The clinical cross-reactivity and immunological cross-antigenicity of wheat and barley

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Abstract

Background: Some patients with a wheat allergy have been reported to show clinical cross-reactivity to barley. However, it is not clear whether the development of barley allergy in patients with a wheat allergy is due to cross-antigenicity between wheat and barley. In our study, we aimed to determine the clinical cross-reactivity and immunological cross-antigenicity of wheat and barley. Methods: We compared the results of barley oral food challenges (OFCs) before oral immunotherapy (OIT) for wheat with those after OIT in nine patients with a wheat allergy to estimate the clinical cross-reactivity of wheat and barley. Moreover, we performed enzyme-linked immunosorbent assay (ELISA) inhibition and immunoblotting inhibition using serum from seven patients allergic to wheat and barley. Results: Nine patients who had positive barley-OFC results performed before OIT for wheat were all negative on barley-OFC performed after OIT. In ELISA inhibition, preincubation of serum from patients allergic to wheat and barley with a high barley extract concentration inhibited binding of IgE to wheat extract by less than 10%. On the other hand, wheat and barley extracts equally inhibited binding to barley sIgE at high concentrations. In the immunoblotting inhibition test, the spots of wheat were inhibited but weakly by barley extracts, and most of the spots of barley were inhibited even by low concentrations of the wheat and barley extract. Conclusion: We showed that barley allergy associated with wheat allergy is caused by cross-reactivity from wheat. The OIT for wheat was one of the promising options for barley allergy.

Introduction

Barley, a member of the grass family, is a major cereal grain rich in dietary fiber. It has been recognized as a health food because barley beta-glucan lowers cholesterol in the blood[1] and is consumed as alcoholic beverages (for example, beer), soups, and cereals worldwide. There are many opportunities to eat cooked barley with rice in childhood in Japan, including school lunch.

Several barley allergies have been reported as cross-reactions to wheat, the third most common cause of immediate food allergy in children in Japan.[2] Poupark et al. reported that the percentage of patients with wheat-allergy who were also allergic to barley was 55%, which was higher than that for rye and oats.[3]Moreover, it has also been reported that 75% of children who showed positivity in the barley oral food challenge test (OFC) were allergic to wheat.[4]

Immunological cross-antigenicity between wheat and barley has been reported in several studies.

Srisuwatchari et al. reported that barley extracts inhibited serum-specific immunoglobulin E (sIgE) bound to wheat gliadin or glutenin by 66% and 53%, respectively, in patients with a wheat allergy, including one defined barley allergy.[6]In addition, Palsou et al. showed that ω-5 gliadin of wheat has cross-antigenicity with γ-3 hordein, a prolamin of barley, by using the sera of patients with wheat-dependent exercise-induced anaphylaxis (WDEIA).[7] Although these two studies showed cross-reactivity between wheat and barley allergen components, neither were examined in sera from patients with a defined barley allergy. No studies have shown clinical cross-reactivity or immunological cross-antigenicity between wheat and barley in patients with barley and wheat allergy. In other words, it is unclear whether allergic reactions to barley in patients with an immediate allergy to wheat are due to individual sensitization or cross-reactivity between both allergens.

Therefore, our study sought to determine the clinical cross-reactivity and immunological cross-antigenicity of wheat and barley. First, to estimate the clinical cross-reactivity of wheat and barley, we compared the results of barley OFCs before oral immunotherapy (OIT) for wheat with those after OIT in patients with a wheat allergy. Next, we evaluated immunological cross-antigenicity by performing enzyme-linked immunosorbent assay (ELISA) inhibition and immunoblotting inhibition using sera from patients allergic to both allergens.

