DOI: 10.1111/pai.13211

ORIGINAL ARTICLE

WILEY

Epicutaneous immunogenicity of partially hydrolyzed whey protein evaluated using tape-stripped mouse model

Hiroshi Iwamoto | Takeshi Matsubara | Tomoyuki Okamoto | Mayu Yoshikawa | Takuya Matsumoto | Gaku Kono | Yasuhiro Takeda

Wellness & Nutrition Science Institute, Morinaga Milk Industry Co., Ltd., Zama, Japan

Correspondence
Takeshi Mastubara, Wellness & Nutrition Science Institute, Morinaga Milk Industry Co., Ltd., 5-1-83 Higashihara, Zama, Kanagawa 252-8583, Japan.
Email: ta-matsubara@morinagamilk.co.jp

Funding information
This study was funded by Morinaga Milk Industry Co., Ltd.

Editor: Philippe Eigenmann

Abstract

Background: Hydrolyzed cow's milk protein formulas are widely used for infants with a history or risk of cow's milk allergy. Based on the current theory that food allergen sensitization occurs via the skin, we investigated the epicutaneous immunogenicity of partially hydrolyzed whey proteins, which are ingredients in infant formulas.

Methods: BALB/c mice were exposed epicutaneously to whey protein concentrate (WPC) or partial whey protein hydrolysates (PWH1 or PWH2) on tape-stripped skin. Sensitization was assessed by evaluating serum β-lactoglobulin (β-LG)-specific antibodies, basophil activation, and cytokine production from β-LG-stimulated lymphoid cells. The anaphylaxis reaction was evaluated by measuring the rectal temperature and plasma level of mouse mast cell protease-1 after oral β-LG challenge. Immune cell accumulation in the skin was also analyzed.

Results: Substantive sensitization and β-LG-induced anaphylaxis reaction were observed in WPC-exposed mice, whereas no significant changes were observed in PWH1- or PWH2-exposed mice. The basophil and eosinophil counts increased in WPC-exposed murine skin, not but in PWH1- or PWH2-exposed mice.

Conclusion: The epicutaneous immunogenicity of PWH1 and PWH2 is markedly decreased, which may reduce the risk of allergen sensitization. Further studies are required to investigate the clinical value of these partial hydrolysates for high-risk infants.

KEYWORDS
cow's milk allergy, epicutaneous immunogenicity, food allergen, infant formulas, whey protein hydrolysate

1 | INTRODUCTION

Food allergy affects 8% of children globally, and its increasing prevalence is now of considerable concern to the general public.[1] Although cow's milk is considered a suitable source of nutrition for infants and young children, cow's milk protein is one of the most common food allergens in these groups of people.[1] Enzymatic hydrolysis is an effective and realistic approach for reducing the antigenicity of proteins in cow's milk,[2] and infant formulas prepared from hydrolyzed cow's milk protein are widely used as breast-milk substitutes for infants with a history or risk of this allergy. The antigenicity of these formula

The peer review history for this article is available at https://publons.com/publon/10.1111/pai.13211

© 2020 EAACI and John Wiley and Sons A/S. Published by John Wiley and Sons Ltd.

products and hydrolyzed protein ingredients has been widely evaluated. The determination of the avidity of specific antibodies to residual antigens in vitro, such as by enzyme-linked immunosorbent assay (ELISA), is the standard approach for these analyses.[3,4] Physiologically, some in vitro models have been established for evaluating the ability of residual antigens to activate sensitized immune cells.[5,6] Animal models enable examination of not only the ability to evoke allergic reactions in sensitized individuals, but also the sensitizing capacity "immunogenicity." Numerous animal studies have evaluated the immunogenicity of hydrolyzed cow's milk protein formulas and their ingredients. Intradermal or intraperitoneal immunization models or, as a mimic of food allergy, per-oral immunization models have been used.[7-9] However, in the past decade, the understanding of the food allergen sensitization mechanism has dramatically changed. Some studies demonstrated that food allergen sensitization mainly occurs through inflamed skin.[10,11] Although the immunogenicity of hydrolyzed cow's milk protein formulas and their ingredients have been evaluated previously, there are no reports of immunogenicity occurring via the skin.

Therefore, we evaluated the epicutaneous immunogenicity of whey protein hydrolysates, which are ingredients in partially hydrolyzed infant formula products, by using a tape-stripping epicutaneous exposure model.

Key Message

Food allergen sensitization occurs through inflamed skin. The immunogenicity of hydrolyzed cow's milk protein formulas have been widely evaluated, but no reports of immunogenicity occurring via the skin. We evaluated the epicutaneous immunogenicity of whey protein hydrolysates, which are ingredients in partially hydrolyzed infant formula products, by using a tape-stripping epicutaneous exposure model. The epicutaneous immunogenicity of the partial hydrolysates was markedly decreased compared to that of native whey protein. Thus, cow's milk protein hydrolysates with reduced epicutaneous immunogenicity and the ability to induce adequate oral tolerance would be suitable for high risk infants.

