



Evaluation of the allergenicity of ω 5-gliadin-deficient Hokushin wheat (1BS-18) in a wheat allergy rat model



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ABSTRACT

We previously developed Hokushin wheat line as a hypoallergenic wheat lacking ω 5-gliadin (1BS-18), a major allergen for wheat-dependent exercise-induced anaphylaxis. However, the allergenicity of 1BS-18 has not been understood completely. In this study, we evaluated the allergenicity of 1BS-18 such as anaphylactic elicitation ability and sensitization ability using rats sensitized with ω 5-gliadin or glutes prepared from Hokushin (Hokushin gluten) or 1BS-18 (1BS-18 gluten). Rats were sensitized by intraperitoneal administration of ω 5-gliadin, Hokushin gluten or 1BS-18 gluten. Immunoglobulin E-mediated systemic anaphylaxis was evaluated by measuring changes in rectal temperature for 30 min after intravenous challenge with ω 5-gliadin or the test glutes in unsensitized rats or rats sensitized with ω 5-gliadin or the test glutes. In ω 5-gliadin-sensitized rats, intravenous challenge with ω 5-gliadin or Hokushin gluten significantly decreased the rectal temperature at 30 min after challenge while challenge with 1BS-18 gluten did not reduce the rectal temperature. Furthermore, intravenous challenge with ω 5-gliadin significantly decreased the rectal temperature in rats sensitized with Hokushin gluten or 1BS-18 gluten. However, the reduced degree observed in 1BS-18 gluten-sensitized rats was smaller than that in Hokushin gluten-sensitized rats. In conclusion, 1BS-18 elicited no allergic reaction in ω 5-gliadin-sensitized rats and had less sensitization ability for ω 5-gliadin than that of Hokushin wheat.

1. Introduction

Wheat is widely cultivated as a staple food around the world. However, many patients suffer from allergy to wheat [1]. There are several clinical types of wheat allergy, including immediate-type wheat allergy, wheat contact dermatitis, bakers' asthma, and wheat-dependent exercise-induced anaphylaxis (WDEIA) [2]. WDEIA is a specific type of wheat allergy that is induced by exercise after the ingestion of wheat. Patients with WDEIA often elicit skin manifestations such as generalized urticaria, dyspnea, and life-threatening anaphylactic shock induced by immunoglobulin (Ig)E-mediated mast cell degranulation [3–5]. Nevertheless, a curative treatment for WDEIA has not been established. The strict elimination of wheat from dietary foods is one of the most reliable types of prophylactic treatments for WDEIA. However, the strict elimination of wheat may lead to a decline in the quality of

life (QOL) for WDEIA patients because wheat is contained in various processed foods [6].

Wheat flour contains 8–12% proteins which is classified as water/salt-soluble proteins (albumin and globulin) and water/salt-insoluble proteins (gluten) [7]. Gluten is composed of more than two proteins such as those of the gliadin and glutenin families. Gliadin family proteins are genetically classified into α/β -, γ -, ω 1,2- and ω 5-gliadins, and the glutenin family proteins are divided into low molecular weight- and high molecular weight-glutenins [7]. Our previous reports have demonstrated that ω 5-gliadin is a major causative allergen for various types of wheat allergy including WDEIA [2,8–10].

Recently, many hypoallergenic foods have been developed to improve the QOL of patients with food allergies [11]. For example, Dodo et al. produced a transgenic peanut with reduced contents of a peanut allergen, Ara h 2 using RNAi technology [12]. Takahashi et al. [13]

Abbreviations: 1BS-18, hypoallergenic wheat lacking ω 5-gliadin; WDEIA, wheat-dependent exercise-induced anaphylaxis; Ig, immunoglobulin; QOL, quality of life; BN, Brown-Norway; OVA, ovalbumin; Ab, antibody; ELISA, enzyme linked immunosorbent assay; PBS, phosphate-buffered saline; PBS-T, phosphate-buffered saline containing 0.1% Tween 20; HRP, horseradish peroxidase