Methods

The first oral food challenge to barley

We examined the factors that influenced the results of all barley-OFCs conducted between August 2012 and November 2020 in patients with a history of wheat allergy. An open OFC to cooked barley (6.2% of protein, based on standards tables of food composition in Japan 2015) was performed according to the Japanese guidelines[1]for patients with a wheat allergy diagnosed by wheat-OFCs; they completely avoided wheat or ingested some amount of wheat products as OIT at the time of barley-OFC. The patients were instructed to ingest 18.6, 62, 186, 620, and 1,860 mg barley protein of cooked barley with 30-minutes intervals until obvious allergic symptoms were observed. Some patients underwent barley-OFC with a different protocol, where they ingested a total amount of at least 236 mg of barley protein. All patients who had negative barley-OFC results were instructed to consume at least 1,860 mg of barley protein at home to confirm that no allergic symptoms were induced.

The second oral food challenge to barley after OIT for wheat

We performed barley-OFCs again for the patients with a barley allergy after rush or slow OIT for wheat[8-10] (desensitized to a daily amount of wheat products: at least 2,000mg of wheat protein).

Assays for IgE

Serum-specific IgE titers to wheat, omega-5 gliadin, and barley were measured within one year before barley-OFCs using ImmunoCAP® (Thermo Fisher Scientific, Uppsala, Sweden). Levels >100 kUA/L were fixed at 100 kUA/L.

ELISA inhibition

Details of the ELISA method were described in a previous report.[11] Pierce Protein-Free blocking buffer (Thermo Fisher Scientific, Uppsala, Sweden) was used for blocking and serum dilution. Proteins were extracted from wheat and barley using a Mammalian cell lysis kit (Sigma-Aldrich, Missouri, America) and added to 2% serum or blocking buffer at a concentration of 0, 0.01, 0.1, 1, and 2 mg/mL and incubated for 1 h at 25degC, and then used for the assay. The inhibition rate was calculated as a ratio to the measured value when no protein was added to the serum.

Immunoblotting and immunoblotting inhibition

Two-dimensional (2D) polyacrylamide gel electrophoresis (PAGE) and immunoblotting were performed by slightly modifying previously reported methods.[12] A total of 25 μg of the extracted protein was subjected to immunoblotting, and Pierce Protein-Free blocking buffer was used for blocking and serum dilution. The

proteins extracted from wheat and barley were added to 2% serum or blocking buffer at a concentration of 0, 0.1, and 2 mg/mL, incubated for 1 h at 25°C, and then used for immunoblotting.

Statistical analysis

All statistical analyses were performed using EZR software (Saitama Medical Center, Jichi Medical University).[13]Continuous and categorical variables were analyzed using the Mann–Whitney U test and Fisher's exact test, respectively.P values <0.05 were considered statistically significant.

Ethical Considerations

This study was conducted in accordance with the Declaration of Helsinki and was approved by the institutional ethical board of Aichi Children's Health and Medical Center (No. 2017001). Rush and slow OIT were also conducted after obtaining approval from the ethical board (No. 201336 and 201427), and written informed consent was obtained.

Results

The first barley-OFC

We performed 77 barley-OFCs for the patients with a defined wheat allergy. The median age at the barley-OFC was 7.4 (range: 2.9–14.9) years, and 65% were male (Table 1). Twenty-eight (36.3%) patients had positive OFC results. Specific IgE titers to wheat, omega-5 gliadin, and barley were significantly higher in the patients with a positive result than a negative result. Moreover, complete avoidance of wheat or a smaller amount of daily-taking wheat product for oral immunotherapy, which means the earlier stage of OIT at the barley-OFC, was associated with positive results (Table 1, Fig 1).

The second barley-OFC after OIT for wheat

Among nine patients with positive results to the first barley-OFCs, two patients enrolled slow OIT while seven enrolled rush OIT for wheat (Table 2). Wheat-OFCs results before OIT are shown in Table S1. All patients achieved desensitized status to a daily amount of wheat products (equivalent to 2,000 mg wheat protein) within three years.

The second barley-OFC was performed at a median of 2 (range: 1.1–5.5) years after the first barley-OFCs (Table 2). The results were negative for all nine patients. The median amount of desensitized wheat protein was 5,580 mg (range: 2046–7800) at the second barley-OFC. The sIgE titers to omega-5 gliadin after wheat OIT significantly decreased compared to before OIT (Table 3). On the other hand, the change in barley-sIgE was not uniform (reduction: 5 patients, elevation: 2 patients, no data: 2 patients).