2 | MATERIALS AND METHODS

2.1 | Materials

Two types of partial whey protein hydrolysates (PWH1 and PWH2) were obtained from Morinaga Milk Industry. Both hydrolysates are ingredients in partially hydrolyzed infant formula products. PWH2 was prepared with a combination of microbial, botanical, and porcine pancreatic enzymes, whereas PWH1 was prepared without the porcine enzymes. The molecular weight profiles of PWH1 and PWH2 provided by the manufacturer's brochure are shown in Table 1. Whey protein concentrate (WPC) was purchased from Milei GmbH. The levels of β-lactoglobulin (β-LG) determined using a commercial ELISA kit (Morinaga Institute of Biological Science) in PWH1, PWH2, and WPC were 2.5×10^3, 2.1×10^3, and 3.3×10^5 ppm, respectively.[12]

2.2 | Animals

BALB/c mice were used, as in the epicutaneous sensitization models in previous studies.[12-16] Seven-week-old female BALB/c specific pathogen-free mice were purchased from CLEA Japan. All mice were maintained under specific pathogen-free conditions and on milk-free chow. The mice were used for study after 1 week of acclimation. All animal experiments were approved by the Institutional Animal Care and Use Committee of Morinaga Milk Industry Co., Ltd. certificated by the Japan Health Science Foundation.

2.3 | Epicutaneous exposure procedure

The epicutaneous exposure procedure was performed by following a previously published method.[12-16] Under anesthesia, the back skin of each mouse was shaved and tape-stripped six times. Three micrograms of each test material (PWH1, PWH2, or WPC, n = 8, respectively) in 200 μL saline was placed on two square (1 × 1-cm) gauze patches. The patches were fixed with transparent dressing (Tegaderm™; 3M) for 1 week. Mice in the sham-exposure (Sham, n = 8) group were exposed to saline in the same manner. The epicutaneous exposure procedure was repeated twice (Experiments 1 and 2) or three times (Experiment 2) at 2-week intervals. Here, Experiment 1 was performed to investigate the sensitization states induced by epicutaneous exposure. Experiment 2 was performed mainly to investigate the impacts of repeated antigen exposure on the skin. The schedules are shown in Figure 1.

2.4 | Antigen-specific antibody measurement

Serum β-LG-specific IgE and IgG1 levels were measured by ELISA. Ninety-six-well plates (Thermo Fisher Scientific) were coated with

TABLE 1 Molecular weight profile of whey protein hydrolysates

Molecular weight	PWH1	PWH2
<200	12	8
200-500	36	36
500-1000	22	20
1000-2000	18	22
2000-5000	10	10
5000-10 000	1	2
10 000<	1	2
Total	100	100

Note: The molecular weight profiles of whey protein hydrolysates evaluated in this study adapted from manufactures brochure. Data are expressed as the percentage of total peptide.

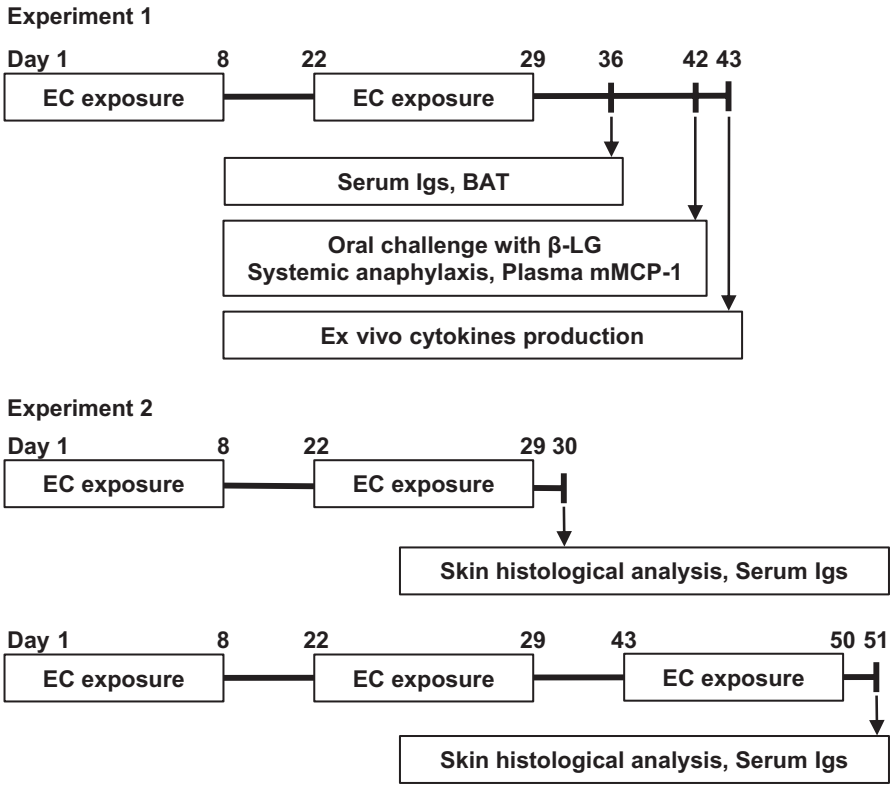

FIGURE 1 Schematic representation of the experimental design. Mice were exposed to test materials epicutaneously twice (Experiments 1 and 2) or three times (Experiment 2) at two-week intervals followed by analysis as described in the diagrams. BAT, basophil activation test; EC exposure, epicutaneous exposure

1 μg/well β-LG (Sigma-Aldrich) overnight at 4°C. After washing the wells, diluted serum samples (1/10 for IgE and 1/1000 for IgG1, respectively; with PBS-Tween containing 1% gelatin) were added and incubated for 1.5 hours at 37°C. The wells were washed again, and biotinylated anti-mouse IgE or biotinylated anti-mouse IgG1 (BD Biosciences; dilution rates were 1/100 and 1/10 000, respectively) was added and incubated for 1 hour at 37°C. After washing, the wells were incubated for 1 hour at 37°C with streptavidin-peroxidase (Roche Diagnostics) and then washed again. The wells were developed with substrate (SIGMAFAST™ OPD; Sigma-Aldrich), and absorbance was measured at 492 nm using 630 nm as reference.

