Allergology International 65 (2016) 400–405

Contents lists available at ScienceDirect

Allergology International

journal homepage: http://www.elsevier.com/locate/alit

Original article

Characterization of a hypoallergenic wheat line lacking ω-5 gliadin

Kunie Kohno [a, b, *], Hitoshi Takahashi [a], Takashi R. Endo [c], Hiroaki Matsuo [d], Kuninori Shiwaku [b, e], Eishin Morita [a]

[a] Department of Dermatology, Shimane University Faculty of Medicine, Shimane, Japan
[b] Center for Community-Based Health Research and Education (COHRE), Organization for the Promotion of Project Research, Shimane University, Shimane, Japan
[c] Department of Plant Life Science, Faculty of Agriculture, Ryukoku University, Shiga, Japan
[d] Department of Pathophysiology and Therapeutics, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan
[e] Shimane University Faculty of Medicine, Shimane, Japan

ARTICLE INFO

Article history:
Received 8 December 2015
Received in revised form
8 March 2016
Accepted 10 March 2016
Available online 18 April 2016

Keywords:
Guinea pig
Hypoallergenic wheat
Wheat allergy
ω-5 gliadin
1BS-18

Abbreviations:
CBB, coomassie brilliant blue;
HRP, horseradish peroxidase;
IP, intraperitoneal; RP-HPLC, reverse phase-high performance liquid chromatography;
SDS-PAGE, sodium dodecyl sulphate −polyacrylamide electrophoresis;
WDEIA, wheat-dependent exercise-induced anaphylaxis

ABSTRACT

Background: There is no curative treatment for wheat-dependent exercise-induced anaphylaxis (WDEIA). ω-5 Gliadin is one of the dominant allergens affecting WDEIA patients. The use of ω-5 gliadin-free wheat flour in the regular diet is considered one of the prophylactic approaches against the elicitation of allergic symptoms and sensitization to ω-5 gliadin. We sought to find hypoallergenic bread wheat (or common wheat) that lacked the genes encoding ω-5 gliadin and to evaluate its *in vitro* allergenicity. We also aimed to evaluate the sensitization ability of one of the selected hypoallergenic wheat lines by using a possible animal model of wheat allergy.
Methods: We screened the deletion lines of bread wheat by western blotting to ascertain common wheat lines lacking the ω-5 gliadin locus. The deletion lines we used have partial deficiency of chromosome 1B (Endo and Gill, 1996). To assess sensitization ability of gluten from the selected deletion line, guinea pigs were fed with either the gluten from the selected deletion line or commercially available gluten, and allergic score was evaluated after challenging the same gluten preparations.
Results: We found that a deletion line 1BS-18 had the least deficiency of chromosome 1B among the deletion stocks lacking the ω-5 gliadin locus. The challenge test using the guinea pigs revealed that the symptoms induced by application of the 1BS-18 gluten were much less than that of commercially available gluten.
Conclusions: The deletion line 1BS-18, which lacked the ω-5 gliadin locus, is likely to have a low sensitization capacity in the guinea pig. The use of the wheat products of the 1BS-18 line in daily life may provide a feasible solution for the onset of wheat allergy.

Introduction

Wheat-dependent exercise-induced anaphylaxis (WDEIA) is a distinct form of wheat allergy typically induced by exercise after the ingestion of wheat products.[1] The estimated prevalence of wheat allergy in European residents is 0.1–3.9% in a meta-analysis, and, is 0.21% in Japanese adults in a cross-sectional study in rural mountainous area in Japan.[2,3] There is no established treatment for WDEIA, and therefore, patients have no choice but to limit their intake of wheat products. Because wheat is one of the staple foods in the human diet, this limitation significantly lowers the quality of life of WDEIA patients. The risk of elicitation of allergic symptoms can be reduced by the limitation of exercise after ingestion of wheat, although this is not always reliable.

Prior to the induction of allergic symptoms, sensitization of wheat allergens is established in some way. Although the mechanism has not been fully clarified, the intestinal transmission capability of wheat allergens[4] and increased serum allergen level following exercise and/or aspirin intake are considered to elicit symptoms in patients with WDEIA.[5–7] Thus, lowering of the oral sensitization ability of wheat products may facilitate an effective prevention measure against the onset of WDEIA.