ELISA inhibition

ELISA inhibition was performed using serum before OIT from seven of the nine patients who underwent the second OFC. Cases 3 and 9 were excluded because of an insufficient volume of serum. The binding of IgE to wheat extract was inhibited by more than 80% after preincubation of the serum with a high concentration of wheat extract, whereas preincubation of serum with a high concentration of barley extract inhibited binding of IgE to wheat extract by less than 10% (Fig 2). On the other hand, wheat and barley extracts equally inhibited binding to barley sIgE at high concentrations. Furthermore, wheat exhibited more inhibition than barley at low concentrations (0.01 mg/ml) of inhibiting extract (p =0.03).

Immunoblotting and immunoblot inhibition

A 2D-PAGE analysis of wheat and barley extract showed multiple bands (Fig 3A). Serum from the seven patients excluding cases 3 and 9 was used for immunoblotting with the wheat extract, and several IgE-binding spots were found. (Fig 3B, Fig S1). In all patients, the 2D-PAGE revealed a strong IgE binding to a 30–50 kDa wheat extract protein, which was estimated to be gliadin based on the molecular weights described in the literature.[14-17] In IgE-immunoblotting using barley extract, strong bands at 30–35, 40–45, 60–80 kDa were detected in all seven patients. In addition, various other spots were detected in each patient.

In immunoblot inhibition, the bands of wheat were inhibited albeit weakly by barley extracts. On the other hand, most of the spots of barley were inhibited even by a low concentration of wheat and barley extract.

Discussion

We found that barley-OFC results were associated with wheat intake at the time of OFC. Based on these results, OIT for wheat was conducted for patients with a wheat allergy who had positive barley-OFC results, and it was found that barley allergy was improved after wheat OIT. Although the cross-reactive protein between wheat and barley was not identified from the results of immunoblotting inhibition, we have shown for the first time, both clinically and immunologically, that barley allergy associated with wheat allergy is caused by cross-reactivity from wheat.

The positive rate of barley-OFC performed at our hospital (36.3%) was lower than previous studies (47.6%, 48.1%)[4,18], which reported that the sIgE titers to wheat and omega-5 gliadin[18] and sIgE titers to barley[4]were useful predictors of the result of a barley-OFC. In addition to these titers, we found that the results of barley-OFC were related to wheat intake at the time of barley-OFC. Therefore, the relatively lower positive rate of barley-OFC is due to the high proportion of cases with high wheat intake at the time of OFC. Moreover, when the results of barley-OFC were examined in patients with low wheat intake (wheat protein, [?]260 mg) at the time of barley-OFC, the sIgE titers to wheat and omega-5 gliadin were higher in patients with a positive barley OFC result (p =0.03 and 0.01, respectively, data not shown).

Based on the barley-OFC results, the change of barley-OFC results in the earlier stage of wheat OIT and after OIT were evaluated. The second barley-OFC, performed after reaching a desensitized state of at least 2,000 mg of wheat protein by wheat OIT, was negative in all patients. These results suggested that barley allergy may be clinically caused by cross-reactivity with a wheat allergy. In addition, it was proven by ELISA inhibition that barley allergy complicated by patients with a wheat allergy was caused by cross-antigenicity of both antigens, and wheat was the source of common antigenic sensitization.

Several components of barley allergy have been reported in the past. The water-soluble fractions of barley, α-amylase/trypsin inhibitor, α-amylase, and β-amylase, are known to be allergenic components of baker's asthma.[19-21] Heat-stable LTP and protein Z4 are the components of beer that can cause allergic symptoms.[22-26] Adult patients with a barley allergy whose allergic symptoms are triggered by beer consumption are rarely complicated by wheat allergy, and the pattern of barley allergy development in children and adults may be different. Using serum from patients with WDEIA, it was reported that γ-3 hordein has cross-antigenicity with omega-5 gliadin.[7]

This study detected various spots in immunoblotting with barley extract using the serum of patients with a barley allergy complicated with the wheat immediate-type allergy. In particular, the spots of 30–35, 40–45, 60–80 kDa were detected in all cases and may be the main component. Furthermore, according to the WHO/IUIS database, the molecular weight of γ-3 hordein is 34 kDa, which may correspond to the 30–35 kDa spot detected in this study. In the future, we plan to identify the barley allergens for the spots detected in this study.