2.5 | Basophil activation test

Antigen-specific basophil activation was evaluated by the surface expression changes of CD200R1 and CD200R3, which are markers of IgE- and IgG1-dependent basophilic activation, respectively.[17] Heparinized whole blood samples were incubated with or without 100 ng/mL β-LG at 37°C for 2 hours. The cells were blocked with 15% horse serum (GE Healthcare) and stained with APC-conjugated anti-mouse IgE (Abcam), PerCP/Cy5.5-conjugated anti-mouse CD49b, PE-conjugated anti-mouse CD200R1 (both from BioLegend), and FITC-conjugated anti-mouse CD200R3 (Hycult Biotech) on ice. Surface expression of CD200R1 and CD200R3 on CD49b+/IgE+ cells was analyzed using a FACSCant flow cytometer equipped with FACSDiva software (BD Biosciences).

2.6 | Oral challenge test and measurement of plasma mouse mast cell protease-1 (mMCP-1)

Mice were orally challenged with 50 mg of β-LG at 13 days after the second exposure. Systemic anaphylactic reactions were evaluated by measuring the rectal temperature using a digital thermometer (TD-300; Natsume Seisakusho Co.). At 60 minutes after the challenge, plasma samples were collected. Plasma levels of mMCP-1 were determined with a commercial ELISA kit (Thermo Fisher Scientific).

2.7 | Lymphoid cell culture and cytokine measurement

On the day after the oral challenge test, the spleens and inguinal lymph nodes (iLNs) were collected from sacrificed mice and macerated through 70-μm cell strainers (Corning). Additionally, hemolysis was performed for spleen cells. Following two washes, the cells (4×10^5 and 1×10^5 cells/well, respectively) were stimulated with or without 1 mg/mL β-LG in complete medium (RPMI 1640, 10% horse serum, 2 mM L-glutamine, 100 IU/mL penicillin, and 100 μg/mL streptomycin). After 96 hours, the supernatants were collected and the levels of interleukin (IL)-2, IL-4, IL-5, IL-10, IL-13, IL-17A, and interferon (IFN)-γ were measured with a Milliplex™ Map Mouse Th17 Magnetic Bead Panel Kit 96-Well Plate Assay (EMD Millipore Corp.). In the supernatant of iLN cells, the levels of IL-10, IL-17A, and IFN-γ were lower than the detection limit. Therefore, commercial ELISA kits (Thermo Fisher Scientific) were used for these cytokines.

2.8 | Histological analysis of immune cells in the skin

Skin tissues were collected from sacrificed mice, fixed in 10% buffered formalin, embedded in paraffin, and stained with hematoxylin. Mast cells were detected by using a commercial esterase-stain kit (Naphthol AS-D chloroacetate Esterase; Muto Pure Chemical). Basophils were stained with anti-mMCP-8 (BioLegend) and probed with anti-rat IgG labeled with peroxidase (Histofine Simple Stain MAX-PO; Nichirei). Eosinophils were identified by Dylon staining (Direct Fast Scarlet 4BS; Muto Pure Chemical). The numbers of mast cells, basophils, and eosinophils were counted in 10 high-power fields at 400× magnification.

2.9 | Statistical analysis

Data are expressed as the mean ± standard error of the mean (SEM). Data were analyzed by Tukey's test using statistical software (JMP version 13.0; SAS Institute). Values of $P < .05$ were considered as statistically significant.

3 | RESULTS

3.1 | No significant increase in antigen-specific IgE and IgG1 in PWH1- or PWH2-exposed mice

Increases in β-LG-specific IgE and IgG1 antibodies were observed in WPC-exposed mice. However, there was no significant increase in the PWH1- or PWH2-exposed mice compared with Sham mice (Figure 2A).

3.2 | No significant activation in basophils in PWH1- or PWH2-exposed mice

Increased CD200R1 expression and decreased CD200R3 expression on basophils indicate IgE- and IgG1-dependent cell activation, respectively.[17] After β-LG stimulation, these changes on basophils were observed in WPC-exposed mice. In PWH1- and PWH2-exposed mice, there were no significant changes in CD200R1 and CD200R3 expression (Figure 2B).

3.3 | No significant anaphylactic reaction in PWH1- or PWH2-exposed mice

Significant decreases in the rectal temperature, which is associated with systemic anaphylaxis, were induced by oral β-LG challenge in WPC-exposed mice. In contrast, there were no significant changes in PWH1- or PWH2-exposed mice. Consistent with this result, the plasma level of mMCP-1 in WPC-exposed mice was significantly higher than that of the Sham group (Figure 3A,B).