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developed hypoallergenic soybean lacking two major allergens, Gly m Bd 28K and 60K by irradiation method with gamma-ray. We previously developed a hypoallergenic wheat (1BS-18) lacking ω 5-gliadin from Hokushin wheat [14]. In 1BS-18 gluten, contents of ω 5-gliadin were ~20% of that in Hokushin (1BS-18, 1.21 ± 0.10 mg/g gluten; Hokushin, 5.17 ± 0.51 mg/g gluten) [15]. However, there were no information regarding the anaphylactic elicitation ability of 1BS-18. In addition, we showed that allergic scores of guinea pigs sensitized with 1BS-18 were slightly lower than those of guinea pigs sensitized with regular wheat in gluten challenge tests [14]. However, the sensitization ability of 1BS-18 could be clarified incompletely because these guinea pigs were orally immunized with each gluten without adjuvant. In this study, we evaluated the allergenicity of 1BS-18 such as its anaphylactic elicitation ability and sensitization ability using rats sensitized with ω 5-gliadin, gluten from Hokushin or gluten from 1BS-18.

2. Materials and methods

2.1. Preparation of gluten from wheat cultivars

Gluten was prepared from Hokushin wheat (Koshoku, Tokyo, Japan) or 1BS-18 wheat produced by Kohno et al. [14] as previously reported [15]. The gluten was ground into powder in a mortar and stored at -30°C until use.

2.2. Animals

Male Brown-Norway (BN) rats (3 weeks old) were obtained from Japan SLC (Shizuoka, Japan). Rats were fed a standard laboratory diet (MF; Oriental Yeast Company, Tokyo, Japan) and water *ad libitum* for more than 1 week before the experiments. All experiments involving animals were carried out in accordance with the Guide for Animal Experimentation of the Committee of Research Facilities for Laboratory Animal Sciences of Hiroshima University (Hiroshima, Japan). This study was approved by the animal ethics committee of Hiroshima University (approval No. A-16-44-3).

2.3. Sensitization protocol

To confirm whether our food-allergic rat model was suitable to evaluate the anaphylactic reaction, rats were sensitized by intraperitoneal injection with 1 ml of physiologic saline (0.9% NaCl) containing 1 mg/ml of typical food allergen ovalbumin (OVA) (Sigma-Aldrich, St Louis, MO, USA) and Imject®Alum [10 mg/ml of $\text{Al}(\text{OH})_3$ and 10 mg/ml of $\text{Mg}(\text{OH})_2$] (Thermo Fisher Scientific, Waltham, MA, USA) at weekly intervals for 4 weeks. Four weeks after the first immunization, blood (0.2 ml) was collected from the jugular vein to check the plasma levels of IgE antibodies (Abs) specific to OVA using an enzyme linked immunosorbent assay (ELISA). Rats with low levels of IgE Abs specific to OVA were given an injection of OVA every week for an additional 2 or 4 weeks and IgE levels were checked again. At 4, 6 or 8 weeks, rats sensitized with OVA were divided randomly into two groups for studies on anaphylaxis. Unsensitized rats were intraperitoneally injected with physiologic saline containing adjuvant alone at weekly intervals for 4 weeks.

To evaluate the ability of anaphylactic elicitation of 1BS-18, rats were sensitized by intraperitoneal injection with 1 ml of physiologic saline (0.9%) containing 5 mmol/l acetic acid, 1 mg/ml of native ω 5-gliadin from Hokushin and Imject®Alum at weekly intervals for 4 weeks in the same protocol as OVA. At 4, 6 or 8 weeks after the first immunization, rats with IgE Abs specific to ω 5-gliadin were divided randomly into five groups for studies on anaphylaxis. Unsensitized rats were intraperitoneally injected with physiologic saline (0.9%) containing 5 mmol/l acetic acid and adjuvant alone at weekly intervals for 4 weeks.

