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Differential induction of allergy responses by low molecular weight wheat proteins from six wheat cultivars

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Abstract Although wheat is a common staple food in the world, some people suffer from a variety of wheat allergies. For example, wheat-dependent exercise-induced anaphylaxis is induced in the gastrointestinal tract by wheat proteins. Relatively high molecular weight proteins that are salt-insoluble induce many wheat allergies. In the present study, we investigated the induction of an allergy response using crude wheat proteins, which are relatively low molecular weight, salt-soluble proteins. The crude antigen used in this study was extracted using phosphate buffered saline. When the antigen extracts from various wheat cultivars were orally administered, differentiable degrees of allergy responses were observed as measured by serum IgE and histamine secretion compared to the control. Serum IgE levels increased following administration of three of the wheat extracts. This evidence suggests that a combination of salt-soluble wheat proteins could be antigens for the induction of various allergy responses.

Keywords Crude antigen · Low MW wheat protein · Serum histamine · Serum IgE · Wheat allergy · Wheat cultivar

Miju Cho, Hyeri Lee, and Min Hee Hwang contributed equally to this study.

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Introduction

Approximately 10,000 years ago, wheat cultivation began (Shewry 2009). Now, it is one of the most popular cereal grains. Wheat constituted 41 % of all cereal calories consumed between 2005-2009 (Shiferaw et al. 2013). Although wheat is a staple food in many countries, in a subset of people, it causes allergic hypersensitivity including wheat-dependent exercise-induced anaphylaxis (WDEIA), atopic eczema/dermatitis syndrome, and baker's asthma (Gómez et al. 1990; Battais et al. 2005).

WDEIA affects a subset of the population performing physical activity after ingesting wheat. Antigens for WDEIA go through the gastrointestinal track and cause severe symptoms in the body, especially the gastrointestinal, cardiovascular, urticarial, and respiratory systems (Hofmann et al. 2012). A few years ago, omega-5 gliadin in wheat was identified as the cause of WDEIA (Palosuo et al. 1999; Morita et al. 2003; Morita et al. 2009). Wheat proteins are divided into salt-soluble proteins and glutens, which include glutenins and gliadins such as omega-5 gliadin (Morita et al. 2009). In the present study, we compared the level of allergy response induced by orally administered crude antigens from six wheat cultivars. These six wheat cultivars varied in their origin, wheat type, and cultivation year. Crude antigens were extracted using phosphate buffered saline (PBS) and used to compare the induction levels of allergic responses.

Materials and Methods

Crude antigen extract

Crude antigens were extracted using the method of Shin et al. (2003). Lipids were removed from 50 mL of ground wheat using 100 mL of ether. After incubation at room temperature for 1 min, the samples were centrifuged and the supernatants were discarded. The remaining ether in the pellet was evaporated through intermittent stirring overnight with an open cap. The dried pellets were suspended

in 350 mL phosphate buffered saline. Then, the suspended mixture was agitated vigorously overnight at 4 °C and was centrifuged at 10,000× *g* for 1 h at 4 °C. The supernatants were filtered using a 3 kDa centrifugal filter (Merck Millipore Inc., Darmstadt, Germany) and the proteins on the centrifugal filter were suspended with distilled water. These aqueous mixtures were lyophilized for 3 days and kept at −80 °C.

SDS-PAGE

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was employed to visualize crude antigens. First, the extracted samples were dissolved in distilled water at a concentration of 5 mg/mL and mixed with 2× Laemmli sample buffer (Bio-Rad, Hercules, California, USA) containing 5 % β-mercaptoethanol. The mixture was denatured at 99 °C for 10 min and loaded onto a 10 % polyacrylamide gel. The amount of loaded protein was 10 μg per well, which was quantified using the Pierce BCA protein assay kit (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Tris-glycine buffer (pH 8.3) was used as the running buffer, and the gel was initially run at 80 V for 30 min and then at 120 V for 120 min. Coomassie brilliant blue staining allowed visualization of the proteins in the gel.