If the amino acid sequences of the epitope sites are highly homologous even for different allergen components, the IgE antibody binds to both, causing cross-antigenicity. Omega-5 gliadin has a repeating structure containing many glutamine residues in the molecule and has many continuous epitopes, QQX_1PX_2QQ (X_1=L, F, S, I; X_2=Q, E, G).[15,27] On the other hand, γ-3 hordein is also a protein-rich in glutamine residues and has an amino acid sequence that is similar but not identical to the epitopes of omega-5 gliadin.[28] Although the amino acid sequence similarity between omega-5 gliadin and γ-3 hordein was not high (23.9%) according to a Basic Local Alignment Search Tool search, the high amino acid homology between the epitopes of both components suggests that cross-antigenicity is likely to occur between wheat and barley allergens. Furthermore, we showed by ELISA inhibition that the response to barley extracts was more inhibited in wheat than in barley at low inhibitor concentrations. This may be because the key amino acid sequences of the epitopes for omega-5 gliadin and γ-3 hordein binding to IgE antibodies are identical, but the epitope sequences are not completely identical, resulting in a difference in affinity, or because the number of similar epitopes is

higher in omega-5 gliadin than in γ-3 hordein. However, in the immunoblotting inhibition, most of the spots of barley disappeared due to inhibition by wheat extracts, and it is likely that other barley proteins are also involved in the cross-antigenicity with wheat proteins. In the future, it is necessary to identify barley allergens and conduct epitope analysis to elucidate the mechanism of cross-reactivity.

There are several limitations to this study. First, no patient underwent a second barley-OFC without OIT for wheat. Hence it cannot be denied that the barley allergy was not improved by the wheat OIT but was the acquisition of tolerance during the natural course. Therefore, in the future, patients with a positive barley OFC result without OIT for wheat will also be evaluated for barley allergy status over time. Second, the protocol for barley-OFC was not consistent in all cases. However, patients with negative OFC results were instructed to consume sufficient amounts of cooked barley at home to confirm the negative result once again.

In conclusion, we showed that wheat OIT increased the symptom-inducing threshold of barley allergy. In addition, barley allergy complicated by patients with an immediate-type wheat allergy was caused by cross-antigenicity of both antigens, and wheat was found to be the source of sensitization. In the future, it is expected that epitopes to which IgE antibodies react in cases of immediate-type wheat and barley allergy will be identified, and the mechanism of cross-antigenicity will be clarified.

Author contributions

SK designed the paper, processed the data, performed the analysis, and drafted the manuscript. YA performed the experiments and drafted the manuscripts. TS performed the experiments. KK and YT contributed to the conception and interpretation of the study and reviewed the paper. TM, SS, MN, KM, and KI conceptualized the study, analyzed and interpreted the data, and supervised the findings of this work. All authors discussed the results and contributed to the final manuscript.

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Impact Statement

We showed for the first time that barley allergy associated with wheat allergy is caused by cross-reactivity from wheat. In this study, we found that wheat OIT increased the symptom-inducing threshold of barley allergy. Although the cross-reactive proteins between wheat and barley were not identified from the results of immunoblotting inhibition, it is expected that the mechanisms of cross-antigenicity of wheat and barley will be clarified in the future.

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Tables

Table 1. Characteristics of barley-OFC positive and negative groups.