To evaluate the ability of sensitization to ω 5-gliadin of 1BS-18, rats

were sensitized by intraperitoneal injection with 1 ml of 5 mmol/l acetic acid containing 1 mg of Hokushin gluten or 1BS-18 gluten and Imject®Alum at weekly intervals for 13 weeks. Thirteen weeks after the first immunization, blood (0.2 ml) was collected from the jugular vein to check the plasma levels of IgE Abs specific to ω 5-gliadin using an ELISA. Unsensitized rats were intraperitoneally injected with physiologic saline (0.9%) containing 5 mmol/l acetic acid and adjuvant alone at weekly intervals for 13 weeks.

2.4. Measurement of plasma levels of IgE Abs specific to OVA or ω 5-gliadin

To confirm the sensitization to OVA or ω 5-gliadin, the plasma levels of IgE Abs specific to OVA or ω 5-gliadin were determined using ELISA. Briefly, the wells of the F8 MaxiSorp Loose Nunc-Immuno™ Modules (Thermo Fisher Scientific) were coated with 100 μ l of OVA (10 μ g/ml) dissolved in phosphate buffered saline (PBS) or ω 5-gliadin (20 μ g/ml) dissolved in 0.1% acetic acid overnight at 4°C . After washing with phosphate buffered saline containing 0.1% Tween 20 (PBS-T) six times, plates were incubated with 1% Block Ace® (DS Pharma Biomedical Osaka, Japan) for 2 h at room temperature. Then, 100 μ l of each sample of rat plasma (diluted 1:10 in 1% Block Ace®) was added to each well and incubated for 2 h at room temperature. After washing with PBS-T, the wells were incubated with 100 μ l of horseradish peroxidase (HRP)-conjugated mouse anti-rat IgE Ab (GeneTex, Irvine, CA, USA) (diluted 1:1000 in PBS) for 2 h at room temperature. The wells were washed with PBS-T and then incubated with 100 μ l of 3,3',5,5'-tetramethylbenzidine solution (KPL, Gaithersburg, MD, USA) at room temperature. After 15 min incubation, the reaction was terminated with 100 μ l of 1 mol/l phosphoric acid. Absorbance was measured at 450 nm against 630 nm as a reference using a Multiskan GO (Thermo Fisher Scientific).

2.5. Evaluation of systemic anaphylaxis

Systemic anaphylaxis was evaluated by measuring changes in rectal temperature for 30 min after intravenous challenge with OVA, ω 5-gliadin, Hokushin gluten or 1BS-18 gluten in unsensitized rats or those sensitized with OVA, ω 5-gliadin, Hokushin gluten or 1BS-18 gluten. In this study, OVA, ω 5-gliadin, Hokushin gluten or 1BS-18 gluten were intravenously administered because we can evaluate their allergenicities while excluding processes of digestion and absorption. Rectal temperature was measured using a specific rectal thermometer for rats (Shibaura Electronics, Saitama, Japan) before and 30 min after intravenous challenge according to a previous report [16]. After measuring the rectal temperature at 0 min, OVA, ω 5-gliadin, Hokushin gluten or 1BS-18 gluten (10 mg/kg) were intravenously injected via a canula inserted in the femoral vein. Then, the rectal temperature was monitored every 5 or 10 min for 30 min.

2.6. Statistical analyses

Data are reported as the mean \pm SEM. Differences in mean values between groups were assessed using Kruskal-Wallis test, followed by a post hoc Tukey test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Evaluation of the anaphylactic reaction in OVA-sensitized rats

To confirm whether our food-allergic rat model was suitable to evaluate the anaphylactic reaction, we measured the rectal temperature for 30 min after intravenous challenge with OVA in OVA-sensitized or unsensitized rats. When OVA was intraperitoneally administered to rats at weekly intervals for 4–8 weeks, plasma levels of OVA-specific IgE Abs were increased higher (ELISA absorbance value; 1.00 ± 0.14 for vehicle group and 1.35 ± 0.14 for OVA group) compared to those in

