be increased as much.

In summary, our findings show that transdermal exposure to HWP1 is sufficient to activate key immune pathways necessary for sensitizing mice for immediate hypersensitivity reactions. Our data also highlight the potential risk of partial

hydrolysis of food proteins, in which hydrolyzed proteins may exhibit increased transdermal permeability and allergenic potency. Additionally, this experimental model using BALB/c mice may be very useful for further basic and applied studies of the sensitizing potential of various hydrolyzed proteins.

Acknowledgments

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Author contributions

All authors were involved in the conception and design of the study as well as in its conduct and the generation of data. The results of these analyses were interpreted and discussed by all authors. Reiko Adachi, Rika Nakamura, and Reiko Teshima coordinated writing of the manuscript with the named authors.

Conflict of interest

The authors have declared that they have no conflicts of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. SDS-PAGE patterns of HWP1 and gluten.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

References

1. Laurière M, Pecquet C, Bouchez-Mahiout I, Snégaroff J, Bayrou O, Raison-Peyron N et al. Hydrolysed wheat proteins present in cosmetics can induce immediate hypersensitivities. *Contact Dermatitis* 2006;**54**:283–289.
2. Fukutomi Y, Itagaki Y, Taniguchi M, Saito A, Yasueda H, Nakazawa T et al. Rhinoconjunctival sensitization to hydrolyzed wheat protein in facial soap can induce wheat-dependent exercise-induced anaphylaxis. *J Allergy Clin Immunol* 2011;**127**: 531–533.
3. Snégaroff J, Bouchez-Mahiout I, Pecquet C, Branlard G, Laurière M. Study of IgE antigenic relationships in hypersensitivity to hydrolyzed wheat proteins and wheat-dependent exercise-induced anaphylaxis. *Int Arch Allergy Immunol* 2006;**139**:201–208.
4. Pelkonen AS, Mäkinen-Kiljunen S, Hilvo S, Siltanen M, Mäkelä MJ. Severe allergic reaction to gluten hydrolysate without reaction to wheat. *Ann Allergy Asthma Immunol* 2011;**106**:343–344.
5. Chinuki Y, Kaneko S, Sakieda K, Murata S, Yoshida Y, Morita E. A case of wheat-dependent exercise-induced anaphylaxis sensitized with hydrolysed wheat protein in a soap. *Contact Dermatitis* 2011;**65**:43–58.
6. Bouchez-Mahiout I, Pecquet C, Kerre S, Snégaroff J, Raison-Peyron N, Laurière M. High molecular weight entities in industrial wheat protein hydrolysates are immunoreactive with IgE from allergic patients. *J Agric Food Chem* 2010;**58**:4207–4215.
7. Laurière M, Pecquet C, Boulenc É, Bouchez-Mahiout I, Snégaroff J, Choudat D et al. Genetic differences in omega-gliadins involved in two different immediate food hypersensitivities to wheat. *Allergy* 2007;**62**:890–896.
8. Leduc V, Moneret-Vautrin DA, Guerin L, Morrisset M, Kanny G. Anaphylaxis to wheat isolates: immunochemical study of a case proved by means of double-blind, placebo-controlled food challenge. *J Allergy Clin Immunol* 2003;**111**:897–899.
9. Hsieh KY, Tsai CC, Herbert Wu CH, Lin RH. Epicutaneous exposure to protein antigen and food allergy. *Clin Exp Allergy* 2003;**33**:1067–1075.
10. Birmingham NP, Parvataneni S, Hassan HMA, Harkema J, Samineni S, Navuluri L et al. An adjuvant-free mouse model of tree nut allergy using hazelnut as a model tree nut. *Int Arch Allergy Immunol* 2007;**144**: 203–210.
11. Teshima R, Okunuki H, Sato Y, Akiyama H, Maitani T, Sawada J. Effect of oral administration of CpG ODN-OVA on WBB6F1-W/Wv mice. *Allergol Int* 2006;**55**:43–48.
12. Strid J, Callrd R, Strobel S. Epicutaneous immunization converts subsequent and

- established antigen-specific T helper type 1 (Th1) to Th2-type responses. *Immunology* 2006;**119**:27–35.
13. Mathias CB, Hobson SA, Garcia-Lioret M, Lawson G, Poddighe D, Freyschmidt EJ et al. IgE-mediated systemic anaphylaxis and impaired tolerance to food antigens in mice with enhanced IL-4 receptor signaling. *J Allergy Clin Immunol* 2011;**127**:795–805.
 14. Zhang T, Pan W, Takebe M, Schofield B, Sampson H, Li XM. Therapeutic effects of a fermented soy product on peanut hypersensitivity is associated with modulation of Th1 and Th2 responses. *Clin Exp Allergy* 2008;**38**:1808–1818.
 15. Shindo T, Kanazawa Y, Saito Y, Kojima K, Ohsawa M, Teshima R. Effective induction of oral anaphylaxis to ovalbumin in mice sensitized by feeding of the antigen with aid of oil emulsion and salicylate. *J Toxicol Sci* 2012;**37**:307–315.
 16. Strid J, Hourihane J, Kimbert I, Callard R, Strobel S. Epicutaneous exposure to peanut protein prevents oral tolerance and enhances allergic sensitization. *Clin Exp Allergy* 2005;**35**:757–766.
 17. Kodama M, Asano K, Oguma T, Kagawa S, Tomomatsu K, Wakaki M et al. Strain-specific phenotypes of airway inflammation and bronchial hyperresponsiveness induced by epicutaneous allergen sensitization in BALB/c and C57BL/6 mice. *Int Arch Allergy Immunol* 2010;**152**(Suppl. 1):67–74.
 18. Inagaki N, Nagai H. Analysis of the mechanism for the development of allergic skin inflammation and the application for its treatment: mouse models for the development of remedies for human allergic dermatitis. *J Pharmacol* 2009;**110**:251–259.
 19. Gao XK, Nakamura N, Fuseda K, Tanaka H, Inagaki N, Nagai H. Establishment of allergic dermatitis in NC/Nga mice as a model for severe atopic dermatitis. *Biol Pharm Bull* 2004;**27**:1376–1381.
 20. Dupont FM, Samoil V, Chan R. Extraction of up to 95% of wheat (*Triticum aestivum*) flour protein using warm sodium dodecyl sulfate (SDS) without reduction or sonication. *J Agric Food Chem* 2008;**56**:7431–7438.
 21. Uchino T, Takezawa T, Ikarashi Y. Reconstruction of three-dimensional human skin model composed of dendritic cells, keratinocytes and fibroblasts utilizing a handy scaffold of collagen vitrigel membrane. *Toxicol In Vitro* 2009;**23**:333–337.
 22. Agner T. Susceptibility of atopic dermatitis patients to irritant dermatitis caused by sodium lauryl sulphate. *Acta Derm Venereol* 1991;**71**:296–300.

Fig.S1