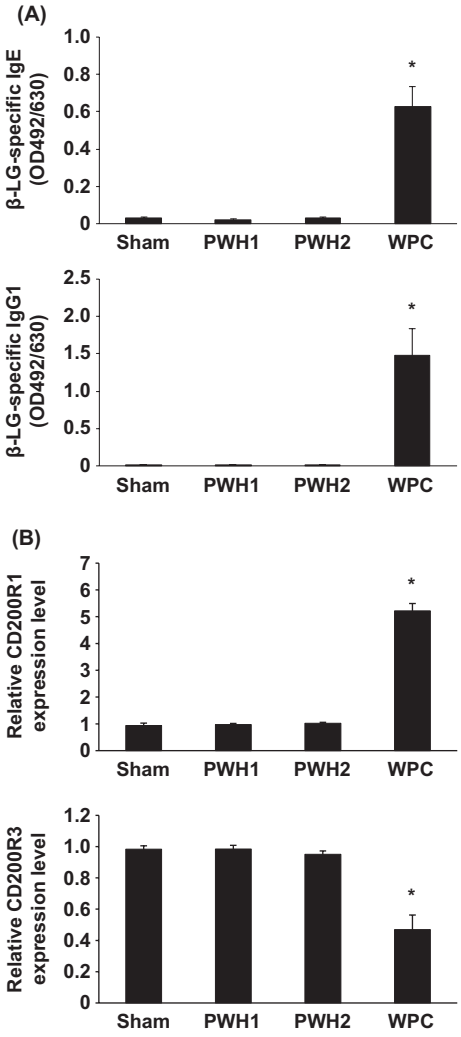

FIGURE 2 Production of antigen-specific antibodies and basophil activation response in epicutaneously exposed mice. A, One week after the second exposure, blood samples were collected and the serum levels of β-LG-specific IgE and IgG1 were measured by ELISA. B, Surface expression of CD200R1 and CD200R3 on basophils from heparinized blood was analyzed by flow cytometry after incubation with or without 100 ng/mL β-LG and presented as relative expression levels. Data are represented as the mean ± SEM (n = 8). *Significant difference from the Sham group; $P < .05$

3.4 | Cytokine production from lymphoid cells was not altered in PWH1- or PWH2-exposed mice

To evaluate the sensitization states of lymphoid cells in epicutaneously exposed mice, cytokine production in the spleen and iLN cells was assessed.

After 96 hours of stimulation with β-LG, the levels of IL-4, IL-5, IL-10, IL-13, IL-17, and IFN-γ in the spleen cell culture supernatants were increased in only WPC-exposed mice. The levels of those cytokines were significantly higher than those in Sham mice, while no significant changes

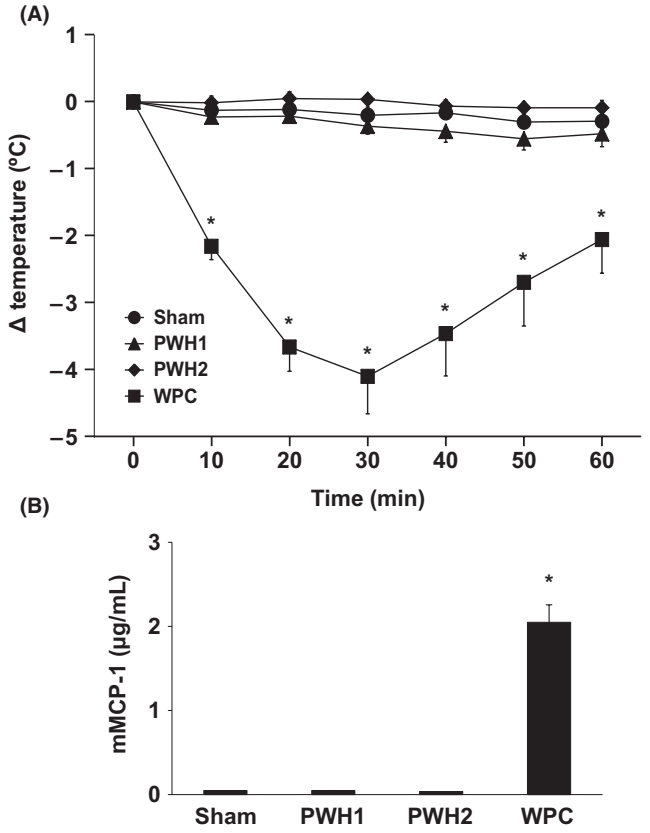

FIGURE 3 Anaphylactic reaction in mice induced by β-LG oral challenge after the second epicutaneous exposure to test materials. A, Rectal temperature changes in mice after challenge with 50 mg of β-LG were analyzed. The asterisk indicates significant differences at each time point compared with Sham group. B, Plasma concentrations of mMCP-1 at 60 min after oral challenge. Data are represented as the mean ± SEM (n = 8). *Significant difference from the Sham group; $P < .05$

were observed in PWH1- or PWH2-exposed mice compared with Sham mice. β-LG induced IL-2 production in all groups, while there were no differences compared with the Sham group (Figure 4A). In the iLN cell culture supernatants, the significant increases of IL-2, IL-4, IL-5, and IL-13 were induced by β-LG stimulation in only WPC-exposed mice. The levels of these cytokines were significantly higher than those in the Sham group, while no significant changes were detected in PWH1- or PWH2-exposed mice. An increasing tendency in IL-17 production induced by β-LG was observed in the WPC-exposed group ($P = .0675$). The level of this cytokine had a higher tendency compared to that in the Sham group ($P = .0675$). No significant changes were observed in the production of IL-10 and IFN-γ (Figure 4B).