Wheat ω-5 gliadin is one of the major allergens in WDEIA[1,8–12]; therefore, wheat cultivars with reduced ω-5 gliadin content can be considered hypoallergenic wheat. Denery-Papini *et al.*[13] and Laurière

* Corresponding author. Department of Dermatology, Shimane University Faculty of Medicine, 89-1 Enya-cho, Izumo, Shimane 693-8501, Japan.
E-mail address: nine_kk@med.shimane-u.ac.jp (K. Kohno).
Peer review under responsibility of Japanese Society of Allergology.

http://dx.doi.org/10.1016/j.alit.2016.03.002

et al.[14] evaluated the ω-gliadin content in wheat cultivars widely consumed in Europe. Altenbach *et al.*[15] reported a genetically modified wheat in which the ω-5 gliadin genes were silenced by a siRNA technique. However, those studies were scant in practical *in vivo* research. Furthermore, while gluten-free bread has been made with glutathione-added rice flour,[16] such bread completely lacks the characteristic flavour and taste of bread made with regular wheat flour. In the present study, we selected a hypoallergenic wheat line that lacks the ω-5 gliadin protein and evaluated its sensitization ability by using a possible animal model of wheat allergy.

Methods

Selection of wheat strains

The ω-5 gliadin gene is located on the short arm of chromosome 1B in wheat.[17] Therefore, we searched for candidate wheat lines among the deletion stocks of common wheat on the website of the National Bioresource Project (NBRP)-WHEAT (www.nbrp.jp/reportProject.jsp?project=wheat) and obtained candidate deletion lines and other lines from NRBP-Wheat (Table 1). These lines were established in a cultivar of common wheat (*Triticum aestivum* L. 2n = 6x = 42; genome formula, AABBDD), namely Chinese Spring. First, to reconfirm the chromosomal location of the gene encoding ω-5 gliadin, we analysed the group-1 nullisomic–tetrasomic series of Chinese Spring wheat. These lines lack a pair of chromosomes (e.g. 1A) and carry 4 doses of either of the homoeologous chromosomes (e.g. 1B or 1D) to compensate the loss of the critical chromosome. Then, we evaluated the *in vitro* allergenicity of the flour from chromosome 1B deletion stocks having partial deficiencies in the long arm, short arm, and satellite of the chromosome.

Table 1
Wheat lines of Chinese Spring wheat obtained from National Bioresource Project (NBRP)-wheat.

Line	NBRP ID	Abbreviation in this study	Characteristics
Euploid	LPGKU2269	CS	Pure line
Nullisomic-1A Tetrasomic-1B	LPGKU0043	NT1A1B	No 1A chromosome, four 1B chromosomes
Nullisomic-1A Tetrasomic-1D	LPGKU0044	NT1A1D	No 1A chromosome, four 1D chromosomes
Nullisomic-1B Tetrasomic-1A	LPGKU0045	NT1B1A	No 1B chromosome, four 1A chromosomes
Nullisomic-1B Tetrasomic-1D	LPGKU0046	NT1B1D	No 1B chromosome, four 1D chromosomes
Nullisomic-1D Tetrasomic-1A	LPGKU0047	NT1D1A	No 1D chromosome, four 1A chromosomes
Nullisomic-1D Tetrasomic-1B	LPGKU0048	NT1D1B	No 1D chromosome, four 1B chromosomes
Deletion 1BL-01	LPGKU1010	1BL-01	Deletion in the 1B long arm (FL = 0.47)[†]
Deletion 1BS-01	LPGKU1027	1BS-01	Deletion in the 1B short arm (FL = 0.35)
Deletion 1BS-04	LPGKU1030	1BS-04	Deletion in the 1B satellite (FL = Sat, 0.52)
Deletion 1BS-18	LPGKU1039	1BS-18	Deletion in the 1B satellite (FL = Sat, 0.50)
Deletion 1BS-19	LPGKU1040	1BS-19	Deletion in the 1B satellite (FL = Sat, 0.31)
Deletion 1BS-22	LPGKU1043	1BS-22	Deletion in the 1B satellite (FL = Sat, 0.24)
Deletion 1BS-23	LPGKU1044	1BS-23	Deletion in the 1B satellite (FL = Sat, 0.30)
Deletion 1BS-24	LPGKU1045	1BS-24	Deletion in the 1B satellite (FL = ?)

[†] FL stands for the fraction length retained in the long, short or satellite (Sat) of chromosome 1B; e.g. FL = 0.47 for 1BL-01 indicates that this line has a pair of deficient 1B chromosomes retaining 47% of the long.