Animals

Nine-week-old male C3H/HeJ mice (Japan SLC, Hamamatsu, Japan) were sensitized with crude antigens from six different varieties of wheat. The mice were housed at 22 °C, 50 % humidity, and a 12/12 h light/dark cycle. The mice were fed a standard diet with continuous free access to water bottles and divided into 8 groups: negative control, positive control, and treatment with the six different wheat extracts. The animals were orally sensitized with a mixture of aluminum potassium sulfate (Sigma-Aldrich Chemicals, St. Louis, Missouri, USA) (1 mg) and crude antigen (5 mg) at days 1, 2, 3, 7, and 21. The mixtures were made by dissolving aluminum potassium sulfate and crude antigen in distilled water. For the positive control, mice were orally sensitized with aluminum potassium sulfate (1 mg). After 28 days, the mice were sacrificed to collect blood from the inferior vena cava. Then, the collected blood was centrifuged at 12,000× *g* (4 °C) for 10 min to obtain the serum, which was used for the evaluation of histamine and IgE concentrations. Korea University Institutional Animal Care and Use Committee approved all experimental procedures for the animals.

Enzyme-linked immunosorbent assay (ELISA)

IgE in the mouse serum was evaluated using mouse IgE ELISA Ready-SET-Go kit (Affymetrix, Waltham, Massachusetts, USA) following the manufacturer's instructions. Histamines in the mouse serum were measured with a histamine ELISA kit (Enzo Life Science, Farmingdale, New York, USA) following the manufacturer's instructions.

Results and Discussion

Wheat protein analysis by SDS-PAGE

The six wheat cultivars are different in their origin, type, and cultivation year. Wheat type refers to the classification of wheat cultivars into hard and soft wheats according to protein content. Hard wheat contains a high percentage of protein (10 to 14 %) whereas soft wheat contains a low percentage of protein (8 to 11 %) (Delcour et al. 2012). The wheat cultivars used are as follows: hard red winter wheat (HRW), Australian hard wheat (AH), Iksan Goso soft wheat (IGSW), and Gyeongnam Jokyung wheat (GJW) cultivated in 2014, and Australian standard white wheat (ASW) as well as Gwangju Kumkang wheat (GKW) cultivated in 2015 (Table 1). ASW and GKW are medium protein wheats.

Wheat proteins are composed of non-gluten proteins, which encompass 15 to 20 % of the total protein, and gluten proteins, which constitute the remaining proteins (Veraverbeke and Delcour 2002). Non-gluten proteins are typically salt-soluble and the major components are albumin and globulin, which range in size from 15 to 100 kDa (Kozai et al. 2006). Gluten proteins include gliadins and glutenins and are salt-insoluble proteins. Gliadins typically are extracted with alcohol and glutenins are soluble in alkali solutions (Kozai et al. 2006). The size of gliadins is between 33 and 67 kDa and that of glutenins is between 75 and 110 kDa (Battais et al. 2003; Kim et al. 2016). Among gliadins, the size of omega-5 gliadin, which is a major inducer of WDEIA, is between 50 and 67 kDa (Comino et al. 2012). By considering the characteristics of wheat proteins, we suggest that crude antigens extracted with PBS mostly include salt-soluble proteins; that is, non-gluten proteins. As shown in Fig. 1, few proteins larger than 75 kDa were obtained. This result indicates that the crude antigen extracts did not contain

Table 1 The six types of wheat cultivars used in the study*

Year	Abbreviation	Name of wheat	Origin	Wheat type
2014	HRW	Hard red winter wheat	America	Hard Wheat
	AH	Australian hard wheat	Australia	Hard Wheat
	IGSW	Iksan Goso soft wheat	Republic of Korea	Soft Wheat
	GJW	Gyeongnam Jokyung wheat	Republic of Korea	Soft Wheat
2015	ASW	Australian standard white wheat	Australia	Medium Wheat
	GKW	Gwangju Kumkang wheat	Republic of Korea	Medium Wheat

*The wheat originated from America, Australia, or Korea. Wheat type is associated with protein content