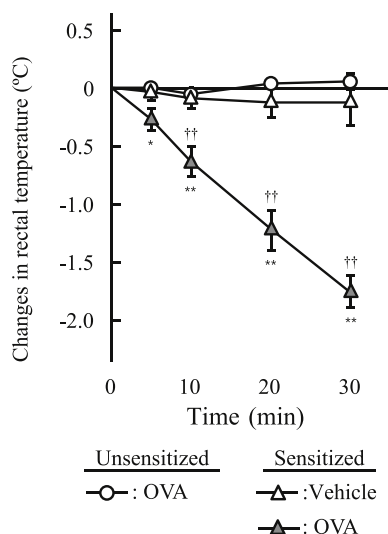


Fig. 1. Changes in rectal temperature after intravenous administration of ovalbumin (OVA) in unsensitized or OVA-sensitized rats. Vehicle alone (1 ml/kg) or OVA (10 mg/kg) was challenged intravenously. Each value represents the mean \pm SEM for five rats. * $P < 0.05$, ** $P < 0.01$: significantly different from unsensitized rats. †† $P < 0.01$: significantly different from vehicle-challenged rats sensitized with OVA.

unsensitized rats (0.01 ± 0.00). When OVA was challenged intravenously in unsensitized rats, their rectal temperatures were not changed significantly. In OVA-sensitized rats, intravenous challenge with OVA significantly reduced the temperature at 30 min after challenge while vehicle alone did not alter the rectal temperature (Fig. 1). Thus, we could confirm that this food-allergic rat model was suitable to evaluate the anaphylactic reaction.

3.2. Evaluation of the anaphylactic elicitation ability of 1BS-18

To evaluate the anaphylactic elicitation ability of 1BS-18, we measured the rectal temperature for 30 min after intravenous challenge with the individual test glutes in ω 5-gliadin-sensitized or unsensitized rats. Before the first immunization with ω 5-gliadin, IgE Abs specific to ω 5-gliadin were not detected in the plasma of all rats. At 4–8 weeks after immunization, the plasma levels of ω 5-gliadin-specific IgE Abs (ELISA absorbance value; 0.09 ± 0.02) were increased in ω 5-gliadin-sensitized rats while their levels were not altered in unsensitized rats (0.01 ± 0.01). When unsensitized rats were challenged intravenously with ω 5-gliadin (10 mg/kg), there were no significant changes in rectal temperature. In ω 5-gliadin-sensitized rats, intravenous challenge with ω 5-gliadin significantly reduced the temperature at 30 min after challenge while vehicle alone did not alter the rectal temperature (Fig. 2A). When ω 5-gliadin-sensitized rats were challenged intravenously with Hokushin gluten, the rectal temperature was significantly decreased to the same levels as those measured in rats challenged with ω 5-gliadin (Fig. 2B). By contrast, challenge with 1BS-18 gluten did not reduce the rectal temperature in ω 5-gliadin-sensitized rats. These results indicate that the anaphylactic elicitation ability of 1BS-18 gluten is much lower than that of Hokushin gluten in rats sensitized with ω 5-gliadin.

3.3. Evaluation of sensitization ability of 1BS-18

We further examined the sensitization ability of 1BS-18. To confirm the sensitization ability of 1BS-18, rats were immunized with Hokushin or 1BS-18 gluten. Systemic anaphylaxis was evaluated by the same challenge method with ω 5-gliadin as described above in unsensitized and gluten-sensitized rats. When rats were immunized by intraperitoneal injection with Hokushin or 1BS-18 gluten at weekly

intervals for 13 weeks, the plasma levels of ω 5-gliadin-specific IgE Abs slightly increased compared with those in unsensitized rats though there were no statistical differences (unsensitized: 0.01 ± 0.01 , Hokushin gluten: 0.02 ± 0.00 , 1BS-18 gluten: 0.02 ± 0.01). In Hokushin gluten-sensitized rats, intravenous challenge with ω 5-gliadin reduced the rectal temperature at 30 min. Intravenous challenge with ω 5-gliadin also reduced the rectal temperature in 1BS-18 gluten-sensitized rats but the degree of reduction was smaller than that in Hokushin gluten-sensitized rats (Fig. 3). These results indicate that the sensitization ability toward ω 5-gliadin of 1BS-18 gluten can be lower than that of Hokushin gluten.