Production of polyclonal IgG against ω-5 gliadin epitope peptides

We synthesized the peptide KQQSPEQQQFPQQQIPQQQ, including 3 IgE-binding epitope sequences of ω-5 gliadin—QQIPQQQ, QQFPQQQ, and QQSPEQQ—and conjugated it with keyhole limpet haemocyanin (KLH).[11] We injected the KLH-conjugated peptide mixed with adjuvant subcutaneously into a New Zealand white rabbit 3 times at 2-week intervals. We confirmed the production of the IgG antibodies against the ω-5 gliadin-epitope peptide by enzyme-linked immunosorbent assay and then purified the IgG antibodies (anti-ω-5 gliadin) using a peptide affinity column. We confirmed the binding of ω-5 gliadin to each of the epitope sequences with synthetic peptide arrays (data not shown). In addition to the specific binding with ω-5 gliadin, we observed several non-specific bands (see Fig. 1a, bottom, lane CS).

Extraction of gliadins

We mashed 4 grains (56–160 mg) of each of the wheat lines into flour using TissueLyser (Qiagen, Venlo, Netherlands) for 30–40 min. Then, we added 1 mL of 50% 1-propanol (Wako Pure Chemical Industries, Osaka, Japan) to the flour and stirred the mixture for 20 min at room temperature. After centrifuging the mixture for 20 min (7000 ×*g*) at room temperature, we dried the supernatant containing gliadins by vacuum drying and stored the dried material at −20 °C until analysis.

Sodium dodecyl sulphate–polyacrylamide electrophoresis and western blotting

We dissolved the wheat proteins into a sodium dodecyl sulphate–polyacrylamide electrophoresis (SDS-PAGE) sample buffer (2% SDS, 10% glycerol, 5% 2-mercaptoethanol, 0.002% bromophenol blue, 62.5 mM Tris–HCl; pH 6.8) and conducted SDS-PAGE in 12.5% acrylamide gels. We stained separated proteins with Coomassie brilliant blue (CBB) to visualize them. For immunoblotting, we electrophoretically transferred the separated proteins to a polyvinylidene difluoride membrane (Immobilon-P; Millipore, Billerica, MA, USA) and blocked the membrane with 5% skim milk in TBST (50 mM Tris–buffered saline, 1% Tween 20; pH 7.4). We washed the membrane thrice with TBST and then probed it with anti-ω-5 gliadin IgG. Next, we incubated the membrane with horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (BioSource International, Camarillo, CA, USA). To visualize the protein bands, we used ECL Plus Western Blotting Detection Reagents (GE Healthcare, Little Chalfont, UK) and autoradiography films.

Reverse phase-high performance liquid chromatography

We dissolved the extracted gliadins in distilled water containing 0.1% trifluoroacetic acid (TFA) (v/v) and analysed the gliadin solution (3.3 mg/mL, 30 μL) with a HPLC system (model Prominence, Shimadzu, Kyoto, Japan) equipped with a Jupiter 5u C18 300A column (4.6 × 250 mm; Phenomenex, Torrance, CA, USA). The gradient of the elution solvents A [0.1% TFA (v/v)] and B [99.9% acetonitrile, 0.1% TFA (v/v)] was linear from 10% to 80% B.

Propagation and flour milling of the hypoallergenic wheat line

We cultivated the 1BS-18 line at the experimental farm of the Graduate School of Agriculture, Kyoto University, to increase the seed. Using the increased seed, we produced 263 kg of grains from 2-year cultivation in a field at the Mountainous Region Research Center of Shimane prefecture. The grains were carefully milled into flour by a flour milling company (Satake, Hiroshima, Japan), and