3.5 | Accumulation of immune cells to skin tissue was not induced in PWH1- or PWH2-exposed mice

In Experiment 2, to investigate the impacts of epicutaneous exposure to test materials on murine skin tissue, back skin specimens were removed following the second or third epicutaneous exposure and immune cells were evaluated histologically.

After the third epicutaneous exposure compared with the second, the numbers of basophils and eosinophils in the skin tissue increased significantly in WPC-exposed mice, while PWH1- or PWH2-exposed mice showed no significant changes. After the third exposure, the number of basophils in WPC-exposed mice was significantly higher than that in the Sham group. A significant difference in the number of eosinophils was observed only between WPC-exposed mice and PWH2-exposed mice ($P = .0057$). There was no significant difference in the numbers of mast cells between groups (Figure 5A,B).

During skin tissue collection, serum samples were obtained, and β-LG-specific antibodies were measured. After the second exposure, β-LG-specific IgE and IgG1 antibodies were increased in the WPC-exposed mice, while there was no significant increase in PWH1- or PWH2-exposed mice, in agreement with the results of Experiment 1. After the third exposure, antibodies in WPC-exposed mice were further increased. In contrast, the absence of antibodies in PWH1- or PWH2-exposed mice was maintained (Figure 5C).

4 | DISCUSSION

In this study, the production of antigen-specific antibodies, activation of basophils, and anaphylactic reactions induced by oral antigen challenge observed in WPC-exposed mice were insignificant in PWH1- or PWH2-exposed mice. These results indicate that the epicutaneous immunogenicity of PWH1 and PWH2 is markedly lower than that of WPC. Although the lowered immunogenicity of some whey protein hydrolysates has been demonstrated in intradermal or per-oral immunized models,[8,9] this is the first report of the epicutaneous immunogenicity of the hydrolysates.

The physical skin barrier, which consists of the stratum corneum and tight junctions, inhibits the penetration of external antigens. Although dermal antigen-presenting cells such as Langerhans cells remain under these barriers, it has been reported that murine Langerhans cells activated by tape-stripping extend their dendrites through the tight junctions and capture external antigens.[18,19] Antigen application through the skin mechanically injured by tape-stripping upregulates thymic stromal lymphopoietin expression and induces Th-2-dominant skin inflammation as is seen in atopic dermatitis (AD).[20,21] In the inflamed skin of patients with AD, Langerhans cells extend dendrites through the tight junctions and are likely to capture external antigens.[22] Therefore, it is considered that AD is a robust risk for epicutaneous sensitization and that the allergy march is driven by food allergen sensitization via lesional skin.[23] In this study, it was presumed that tape-stripping induced stratum corneum damage and Langerhans cell activation and that the WPC was captured by the Langerhans cells and presented to T cells. Although PWH1 and PWH2 may have been captured and presented by activated Langerhans cells in the same manner, the immunogenicity of the hydrolysates may not have been high enough to sensitize the epicutaneously exposed mice.

The accumulation of immune cells, such as mast cells,[14] basophils,[24] or eosinophils,[14,15] in skin tissues following epicutaneous antigen exposure has been reported. In our study, increased numbers