	Total (n=77)	Positive (n=28)	Negative (n=49)	P
Median age at barley OFC (years)	7.4 (2.9–14.9)	6.2 (2.9–11.5)	8.1 (4.4–14.9)	0.003
Male sex, n (%)	50 (65%)	16 (57%)	34 (69%)	0.32
History of atopic dermatitis, n (%)	55 (71%)	20 (71%)	35 (71%)	1
History of bronchial asthma, n (%)	35 (45%)	13 (46%)	22 (45%)	1
History of allergic rhinitis, n (%)	39 (51%)	13 (46%)	26 (53%)	0.64
History of anaphylaxis due to wheat ingestion, n (%)	74 (96%)	27 (96%)	47 (96%)	1
Wheat intake at barley OFC (mg of wheat protein)	52 (0–11,160)	5.2 (0–260)	1,029 (0–11,160)	<0.001
Total IgE (IU/ml)	697 (100–4,593)	827 (147–4,593)	596 (100–3,621)	0.16
Wheat-sIgE (kUA/L)	50.9 (2.6–100)	91.0 (2.8–100)	38.7 (2.6–100)	0.01
Omega-5 gliadin-sIgE (kUA/L)	1.7 (0.34–100)	6.2 (0.34–100)	1.11 (0.34–20.1)	<0.001
Barley-sIgE (kUA/L)	18.9 (0.59–100)	24.8 (0.59–100)	17.2 (0.68–100)	0.02

Values are presented as the number (%) or median (range).

Levels of specific IgE titers [?]100 kUA/L and [?]0.34 kUA/L were set to 100 kUA/L and 0.34 kUA/L, respectively.

Abbreviations: OFC: oral food challenge, sIgE: specific IgE (kUA/L)

Continuous and categorical variables were analyzed using the Mann–Whitney U test and Fisher's exact test, respectively.

Abbreviations: OFC: oral food challenge

Table 2. Changes in barley allergy caused by wheat OIT.

Case No.	Sex/ Age (years)	Baseline barley-OFC	Baseline barley-OFC	Baseline barley-OFC	Secondary barley-OFC	Secondary barley-OFC	Secondary barley-OFC	OFC interval (years)	Ty OI
		Wheat intake at OFC (mg protein)	Total dose of barley (mg protein)	Symptoms to barley	Wheat intake at OFC (mg protein)	Total dose of barley (mg protein)	Symptoms to barley		

Case No.	Sex/ Age (years)	Baseline barley-OFC	Baseline barley-OFC	Baseline barley-OFC	Secondary barley-OFC	Secondary barley-OFC	Secondary barley-OFC	OFC interval (years)	Ty OI
1	M/4.4	47	2,747	Intermittent coughing	2,080	2,747	None	1.5	Slo
2	M/5.6	2	1,116	Wheezing, multiple hives, sleep (not usual)	5,580	1,352	None	2	Ru
3	M/5.9	0	1,116	Difficulty in breathing, multiple hives, abdominal pain, vomiting	7,800	3,100	None	5.5	Ru
4	F/6	7	887	Transient coughing, runny nose	2,046	2,747	None	1.4	Slo
5	F/6.1	13	527	Transient coughing, local hives	3,720	2,747	None	2.3	Ru
6	M/6.2	2	2,747	Intermittent coughing, local hives	7,440	2,747	None	1.1	Ru
7	M/6.4	0	1,116	Wheezing, local hives, abdominal pain, nausea, tendency to lay down	5,580	2,747	None	3.2	Ru
8	M/6.6	4	267	Intermittent coughing, multiple hives	5,580	2,747	None	1.4	Ru

Case No.	Sex/ Age (years)	Baseline barley-OFC	Baseline barley-OFC	Baseline barley-OFC	Secondary barley-OFC	Secondary barley-OFC	Secondary barley-OFC	OFC interval (years)	Ty OI
9	F/8.4	21	2,747	Multiple hives	5,394	2,747	None	2.3	Ru

Baseline OFC was performed before or at an early stage of wheat OIT. The secondary OFC was performed when patients achieved desensitization with at least 2,000 mg of wheat protein by wheat OIT.

Abbreviations: OIT: oral immunotherapy, OFC: oral food challenge; M: male; F: female.

Table 3. Changes in each sIgE after OIT of wheat.