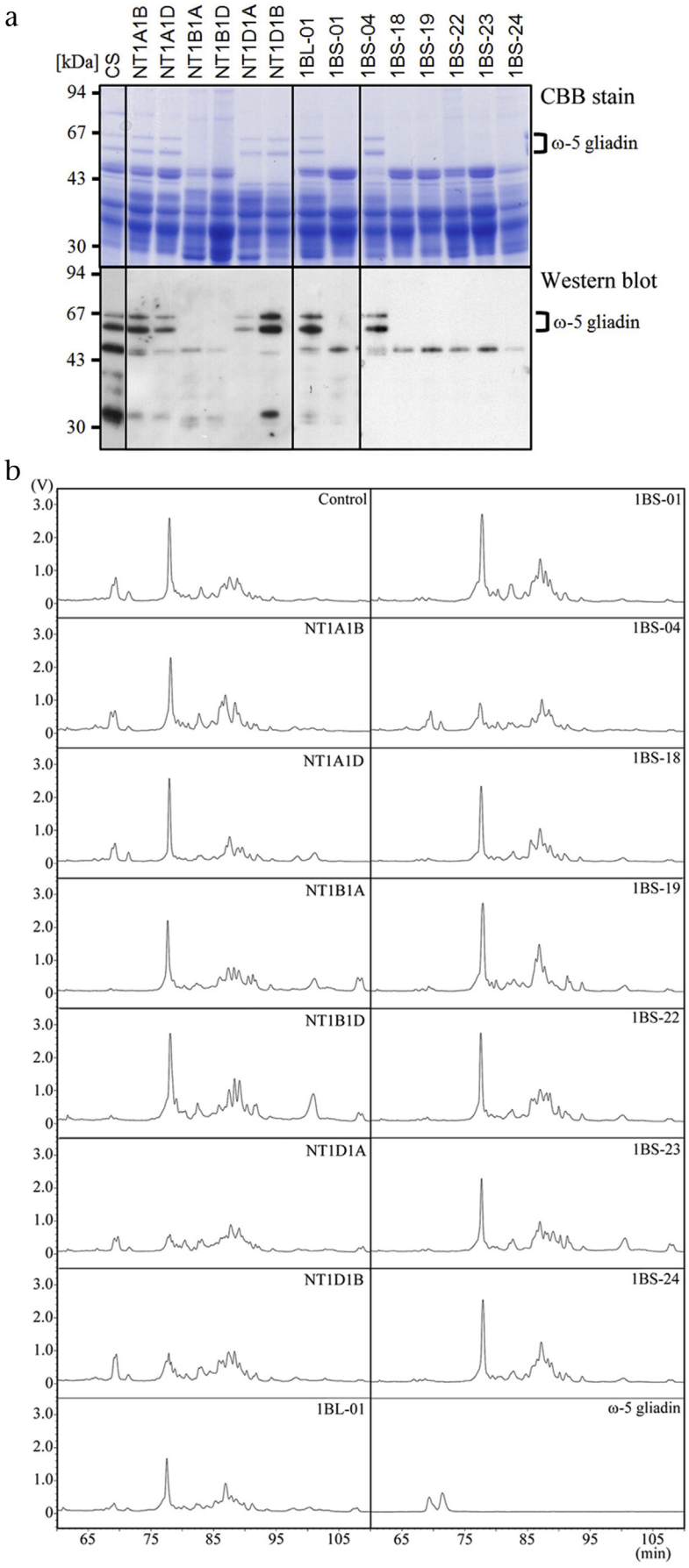

Fig. 1. Analysis of the gluten fraction. Gluten fractions from the euploid (CS), nullisomic–tetrasomic lines (from NT1A1B to NT1D1B), and 1B deletion lines (from 1BL to 01 to 1BS-24) of Chinese Spring. **(a)** top: coomassie brilliant blue (CBB) visualization of sodium dodecyl sulphate–polyacrylamide electrophoresis (SDS-PAGE), **(a)** bottom: western blotting; **(b)** reverse-phase high-performance liquid chromatography (RP-HPLC).

care was taken to avoid contamination. We confirmed by western blotting that the flour from the 1BS-18 strain had no ω-5 gliadin contamination.

Evaluation of sensitization ability of the 1BS-18 gluten in a guinea pig wheat challenge model

We obtained an average of 37.7 g gluten from 100 g of the 1BS-18 flour by kneading the flour with water to remove water-soluble proteins; we repeated this process to accumulate sufficient gluten for subsequent experiments. We used commercially available regular gluten (Ezaki Glico, Osaka, Japan) as a control. To prepare application solutions of 1BS-18 gluten and control gluten, we added 1400 mL of 1% of acetic acid to 200 g of gluten and mixed it for 16 h at room temperature. We collected the supernatant into a new tube after centrifugation (20,000 ×*g* for 15 min at 4 °C) and emulsified the gluten solution (80 mg/mL) by adding an equal volume of coconut oil–lecithin (4:1) mixture.