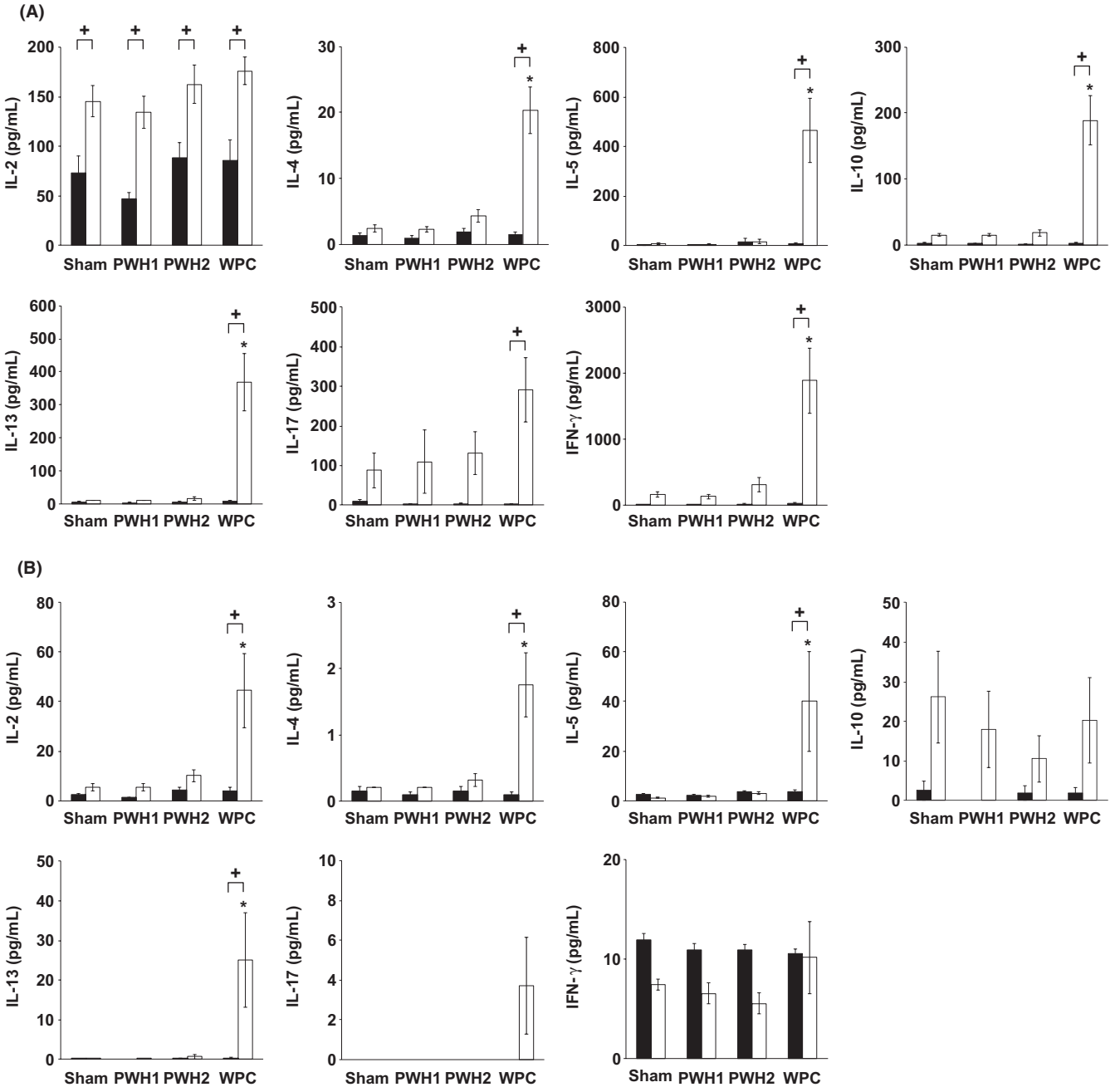

FIGURE 4 Ex vivo cytokine production from lymphoid cells with β-LG after second epicutaneous exposure to test materials. Spleen (A) and inguinal lymph node (B) cells were stimulated with (white bars) or without (black bars) 1 mg/mL β-LG for 96 h. Levels of cytokines in the supernatants were measured. Data are represented as the mean ± SEM (n = 8). *Significant difference from the Sham group; $P < .05$. +Significant difference attributed to the presence of β-LG; $P < .05$

of basophils and eosinophils but not mast cells were observed in the skin of WPC-exposed mice. These differences in the behavior of skin immune cells may be related to differences in experimental designs among studies, such as the procedure used for antigen exposure and the timing of histological observation. In this study, the increase in basophils and eosinophils was observed only after the third epicutaneous exposure; however, anaphylaxis reactions were observed after the second exposure. Therefore, interestingly, epicutaneous sensitization was established sufficiently prior to the accumulation of immune cells in the skin. This finding suggests that epicutaneous sensitization occurs without immune-related histological changes in the skin.

In this study, the epicutaneous exposure was performed with only a single dose of test materials. Therefore, one of the limitations of this study is that dose-dependent effects were not evaluated. However, sensitization induced by WPC but not by PWH1 or PWH2 indicates substantial differences in immunogenicity between these materials.

It is believed that food allergen sensitization occurs through inflamed skin, whereas per-oral consumption of food allergens induces tolerance.[10] Several studies have shown that early introduction rather