4. Discussion

The strict elimination of wheat from dietary foods is one of the most reliable types of prophylactic treatments for WDEIA. However, the strict elimination of wheat may lead to a decline in the QOL for WDEIA patients because wheat is contained in various processed foods [6]. Previous reports have shown that hypoallergenic foods improve the QOL of patients with food allergies [11]. Kohno et al. [14] developed a 1BS-18 which lacks ω 5-gliadin from Hokushin wheat. In this study, we evaluated the allergenicity of 1BS-18 such as its anaphylactic elicitation ability and sensitization ability using rats sensitized by ω 5-gliadin or gluten from Hokushin or 1BS-18.

At first, we measured rectal temperature after intravenous OVA challenge to sensitized rats. As shown in Fig. 1, rectal temperature decreased by ~ 1.5 – 2.0 °C after intravenous injection of OVA in OVA-sensitized rats whereas there were no changes in unsensitized rats and in OVA-sensitized rats challenged with vehicle. We further measured rectal temperature after intravenous challenge with ω 5-gliadin or individual test glutes in ω 5-gliadin sensitized rats. In ω 5-gliadin-sensitized rats, intravenous challenge with ω 5-gliadin decreased the rectal temperature by ~ 0.5 °C significantly, but this change had not observed in unsensitized rats (Fig. 2A). Thus, we confirmed that this ω 5-gliadin-sensitized rat model is suitable to evaluate the anaphylactic elicitation ability of ω 5-gliadin as well as OVA-sensitized rat model though the severity of anaphylactic reaction is milder than OVA. Next, we evaluated anaphylactic elicitation ability of Hokushin and 1BS-18 using this model. By intravenous challenge with Hokushin gluten, the rectal temperature of ω 5-gliadin-sensitized rats was decreased significantly (Fig. 2B). On the other hands, challenge with 1BS-18 did not reduce the rectal temperature in ω 5-gliadin-sensitized rats. Although we could not evaluate the effects of higher-dose gluten on the rectal temperature in this study due to poor water-solubility of gluten (gliadins), these results suggest that the anaphylactic elicitation ability of 1BS-18 gluten is much lower than that of Hokushin gluten in rats sensitized with ω 5-gliadin.

Next, we evaluated sensitization ability of 1BS-18. In Hokushin gluten sensitized-rats, intravenous challenge with ω 5-gliadin decreased the rectal temperature significantly (Fig. 3). However, intravenous challenge with ω 5-gliadin decreased the rectal temperature weakly in 1BS-18 gluten-sensitized rats than that of in Hokushin gluten sensitized rats. These results indicated that 1BS-18 could have lower sensitization ability toward ω 5-gliadin than Hokushin. Our previous reports showed that content of ω 5-gliadin was 1.21 ± 0.10 mg/g in 1BS-18 gluten [15]. However, we confirmed 1BS-18 gluten contained no ω 5-gliadin by preliminary immunoblot assay. Thus, the slight detection in 1BS-18 gluten may be due to the cross-reactivity of anti- ω 5-gliadin Abs to the other gliadin components in ELISA. Although 1BS-18 gluten contains none or much small amounts of ω 5-gliadin, challenge with ω 5-gliadin induced the anaphylactic reaction in 1BS-18 gluten-sensitized rats. This result may be explained by cross-reaction of IgE Abs specific to α / β -gliadin, γ -gliadin, or ω 1,2-gliadin produced in 1BS-18 gluten-sensitized rats to ω 5-gliadin. This potential cross-reactivity is supported by experiments where IgE Abs from patients with wheat allergy strongly bound to the QX₁PX₂QQ consensus motif found in ω 5-gliadin and the