All animal experiments were performed at LSI Medience Corporation (LSI Medience Corporation, Kumamoto, Japan). To evaluate the sensitization ability of 1BS-18 gluten, a guinea pig wheat challenge model was employed according to a mouse model of food allergy reported previously.[18,19] According to the application and challenge protocol shown in Figure 2 and 20 6-week-old male Kud:Hartley guinea pigs purchased from Kudo (Saga, Japan) were randomly divided into 2 groups: control gluten (n = 10) and 1BS-18 gluten (n = 10). The guinea pigs were fasted for 16 h prior to application. On day 1, 7% sodium hydrogen carbonate (20 mL/kg) and salicylic acid (10 mg/mL in 0.5% methylcellulose, 20 mL/kg) were administered through per-oral gastric tube to neutralize gastric acid, and 1 h later the gluten solution (40 mg/mL, 20 mL/kg) was injected into stomach through the gastric tube. From day 2–10, the application process was repeated every day. The application was continued until day 14 and a gluten/exercise challenge test was performed on day 15. If half of the animals showed allergy scores (calculated as below) more than 1, the application was stopped and the gluten/exercise challenge test was done at the next day. For the gluten/exercise challenge test, sodium hydrogen carbonate, salicylic acid, and gluten solution was administered, then the animals were loaded with exercise using a treadmill for 30 min at condition of 15 m/min, 20% gradients. Allergy scores were calculated by the summation of the following index, which was a slight modification of previously described by Cueto-Sola *et al.*[20]: 0 = no symptoms 1 = pilar erecti, scratching and rubbing around the nose and head; 2 = redness and puffiness reaction, diarrhoea; 3 = wheezing, laboured respiration, and cyanosis around the mouth and tail; 4 = death. For instance, if a guinea pig had symptoms of scratching around the nose and head (1), redness and puffiness reaction and diarrhoea (2) and wheezing, laboured respiration, and cyanosis (3), the allergic score is 1 + 2 + 3 = 6. After this, the same gluten solution (20 mL/kg) was additionally administered by intraperitoneal (IP) injection, and 30 min later, the allergy scores were recorded in the same way.

Statistical analysis

We performed two-sided *t*-tests using SPSS ver. 20 (IBM, Armonk, NY, USA) to analyse the allergy scores in the animal experiment *P*-values less than 0.05 were considered statistically significant.

Ethics

We performed all the animal experiments with the approval of the ethics committee for animal studies of LSI Medience Corporation (Tokyo, Japan).

Results

Screening for ω-5 gliadin-deficient lines

We detected ω-5 gliadin by SDS-PAGE (Fig. 1a, top) and western blotting (Fig. 1a, bottom) in NT1A1B, NT1A1D, NT1D1A, and NT1D1B but not in NT1B1A or N1B1D. This result indicated that

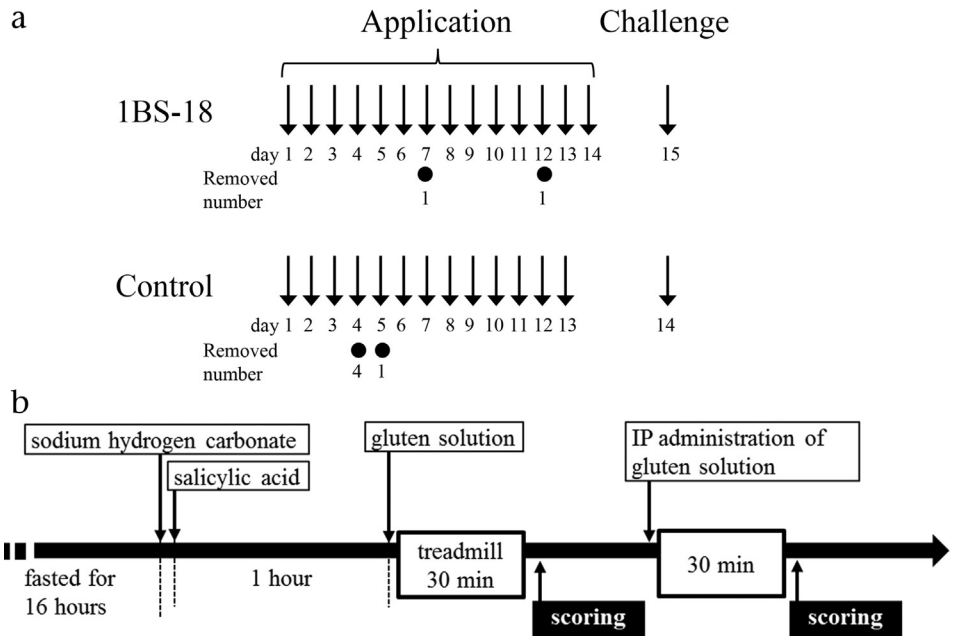

Fig. 2. Scheme of the application and challenge tests using gluten from 1BS-18 and control flour. **(a)** Gluten application schedule for guinea pigs: 1BS-18 group, from day 0–14; control group, from day 0–13 (see the text for the detailed steps). During the application, 2 and 5 of animals were removed from the 1BS-18 group and the control group, respectively. **(b)** Protocol for the gluten and exercise challenge test and intraperitoneal (IP) administration (see the text for the detailed steps).

the gene encoding ω-5 gliadin is present on chromosome 1B. Then, we investigated 1 deletion line for the 1BL arm and 7 deletion stocks for the 1BS arm and satellite. We found that all the deletion stocks for the satellite except 1BS-04 lacked the ω-5 gliadin protein (Fig. 1a), and we confirmed the same result by reverse phase-high performance liquid chromatography (RP-HPLC) (Fig. 1b). Since the 1BS-18 line has the smallest deletion of the 1BS satellite (Table 1), we employed this line as a representative hypoallergenic wheat line for the gluten allergenicity test described below.