		Before OIT of wheat	Before OIT of wheat	Before OIT of wheat	Before OIT of wheat	After OIT
Case No.	tIgE	tIgE	Wheat-sIgE	ω-5 gliadin-sIgE	Barley-sIgE	tIgE
1	697	697	100	4.57	100	290
2	3,621	3,621	100	33.7	100	2,449
3	2,092	2,092	100	16.2	ND	1,230
4	202	202	66.2	6.35	16.5	284
5	974	974	100	19.0	98.7	1,372
6	1,332	1,332	100	30.3	100	764
7	579	579	100	11.2	67.4	2,328
8	752	752	55.3	7.03	26.6	337
9	1,014	1,014	42.6	0.82	6.26	ND

Abbreviations: OIT: oral immunotherapy, tIgE: total IgE (IU/ml), sIgE: specific IgE (kUA/L), ND: no data

Figure legends

Figure 1. Distribution of wheat intake at barley-oral food challenge (OFC) among OFC positive (n=28) and negative (n=49) patients.

Figure 2. ELISA inhibition assay of wheat and barley coated plates.

Serum from seven patients with wheat and barley allergy who underwent OIT for wheat was used for enzyme-linked immunosorbent assay inhibition. The inhibition percent of seven patients is shown in the graph as mean ± standard deviation. At low concentrations (0.01 mg/ml) of inhibiting extract, wheat inhibited more than barley (p =0.03).

Figure 3. Results of immunoblotting of wheat and barley using IgE antibody from the patient's serum.

(A) Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) and (B) immune blotting analysis of protein extracts of wheat and barley with the serum of a case 6 and healthy. For wheat, the spots in the solid line were estimated to be gliadin based on the molecular weights described in the literature. All spots of wheat were inhibited by the wheat extract but not by the barley extract. For barley, the white triangle represents spots corresponding to 60–80 kDa proteins in the barley extracts recognized by immunoglobulin (Ig)E antibodies in the patient's serum. The black arrow represents spots corresponding to 40–45 kDa proteins, and the black triangle represents spots corresponding to 30–35 kDa proteins in the barley recognized by the IgE antibodies in the patient's serum. All spots of barley were suppressed for both wheat and barley extracts.

Appendices: NA

Fig 1.

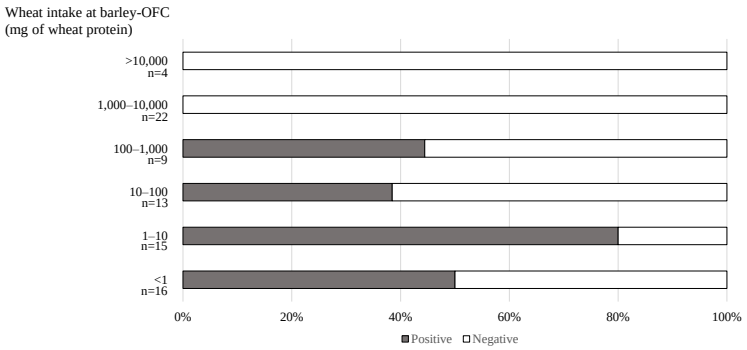

Fig 2.

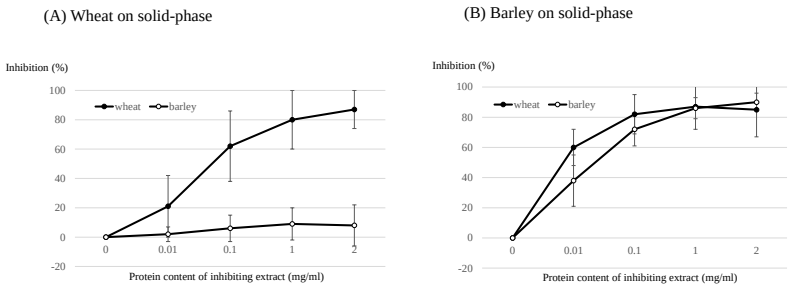

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Figure 3.pdf available at https://authorea.com/users/368767/articles/549078-the-clinical-cross-reactivity-and-immunological-cross-antigenicity-of-wheat-and-barley