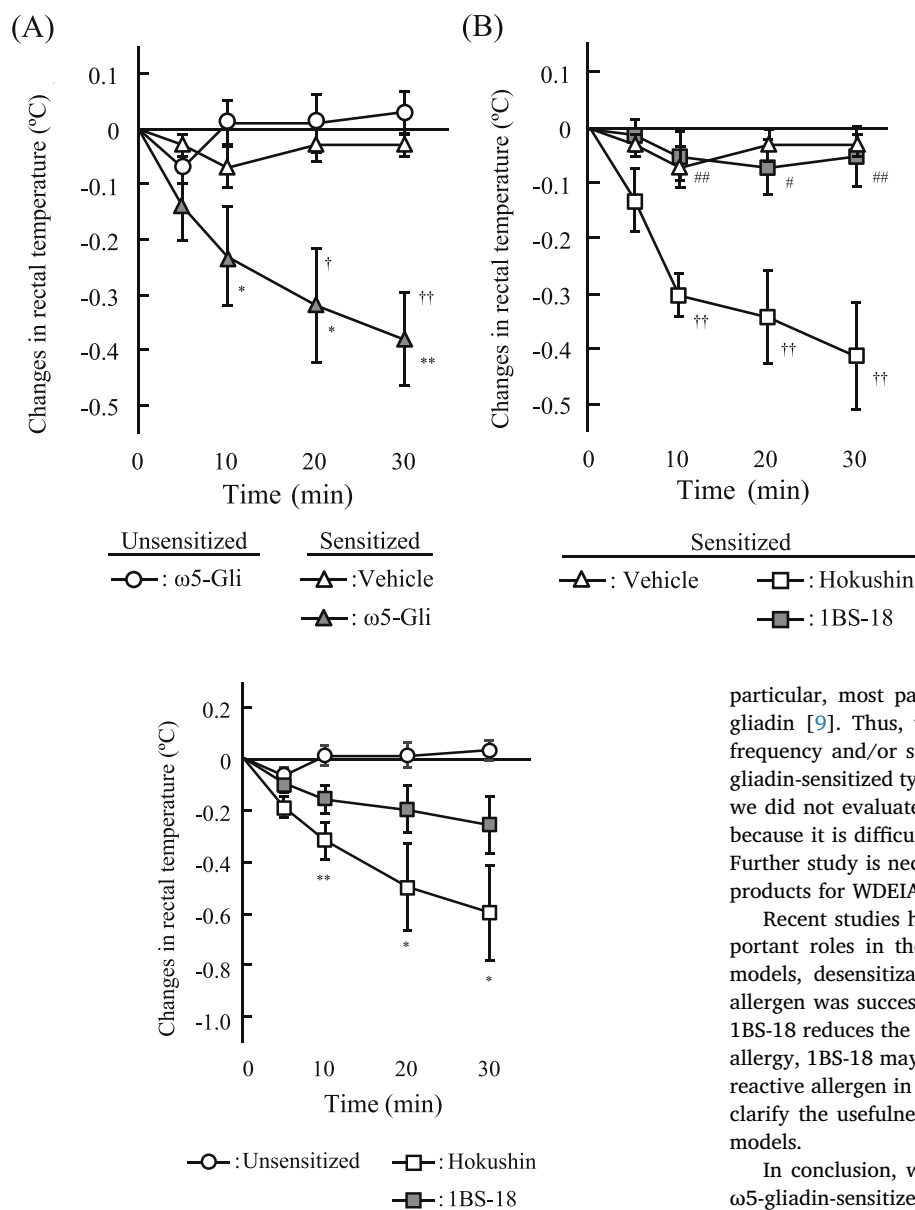


Fig. 2. Changes in rectal temperature after intravenous administration of ω5-gliadin (A) or gluten from Hokushin wheat or 1BS-18 wheat (B) in unsensitized or ω5-gliadin-sensitized rats. Vehicle alone (1 ml/kg), ω5-gliadin (10 mg/kg), Hokushin gluten (10 mg/kg) or 1BS-18 gluten (10 mg/kg) was challenged intravenously. Each value represents the mean ± SEM for five rats. *P < 0.05, **P < 0.01: significantly different from unsensitized rats. †P < 0.05, ††P < 0.01: significantly different from vehicle-challenged rats sensitized with ω5-gliadin. #P < 0.05, ##P < 0.01: significantly different from Hokushin gluten-challenged rats sensitized with ω5-gliadin. ω5-Gli, ω5-gliadin; Hokushin, Hokushin wheat; 1BS-18, ω5-gliadin-deficient Hokushin wheat.