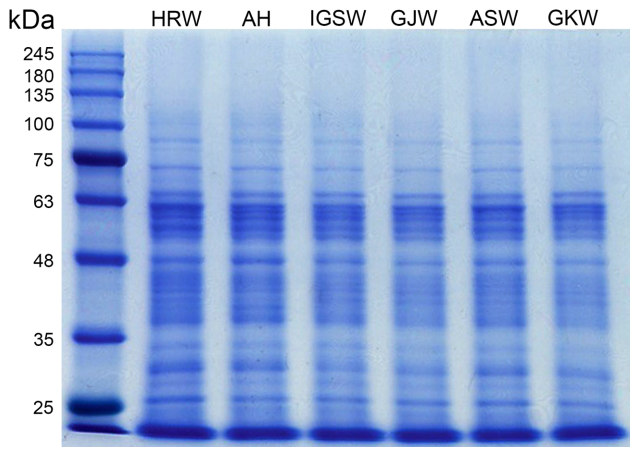

Fig. 1 SDS-PAGE of crude wheat antigens. Coomassie brilliant blue staining of proteins from hard red winter wheat (HRW), Australian hard wheat (AH), Iksan Goso soft wheat (IGSW), Gyeongnam Jokyung wheat (GJW), Australian standard white wheat (ASW), and Gwangju Kumkang wheat (GKW) are shown. BLUeye pre-stained protein ladder (GeneDirex Inc., Taiwan) was used to mark the protein sizes

glutenins (75 to 110 kDa). In addition, alcohol-soluble gliadins were not included in the crude antigen extracts. Gliadins are a major cause of WDEIA and glutenins also are implicated in WDEIA, although to a lesser extent (Kozai et al. 2006). However, in the present study, we used the salt-soluble proteins to sensitize mice and found induction of allergy responses.

Analysis of IgE and histamine by ELISA

Histamine and IgE are significant factors in allergy reactions. Histamine is secreted by mast cells or basophils causing many different allergy symptoms. Many patients threatened by various degrees of allergenic responses have elevated levels of histamine (Lin et al. 2000). When IgE is activated by specific allergens and attaches to the Fcε receptor on mast cells or basophils, it acts as a trigger for histamine release. Aluminum potassium sulfate was used as an adjuvant that promotes an immune response by stimulating the immune system or prolonging existence of the antigen. Aluminum potassium sulfate induces antigen precipitation, which provides the immune system with longer exposure to the antigen to mount an increased immune response (HogenEsch 2002; Marrack et al. 2009).

We checked body weight and food intake once every 3 days during the experimental period (Table 2). Because body weight and food intake reflect the condition of the animals, these results show the health of the animals. No statistical difference was found between the initial and final body weight, body weight gain, or food intake among tested groups. Thus, the condition of the mice most likely did not influence the results obtained in this experiment.

The IgE concentrations in mouse blood induced in response to the six different wheat cultivars were not statistically different compared to the negative control via ELISA analysis. However, the IgE concentrations for HRW and ASW (p-value of 0.01) as well as AH (p-value of 0.05) increased significantly compared to the positive control, which was treated with aluminum potassium sulfate (Fig. 2A). These three wheat cultivars as well as GKW are hard or medium wheats, suggesting that the amount of protein in wheat could have a significant effect on IgE induction and possibly allergy induction. The hard wheats employed in this study have higher protein content than the soft wheats as described above. In Fig. 2A, the negative control group had no treatment and the positive control group was treated with aluminum potassium sulfate. However, unexpectedly, no statistical difference was observed between the negative and positive control. This might mean that the treatment of aluminum potassium sulfate in the positive control did not induce any hypersensitivity or immune response. Thus, in this experimental condition, the role of aluminum potassium sulfate to prolong the antigen was diminished and it did not sufficiently stimulate the immune system to a detectable difference.

Based on the ELISA analysis of IgE in Fig. 2A, an additional histamine ELISA was analyzed with the serum from the mice treated with IGSW and GJW cultivars. These cultivars induced relatively low concentrations of IgE secretion and thus were expected to induce low levels of histamine secretion (Fig. 2B). There was no statistical difference in the concentration of serum histamine between the positive control, IGSW, and GJW. However, the histamine concentration of the IGSW and GJW groups were higher than that of the negative control. This result indicates that these two wheat cultivars might induce lower histamine secretion as well as IgE secretion.

Table 2 Body weight and food intake of each group treated with different wheat cultivars*

Mean ± SD	Con	+Con	HRW	AH	IGSW	GJW	ASW	GKW
Initial weight (g)	20.41±0.42	20.00±0.90	19.85±1.06	19.56±0.95	19.82±1.33	19.82±0.96	19.12±1.03	19.59±0.89
Final weight (g)	21.75±0.32	21.12±1.25	20.78±0.96	20.30±0.86	20.95±1.14	20.89±1.16	20.53±0.94	21.09±1.60
Body weight gain (g)	5.94±2.25	5.40±2.27	3.60±3.92	1.22±2.26	4.47±2.76	4.25±1.09	2.69±2.74	5.34±4.59
Food Intake (g/day)	2.58±0.28	2.73±0.23	0.23±0.32	2.97±0.41	3.06±0.42	2.77±0.27	2.95±0.38	3.02±0.25