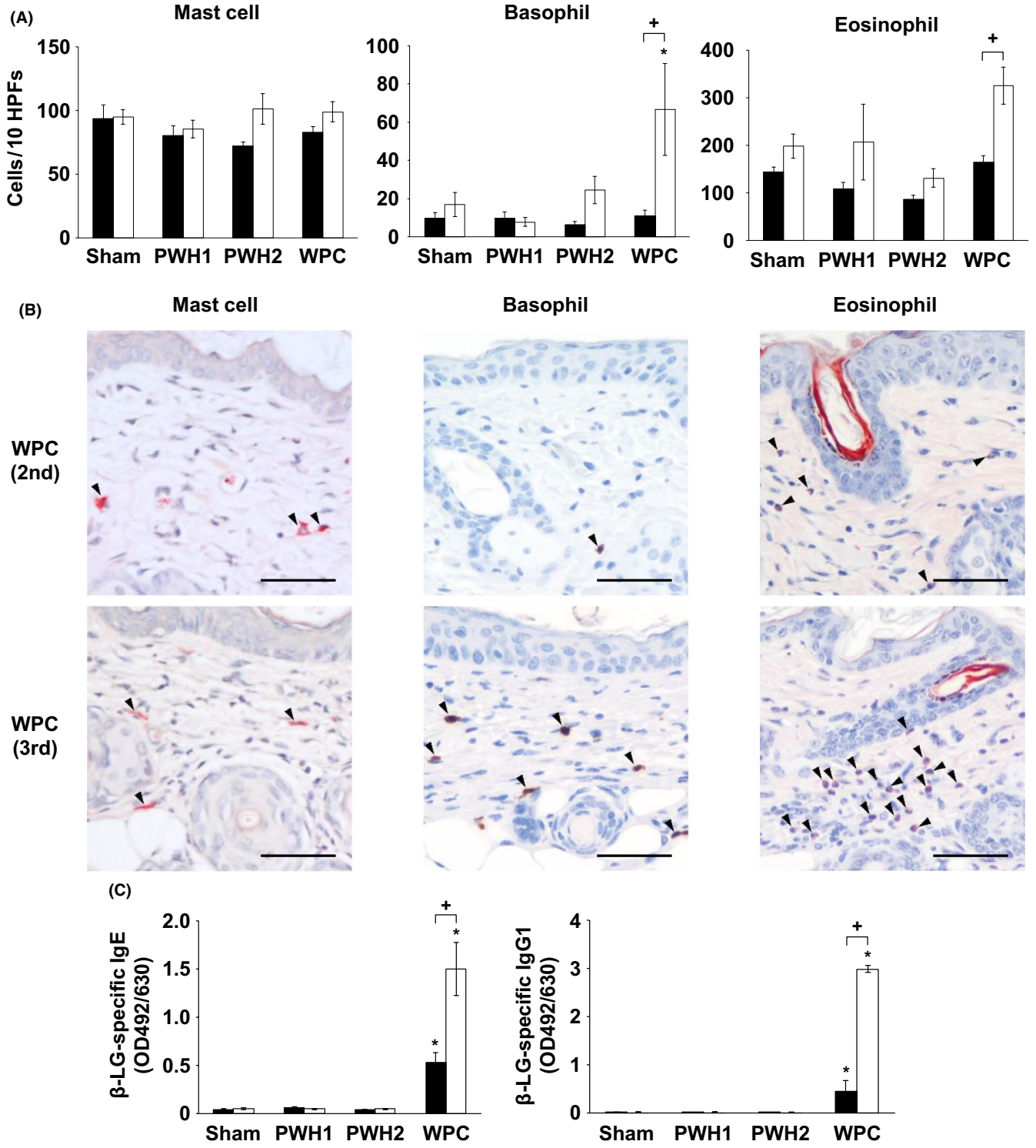

FIGURE 5 Impacts of repeated epicutaneous exposure of test materials on skin tissue and serum β-LG-specific antibodies. A, The numbers of mast cells, basophils, and eosinophils were counted in 10 high-power field at 400× magnification. B, Histological staining of mast cells, basophils, and eosinophils in WPC-exposed murine skin after second (upper) and third (lower) exposure. Scale bar = 50 μm. C, Blood samples were obtained during skin tissue collection. The serum levels of β-LG-specific IgE and IgG1 were measured by ELISA. (A, C) Data are represented as the mean ± SEM (n = 8). The black and white bars indicate the means of the data from mice after second and third exposures, respectively. *Significant difference from the Sham group; $P < .05$. ⁺Significant difference between second and third exposures; $P < .05$

than avoidance of potentially allergenic foods reduces the prevalence of food allergy.[25-27] Therefore, inducing oral tolerance at an appropriate time and avoiding epicutaneous sensitization is extremely important for preventing food allergy.[11] However, substantial per-oral consumption without epicutaneous exposure is unrealistic manner for early childhood, as the hands and faces of children frequently contact spat milk

and leftover meals. Particularly, food allergens attached to the face or hands will be a high risk of sensitization in subjects with AD. Recently, we reported that the ingestion of whey protein, not only in an intact state but also in its partial hydrolysates (PWH1 and PWH2), induced oral tolerance in a β-LG epicutaneously sensitized mouse model.[12] The present study demonstrated that the epicutaneous immunogenicity of the same partial hydrolysates was markedly decreased compared to that of native whey protein. Because the balance of sensitization and tolerance is important, cow's milk protein hydrolysates with reduced epicutaneous immunogenicity and the ability to induce adequate tolerance would be suitable as breast-milk substitutes for infants at risk of food allergy. Further studies focusing on allergy prevention are required to investigate the clinical significance of these partial hydrolysates in nutrition administration to high-risk infants such as subjects with AD.

ACKNOWLEDGEMENTS

The authors thank their laboratory colleagues, M. Ikeda, H. Sonoki, T. Koyama, S. Morita, and E. Karita-Kokubo for helpful assistance in experiments. Histological analysis was technically supported by Sept. Sapie Co., Ltd. The authors thank Editage (www.editage.com) for English language editing.

CONFLICT OF INTEREST

All authors are employed by Morinaga Milk Industry Co., Ltd., and declare no conflict of interest.

ORCID

Hiroshi Iwamoto https://orcid.org/0000-0002-3238-2192
Takeshi Matsubara https://orcid.org/0000-0001-7407-2451