Fig. 3. Changes in rectal temperature after intravenous administration of ω5-gliadin in unsensitized rats or rats sensitized with gluten from Hokushin or 1BS-18 wheat. ω5-Gliadin (10 mg/kg) was challenged intravenously to unsensitized rats or rats sensitized with gluten from Hokushin or 1BS-18 wheat. Each value represents the mean ± SEM for five rats. *P < 0.05, **P < 0.01: significantly different from unsensitized rats. Hokushin, Hokushin wheat; 1BS-18, ω5-gliadin-deficient Hokushin wheat.

other gliadins (where X₁ being either L, F, S or I and X₂ being either Q, E or G) [17].

In this study, we did not examine the effect of challenge with 1BS-18 gluten on changes in rectal temperature in Hokushin gluten-sensitized rats. We have reported that the amount of total gliadins including γ-gliadin mainly in 1BS-18 gluten was almost the same as those in Hokushin gluten [15]. We speculate that IgE Abs specific to various gliadins and glutenins as well as ω5-gliadin in Hokushin gluten were produced when rats were immunized with Hokushin gluten. Thus, challenge with 1BS-18 gluten may elicit the anaphylaxis in Hokushin gluten-sensitized rats. However, our results showed that the anaphylactic elicitation ability of 1BS-18 gluten is lower than that of Hokushin gluten in rats sensitized with ω5-gliadin. The major causative allergens for wheat allergy are reportedly different among their clinical types. In

particular, most patients with WDEIA have IgE Abs binding to ω5-gliadin [9]. Thus, we speculate that 1BS-18 gluten may reduce the frequency and/or severity of anaphylaxis among patients with a ω5-gliadin-sensitized type of wheat allergy including WDEIA. In this study, we did not evaluate the anaphylactic reactions using a WDEIA model because it is difficult to measure the rectal temperature after exercise. Further study is necessary to clarify the effectiveness of 1BS-18 wheat products for WDEIA patients.

Recent studies have shown that appropriate oral exposure has important roles in the establishment of oral tolerance [18]. In mouse models, desensitization therapy using a hypoallergenic recombinant allergen was successful in the treatment of food allergy [19]. Because 1BS-18 reduces the allergenicity toward a ω5-gliadin-sensitized type of allergy, 1BS-18 may be useful to safely acquire oral tolerance by cross-reactive allergen in patients with WDEIA. Further study is necessary to clarify the usefulness of 1BS-18 for desensitization therapy in animal models.

In conclusion, we clarified that 1BS-18 elicited no anaphylaxis in ω5-gliadin-sensitized rats. These results suggest that 1BS-18 is useful as an alternative type of wheat for patients with a ω5-gliadin-sensitized type of wheat allergy such as WDEIA. Additionally, 1BS-18 has less sensitization ability for ω5-gliadin than that of Hokushin wheat. Thus, 1BS-18 may be useful as a prophylactic food instead of normal wheat to prevent the development of WDEIA. In addition, 1BS-18 can be applied to oral immunotherapy without side effects such as an anaphylactic reaction.

Declaration of competing interest

The authors have no conflict of interest to declare.

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Transparency document

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Appendix A. Supplementary data