*The values in the table are expressed as mean value and standard deviation. There was no statistical difference between the groups. Con is the negative control; +Con is the positive control, which received aluminum potassium sulfate (1 mg); each experimental groups were treated with aluminum potassium sulfate (1 mg) and crude antigen (5 mg). Wheat varieties include hard red winter wheat (HRW), Australian hard wheat (AH), Iksan Goso soft wheat (IGSW), Gyeongnam Jokyung wheat (GJW), Australian standard white wheat (ASW), and Gwangju Kumkang wheat (GKW)

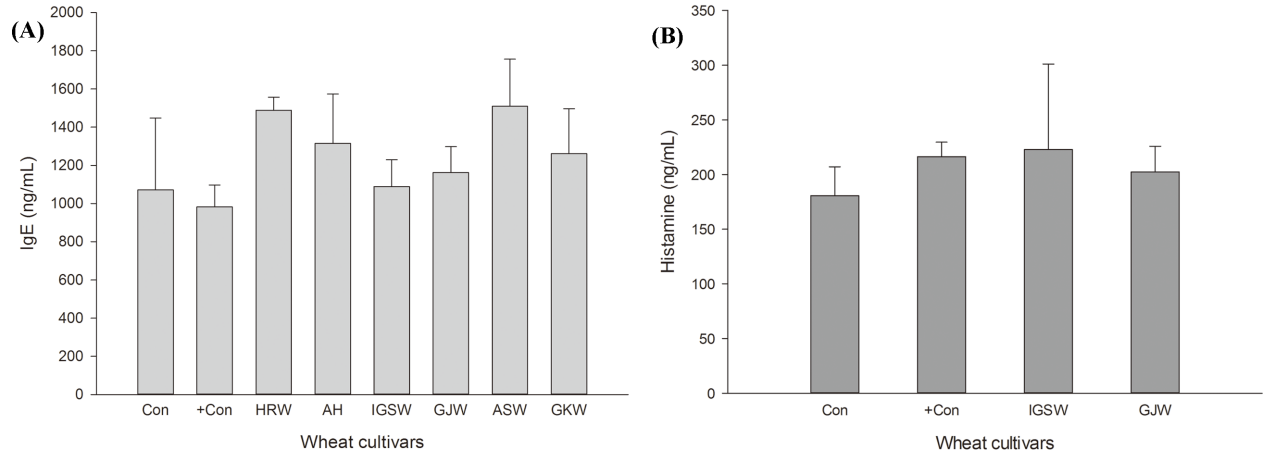

Fig. 2 ELISA analysis for the determination of IgE and histamine concentrations in mouse blood samples. The mice were treated with crude antigens from six wheat cultivars. IgE levels in HRW and ASW (p <0.01) and AH (p <0.05) increased significantly compared to the positive control (A). ELISA analysis for histamine concentrations of IGSW and GJW showed no statistically significant differences compared to controls (B). Negative control (Con), positive control (+Con), hard red winter wheat (HRW), Australian hard wheat (AH), Iksan Goso soft wheat (IGSW), Gyeongnam Jokyung wheat (GJW), Australian standard white wheat (ASW), and Gwangju Kumkang wheat (GKW)

In this study, we have compared the levels of allergy response induction after administration of the crude antigen extract of different wheat cultivars by IgE level. IgE is one of the signature factors of hypersensitivity and is utilized for specific allergy tests (Asero et al. 2007). However, our current results show that the levels of serum IgE and serum histamine are not equivalent, suggesting there could be another variable that modulates the interaction between IgE and histamine secretion.

In future studies, the quantity of allergy inducible proteins in whole wheat needs to be considered, including whether they are gluten or non-gluten proteins. In this study, salt-soluble proteins were extracted from various wheat cultivars. The differential allergy responses observed for IgE and histamine secretion showed that the salt-soluble wheat proteins also could be involved in inducing allergy responses, especially in hard wheat containing a high concentration of protein. Although the protein component is a major factor in inducing allergy responses, the other components in wheat extracts might be involved in the phenomenon observed in our results. Additional studies with other wheat cultivars are needed to ensure that the results in this study are a general phenomenon with wheat.

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