REFERENCES

1. Sicherer SH, Sampson HA. Food allergy: epidemiology, pathogenesis, diagnosis, and treatment. *J Allergy Clin Immunol*. 2014;133:291-307.
2. Ena JM, von Beresteijn ECH, Robben AJPM, Schmidt DG. Whey protein antigenicity reduction by fungal proteinases and a pepsin/pancreatin combination. *J Food Sci*. 1995;60:104-116.
3. Mäkinen-Kiljunen S, Sorva R. Bovine beta-lactoglobulin levels in hydrolysed protein formulas for infant feeding. *Clin Exp Allergy*. 1993;23:287-291.
4. Rosendal A, Barkholt V. Detection of potentially allergenic material in 12 hydrolyzed milk formulas. *J Dairy Sci*. 2000;83:2200-2210.
5. Iwamoto H, Matsubara T, Nakazato Y, Namba K, Takeda Y. Evaluation of the antigenicity of hydrolyzed cow's milk protein formulas using the mouse basophil activation test. *Toxicol Lett*. 2016;242:53-59.
6. Knipping K, van Roest M, Kruijssen L, et al. Intra- and inter-laboratory validation of an innovative huFcepsilonRIalpha-RBL-2H3 degranulation assay for in vitro allergenicity assessment of whey hydrolysates. *Toxicol Vitro*. 2016;33:29-34.
7. Bogh KL, Barkholt V, Madsen CB. Characterization of the immunogenicity and allergenicity of two cow's milk hydrolysates–a study in brown Norway rats. *Scand J Immunol*. 2015;81:274-283.
8. Cordle CT, Mahmoud MI, Moore V. Immunogenicity evaluation of protein hydrolysates for hypoallergenic infant formulae. *J Pediatr Gastroenteral Nutr*. 1991;13:270-276.
9. van Esch B, van Bilsen J, Jeurink PV, et al. Interlaboratory evaluation of a cow's milk allergy mouse model to assess the allergenicity of hydrolysed cow's milk based infant formulas. *Toxicol Lett*. 2013;220:95-102.
10. Lack G. Epidemiologic risks for food allergy. *J Allergy Clin Immunol*. 2008;121:1331-1336.
11. Natsume O, Ohya Y. Recent advancement to prevent the development of allergy and allergic diseases and therapeutic strategy in the perspective of barrier dysfunction. *Allergol Int*. 2018;67:24-31.
12. Matsubara T, Iwamoto H, Nakazato Y, et al. Ingestion of partially hydrolyzed whey protein suppresses epicutaneous sensitization to beta-lactoglobulin in mice. *Pediatr Allergy Immunol*. 2018;29:433-440.
13. Wang LF, Lin JY, Hsieh KH, Lin RH. Epicutaneous exposure of protein antigen induces a predominant Th2-like response with high IgE production in mice. *J Immunol*. 1996;156:4079-4082.
14. Wang G, Savinko T, Wolff H, et al. Repeated epicutaneous exposures to ovalbumin progressively induce atopic dermatitis-like skin lesions in mice. *Clin Exp Allergy*. 2007;37:151-161.
15. Jin H, Oyoshi MK, Le Y, et al. IL-21R is essential for epicutaneous sensitization and allergic skin inflammation in humans and mice. *J Clin Invest*. 2009;119:47-60.
16. Iwamoto H, Matsubara T, Okamoto T, Matsumoto T, Yoshikawa M, Takeda Y. Ingestion of casein hydrolysate induces oral tolerance and suppresses subsequent epicutaneous sensitization and development of anaphylaxis reaction to casein in mice. *Int Arch Allergy Immunol*. 2019;179:221-230.
17. Iwamoto H, Matsubara T, Nakazato Y, Namba K, Takeda Y. Decreased expression of CD200R3 on mouse basophils as a novel marker for IgG1-mediated anaphylaxis. *Immun Inflamm Dis*. 2015;3:280-288.
18. Kubo A, Nagao K, Yokouchi M, Sasaki H, Amagai M. External antigen uptake by Langerhans cells with reorganization of epidermal tight junction barriers. *J Exp Med*. 2009;206:2937-2946.
19. Nakajima S, Igyártó BZ, Honda T, et al. Langerhans cells are critical in epicutaneous sensitization with protein antigen via thymic stromal lymphopoietin receptor signaling. *J Allergy Clin Immunol*. 2012;129:1048-1055.e1046.
20. Kondo H, Ichikawa Y, Imokawa G. Percutaneous sensitization with allergens through barrier-disrupted skin elicits a Th2-dominant cytokine response. *Eur J Immunol*. 1998;28:769-779.
21. Oyoshi MK, Larson RP, Ziegler SF, Geha RS. Mechanical injury polarizes skin dendritic cells to elicite a T_H2 response by inducing cutaneous thymic stromal lymphopoietin expression. *J Allergy Clin Immunol*. 2010;126:976-984.
22. Yoshida K, Kubo A, Fujita H, et al. Distinct behavior of human langerhans cells and inflammatory dendritic epidermal cells at tight junctions in patients with atopic dermatitis. *J Allergy Clin Immunol*. 2014;134:856-864.
23. Matsumoto K, Saito H. Epicutaneous immunity and onset of allergic diseases-pre-"eczema"tous sensitization drives the allergy march. *Allergol Int*. 2013;62:291-296.
24. Muto T, Fukuoka A, Kabashima K, et al. The role of basophils and proallergic cytokines, TSLP and IL-33, in cutaneously sensitized food allergy. *Int Immunol*. 2014;26:539-549.
25. Katz Y, Rajuan N, Goldberg MR, et al. Early exposure to cow's milk protein is protective against IgE-mediated cow's milk protein allergy. *J Allergy Clin Immunol*. 2010;126:77-82.
26. Koplin JJ, Osborne NJ, Wake M, et al. Can early introduction of egg prevent egg allergy in infants? A population-based study. *J Allergy Clin Immunol*. 2010;126:807-813.
27. Du Toit G, Roberts G, Sayre PH, et al. Randomized trial of peanut consumption in infants at risk for peanut allergy. *N Engl J Med*. 2015;372:803-813.

How to cite this article: Iwamoto H, Matsubara T, Okamoto T, et al. Epicutaneous immunogenicity of partially hydrolyzed whey protein evaluated using tape-stripped mouse model. *Pediatr Allergy Immunol*. 2020;00:1–8. https://doi.org/10.1111/pai.13211