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References

- [1] J. Savage, C.B. Johns, Food allergy: epidemiology and natural history, *Immunol. Allergy Clin. N. Am.* 35 (2015) 45–59.
- [2] H. Matsuo, T. Yokooji, T. Taogoshi, Common food allergens and their IgE-binding epitopes, *Allergol. Int.* 64 (2015) 332–343.
- [3] W. Barg, W. Medrala, A. Wolanczyk-Medrala, Exercise-induced anaphylaxis: an update on diagnosis and treatment, *Curr. Allergy Asthma Rep.* 11 (2011) 45–51.
- [4] M.C. Berin, H.A. Sampson, Food allergy: an enigmatic epidemic, *Trends Immunol.* 34 (2013) 390–397.
- [5] E. Morita, K. Kunie, H. Matsuo, Food-dependent exercise-induced anaphylaxis, *J. Dermatol. Sci.* 47 (2007) 109–117.
- [6] A.J. Cummings, R.C. Knibb, R.M. King, J.S. Lucas, The psychosocial impact of food allergy and food hypersensitivity in children, adolescents and their families: a review, *Allergy* 65 (2010) 933–945.
- [7] H. Wieser, Chemistry of gluten proteins, *Food Microbiol.* 24 (2007) 115–119.
- [8] H. Matsuo, K. Kohno, E. Morita, Molecular cloning, recombinant expression and IgE-binding epitope of ω -5 gliadin, a major allergen in wheat-dependent exercise-induced anaphylaxis, *FEBS J.* 272 (2005) 4431–4438.
- [9] T. Yokooji, S. Kurihara, T. Murakami, Y. Chinuki, H. Takahashi, E. Morita, S. Harada, K. Ishii, M. Hiragun, M. Hide, H. Matsuo, Author information, Characterization of causative allergens for wheat-dependent exercise-induced anaphylaxis sensitized with hydrolyzed wheat proteins in facial soap, *Allergol. Int.* 62 (2013) 435–445.
- [10] H. Matsuo, E. Morita, A.S. Tatham, K. Morimoto, T. Horikawa, H. Osuna, Z. Ikezawa, S. Kaneko, K. Kohno, S. Dekio, Identification of the IgE-binding epitope in omega-5 gliadin, a major allergen in wheat-dependent exercise-induced anaphylaxis, *J. Biol. Chem.* 279 (2004) 12135–12140.
- [11] M.C. van Putten, L.J. Frewer, L.J.W.J. Gilissen, B. Gremmen, A.A.C.M. Peijnenburg, H.J. Wichers, Novel foods and food allergies: a review of the issues, *Trends Food Sci. Technol.* 17 (2006) 289–299.
- [12] H.W. Dodo, K.N. Konan, F.C. Chen, M. Egnin, O.M. Viquez, Alleviating peanut allergy using genetic engineering: the silencing of the immunodominant allergen Ara h 2 leads to its significant reduction and a decrease in peanut allergenicity, *Plant Biotechnol. J.* 6 (2008) 135–145.
- [13] K. Takahashi, H. Banba, A. Kikuchi, Miwako Ito, Shigeki Nakamura, An induced mutant line lacking the alpha subunit of beta-conglycinin in soybean (*Glycine max* (L.) Merrill), *Breed. Sci.* 44 (1994) 65–66.
- [14] K. Kohno, H. Takahashi, T.R. Endo, H. Matsuo, K. Shiwaku, E. Morita, Characterization of a hypoallergenic wheat line lacking ω -5 gliadin, *Allergol. Int.* 65 (2016) 400–405.
- [15] T. Yokooji, H. Nouma, R. Ogino, T. Taogoshi, E. Morita, H. Matsuo, Quantification of the ω 5- and γ -gliadin content in wheat flour and rat plasma with an enzyme-linked immunosorbent assay using antibodies specific to their IgE-binding epitopes, *Allergol. Int.* 68 (2019) 112–113.
- [16] T. Yokooji, H. Matsuo, Sodium cromoglycate prevents exacerbation of IgE-mediated food-allergic reaction induced by aspirin in a rat model of egg allergy, *Int. Arch. Allergy Immunol.* 167 (2015) 193–202.
- [17] F. Battais, T. Mothes, D.A. Moneret-Vautrin, F. Pineau, G. Kanny, Y. Popineau, M. Bodinier, S. Denery-Papini, Identification of IgE-binding epitopes on gliadins for patients with food allergy to wheat, *Allergy* 60 (2005) 815–821.
- [18] G. Lack, Epidemiologic risks for food allergy, *J. Allergy Clin. Immunol.* 121 (2008) 1331–1336.
- [19] X.M. Li, K. Srivastava, J.W. Huleatt, K. Bottomly, A.W. Burks, H.A. Sampson, Engineered recombinant peanut protein and heat-killed *Listeria monocytogenes* coadministration protects against peanut-induced anaphylaxis in a murine model, *J. Immunol.* 170 (2003) 3289–3295.