Comparison of gluten allergenicity between the regular and 1BS-18 wheat flour in the guinea pig wheat challenge model

The control guinea pig group was fed with regular gluten and 5 of the 10 animals were withdrawn during the application period because of unhealthy condition (Fig. 2). The autopsy of the sacrificed guinea pigs showed bleeding in the duodenum, suggesting the mucosal damage in the gastrointestinal tract. In the control gluten group, more than half of animals in this group showed allergy score more than 1 on day 13, then we performed gluten/exercise challenge test with the 5 surviving guinea pigs on day 14 by forcing them to exercise after oral administration of the regular gluten. All of them had allergy scores 1 to 3, suggesting that the animals had been sensitized with the regular gluten (Table 2). The sensitization was further tested by IP injection of the gluten, and higher allergy scores were seen (4 to 6). Although the effect of exercise in the challenge test was not observed, these results suggest that these guinea pigs were sensitized with the regular gluten.

The same experiment was performed with 1BS-18 gluten. During the application period, 2 of the 10 tested guinea pigs were withdrawn because of the similar unhealthy condition as seen in the regular gluten group. Challenge test was performed with the remaining 8 guinea pigs on day 15, forcing them to exercise after oral administration of 1BS-18 gluten. As summarized in Table 2, the average allergy score of the 1BS-18 group was 0.0 in the case of oral gluten administration and 0.4 in the case of IP gluten injection; these scores were significantly lower than those of the control group: 1.4 in the case of oral gluten administration and 4.4 in the case of IP gluten injection.

Table 2
Allergic scores of guinea pig in the application and challenge tests of WDEIA.

Animal no.	Score in application (index)	Score in challenge test (index)	
		Gluten + treadmill	Additional IP injection
Control gluten sensitized group			
1	1 (1 on day 13)	1 (1)	4 (1 + 3)
5	1 (1 on day 13)	1 (1)	4 (1 + 3)
7	3 (1 + 2 on day 13)	3 (1 + 2)	6 (1 + 2+3)
8	1 (1 on day 13)	1 (1)	4 (1 + 3)
9	1 (1 on day 13)	1 (1)	4 (1 + 3)
Mean (SD)	1.4 ± 0.9	1.4 ± 0.9	4.4 ± 0.9
1BS-18 gluten sensitized group			
3	0 (day 14)	0	0
4	0 (day 14)	0	1 (1)
5	0 (day 14)	0	0
6	0 (day 14)	0	1 (1)
7	0 (day 14)	0	1 (1)
8	0 (day 14)	0	0
9	0 (day 14)	0	0
10	0 (day 14)	0	0
Mean ± SD	0 ± 0.0	0 ± 0.0	0.4 ± 0.5

The allergic scores in application and both challenge tests were significantly lower ($P < 0.05$) in the 1BS-18 group than in the control group.

Discussion

In the present study, we demonstrated by the protein analysis that most of the wheat deletion lines with deficiencies in the satellite of chromosome 1B lacked genes encoding ω-5 gliadin. Based on the observation, we selected a deletion line, 1BS-18, as the best candidate for the breeding of hypoallergenic wheat, because it has the smallest deletion involving the ω-5 gliadin gene locus. The smaller deletion of chromosome 1B is desirable for the growth and harvest of wheat.

We evaluated the hypoallergenic nature of this line by *in vivo* experiments using the guinea pig wheat challenge model, and found that 1BS-18 gluten-induced allergic scores were milder than those induced with regular gluten, suggesting a low allergenicity of 1BS-18 compared with regular wheat. During the application process of gluten with sodium hydrogen carbonate and salicylic acid, 5 animals in the control group and 2 animals the 1BS-18 group were removed because of unhealthy conditions (Fig. 2). It is unlikely that the condition seen in the application period is due to allergic reaction because sensitization needs fundamentally a couple of weeks. In a preliminary experiment, we found that enough amount of gluten (800 mg/kg body weight) is necessary to induce allergic symptoms for guinea pigs in the presence of sodium hydrogen carbonate and salicylic acid. In addition, absorption of allergens is known to increase after the administration of aspirin, which is metabolically converted to salicylic acid.[1,21] Therefore, we used salicylic acid for gluten challenging. But the administration of salicylic acid in this study may have caused the gastrointestinal damage in combination to relatively large amount of gluten application. The hyposensitization ability of 1BS-18 gluten is supported by the fact that low allergy scores were seen in the 1BS-18 group compared to those in the control group in the challenge tests as well as IP administration. As in humans, ω-5 gliadin sensitization is probably implicated in the development of wheat allergy in guinea pigs. The fact that we have failed to measure the antigen-specific IgE of guinea pigs was a limitation of our study.

Several studies on hypoallergenic wheat have been reported. Kumagai *et al.*[22] revealed that deamination by hydrolysis with an ion-exchange regime is useful to reduce the allergenicity of wheat protein and that the deaminated wheat proteins induce oral immunological tolerance in rats allergic to wheat.[23] However, deamination is far from practical use because of the difficulty of its application on a large scale. The enzymatic fragmentation of proteins in wheat flour by proteases also reduces wheat allergenicity.[24] However, the use of such flour for wheat products such as bread and noodles is difficult owing to the state losing specific viscous elasticity of gluten and altered processing characteristics of wheat gluten after the enzymatic degradation.

1BS-18 flour differed from regular flour only in the lack of ω-5 gliadin, i.e. it had not been subjected to any chemical or enzymatic treatments. In addition, the 1BS-18 line does not meet the definition of genetically modified crops because there is no insertion of foreign genes.[17] Thus, the 1BS-18 line can be an excellent parent for the development of commercial hypoallergenic wheat cultivars. Although the 1BS-18 line is an aneuploid of a low-yielding cultivar, Chinese Spring, commercial hypoallergenic wheat can be cross-bred for better yield and quality by repeated backcrossing of the 1BS-18 line to elite commercial cultivars.

We selected a deletion line of Chinese Spring 1BS-18 as a representative hypoallergenic wheat line lacking ω-5 gliadin. We demonstrated the hyposensitization ability of 1BS-18 gluten in a guinea pig wheat challenge model, suggesting that the use of the flour of such hypoallergenic wheat lacking ω-5 gliadin possibly be a prophylactic approach for wheat allergy including WDEIA.

Acknowledgement

The authors would like to thank Dr. Shuji Horie for advice on this study; Tetsuya Murakami, Kiyoaki Ikeda, and Kenichi Kurita, Research & Development Division, Glico Nutrition Co., Ltd., for their help in extracting the gluten fraction; Shigehito Matumoto and Kazumi Tatewaki, Shimane Prefecture Mountainous Region Research Center, for wheat cultivation; and Kiyoe Ueda and Kanako Yano, Laboratory Specialists at the Department of Dermatology, Faculty of Medicine, Shimane University, for their technical assistance in the experiments. This research is (partially) supported by the Practical Research Project for Allergic Diseases and Immunology (Research on Allergic Diseases and Immunology) from Japan Agency for Medical Research and Development, AMED.

Conflict of interest

The authors have no conflict of interest to declare.

Authors' contributions

EM, TE, and KS designed the research. KK and HT conducted the research. KK summarized the data and wrote the article. HM, TE, KS, and EM revised the article. KK had the primary responsibility for the final content. All authors read and approved the final manuscript.

References

1. Morita E, Kunie K, Matsuo H. Food-dependent exercise-induced anaphylaxis. *J Dermatol Sci* 2007;**47**:109–17.
2. Nwaru BI, Hickstein L, Panesar SS, Roberts G, Muraro A, Sheikh A, et al. Prevalence of common food allergies in Europe: a systematic review and meta-analysis. *Allergy* 2014;**69**:992–1007.
3. Morita E, Chinuki Y, Takahashi H, Nabika T, Yamasaki M, Shiwaku K. Prevalence of wheat allergy in Japanese adults. *Allergol Int* 2012;**61**:101–5.
4. Bodinier M, Legoux MA, Pineau F, Triballeau S, Segain JP, Brossard C, et al. Intestinal translocation capabilities of wheat allergens using the Caco-2 cell line. *J Agric Food Chem* 2007;**55**:4576–83.
5. Matsuo H, Morimoto K, Akaki T, Kaneko S, Kusatake K, Kuroda T, et al. Exercise and aspirin increase levels of circulating gliadin peptides in patients with wheat-dependent exercise-induced anaphylaxis. *Clin Exp Allergy* 2005;**35**:461–6.
6. Matsuo H, Kaneko S, Tsujino Y, Honda S, Kohno K, Takahashi H, et al. Effects of non-steroidal anti-inflammatory drugs (NSAIDs) on serum allergen levels after wheat ingestion. *J Dermatol Sci* 2009;**53**:241–3.
7. Kohno K, Matsuo H, Takahashi H, Niihara H, Chinuki Y, Kaneko S, et al. Serum gliadin monitoring extracts patients with false negative results in challenge tests for the diagnosis of wheat-dependent exercise-induced anaphylaxis. *Allergol Int* 2013;**62**:229–38.
8. Palosuo K, Alenius H, Varjonen E, Koivuluhta M, Mikkola J, Keskinen H, et al. A novel wheat gliadin as a cause of exercise-induced anaphylaxis. *J Allergy Clin Immunol* 1999;**103**:912–7.
9. Palosuo K, Alenius H, Varjonen E, Kalkkinen N, Reunala T. Rye gamma-70 and gamma-35 secalins and barley gamma-3 hordein cross-react with omega-5 gliadin, a major allergen in wheat-dependent, exercise-induced anaphylaxis. *Clin Exp Allergy* 2001;**31**:466–73.
10. Morita E, Matsuo H, Mihara S, Morimoto K, Savage AW, Tatham AS. Fast omega-gliadin is a major allergen in wheat-dependent exercise-induced anaphylaxis. *J Dermatol Sci* 2003;**33**:99–104.
11. Matsuo H, Morita E, Tatham AS, Morimoto K, Horikawa T, Osuna H, et al. Identification of the IgE-binding epitope in omega-5 gliadin, a major allergen in wheat-dependent exercise-induced anaphylaxis. *J Biol Chem* 2004;**279**:12135–40.
12. Matsuo H, Dahlström J, Tanaka A, Kohno K, Takahashi H, Furumura M, et al. Sensitivity and specificity of recombinant omega-5 gliadin-specific IgE measurement for the diagnosis of wheat-dependent exercise-induced anaphylaxis. *Allergy* 2008;**63**:233–6.
13. Denery-Papini S, Lauriére M, Branlard G, Morisset M, Pecquet C, Choudat D, et al. Influence of the allelic variants encoded at the Gli-B1 locus, responsible for a major allergen of wheat, on IgE reactivity for patients suffering from food allergy to wheat. *J Agric Food Chem* 2007;**55**:799–805.
14. Laurière M, Pecquet C, Boulenc E, 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**:890–6.
15. Altenbach SB, Allen PV. Transformation of the US bread wheat 'Butte 86' and silencing of omega-5 gliadin genes. *GM crops* 2011;**2**:66–73.
16. Yano H. Improvements in the bread-making quality of gluten-free rice batter by glutathione. *J Agric Food Chem* 2010;**58**:7949–54.
17. Endo TR, Gill BS. The deletion stocks of common wheat. *J Hered* 1996;**87**:295–307.
18. Shindo T, Kanazawa Y, Furuya M, Tazura Y, Kojima K, Teshima R. [Murine food allergy model with oral sensitization and oral challenge]. *Hatano Kenkyu-sho Nenpou* 2007;**30**:9–16 (in Japanese).
19. 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**:307–15.
20. Cueto-Sola M, Bailon E, Utrilla P, Rodríguez-Ruiz J, Garrido-Mesa N, Zarzuelo A, et al. Active colitis exacerbates immune response to internalized food antigens in mice. *Int Arch Allergy Immunol* 2013;**162**:214–24.
21. Yokooji T, Hamura K, Matsuo H. Intestinal absorption of lysozyme, an egg-white allergen, in rats: kinetics and effect of NSAIDs. *Biosci Biotechnol Biochem* 2013;**438**:61–5.
22. Kumagai H, Suda A, Sakurai H, Kumagai H, Arai S, Inomata N, et al. Improvement of digestibility, reduction in allergenicity, and induction of oral tolerance of wheat gliadin by deamidation. *Biosci Biotechnol Biochem* 2007;**71**:977–85.
23. Ikezawa Z, Yokota S, Tsubaki K, Kohno H, Sugiyama H, Ikeda K, et al. Hypoallergenic wheat preparation, process for producing the same, and processed food including the same. Patent US, No: 5480660, 1996.
24. Watanabe M, Watanabe J, Sonoyama K, Tanabe S. Novel method for producing hypoallergenic wheat flour by enzymatic fragmentation of the constituent allergens and its application to food processing. *Biosci Biotechnol Biochem* 2000;**64**:2663–7.