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Letter to the Editor

In vitro basophil activation is reduced by short-term omalizumab treatment in hydrolyzed wheat protein allergy

Dear Editor,

Food allergies are IgE-mediated immediate-type allergy commonly seen in children and adults. The most common cause of food allergies is hen-egg in children, whereas wheat is the most frequent culprit food in adult patients with food-dependent exercise-induced anaphylaxis.¹ In Japan, an outbreak of wheat allergy has been recently noted which was caused by cutaneous sensitization to hydrolyzed wheat protein (HWP) supplemented in a soap, and over 2000 subjects were affected.^{2–6} Even after 5 years have passed since the cessation of using the soap, some patients have positive IgE test results against wheat and/or gluten and occasionally develop allergic reactions, such as facial edema and urticaria, when wheat products are ingested. Allergic symptoms in the HWP allergy is elicited by reaction of their IgE to natural wheat proteins ingested. We have reported that the IgE against HWP react mainly to wheat γ -gliadin and other gliadins with lower extent, indicating a variation of the IgE-reaction among the HWP allergy patients.⁷

The objective of the study was to assess the efficacy of omalizumab (Xolair, Novartis Pharma, Tokyo) for HWP-allergy by comparing the wheat allergen-induced basophil activation before and after the omalizumab treatment by using water soluble and water insoluble wheat fractions.

The inclusion criteria of subjects for this study were as follows: fulfilled the diagnostic criteria for immediate wheat allergy to the hydrolyzed wheat (Glupearl 19S) contained in “Cha-no-Shizuku” soap and some other products by the Special Committee for the Safety of Protein Hydrolysate in Cosmetics⁵; aged older than 20 years; and with a history of anaphylaxis after wheat ingestion within 12 months or avoiding wheat products because of positive results of an IgE test against wheat and/or gluten performed within 4 weeks. A total of 12 subjects were enrolled in this study at 5 sites across Japan: Shimane University Hospital, Kakogawa Prefectural Hospital, Fujita Health University Hospital, Osaka University Hospital and Tokyo Medical and Dental University Hospital. Their characteristics are described in Table 1. The subjects were administered 150 mg of omalizumab by subcutaneous administration 3 times with 4 weeks-interval (Supplementary Fig. 1). The primary endpoint of the study was evaluated as a reduction in allergen-induced CD203c expression-based basophil activation test (BAT) results 4, 8, and 12 weeks after the omalizumab treatment, because we had previously described that the BAT clearly differentiates sensitization conditions against wheat allergens.⁶ The activation agents used for the BAT were: (A) HWP (final concentrations 0.1 and 1 μ g/mL), (B) soluble

fraction of wheat protein (PBS) (final concentrations 1 and 10 μ g/mL), (C) ethanol extraction fraction of wheat protein (EtOH) (final concentrations 1 and 10 μ g/mL), and (D) alkali extraction fraction of wheat protein (Alkali) (final concentrations 1 and 10 μ g/mL). Wheat fractionation was performed according to a previous described method (Supplementary Methods).⁸ Using these agents, the CD203c expression on the basophil surface was monitored by fluorescence-activated cell sorting (FACS). The activation rate was examined by comparing the anti-IgE activation, and the value was expressed as a percentage against that of anti-IgE activation. Previous report showed that the patients with wheat allergy displayed activation rates greater than 10% on CD203c expression-based BAT, on the other hand, the patients with tolerant to wheat displayed activation rates lower than 10%.⁸ Therefore, we decided the activation rate greater than 10% in the BAT was positive in this study. This study was approved by the ethics committee of Shimane University and the Dean of the Faculty of Medicine (approval nos. 1515 and 1631) and preregistered to a public registry (UMIN000014272).

Before omalizumab treatment, the percentages of the 12 subjects who displayed activation rates greater than 10% in the BAT were 100% with HWP (0.1 μ g/mL), 66.7% with PBS (10 μ g/mL), 50% with EtOH (10 μ g/mL), and 75% with Alkali (10 μ g/mL). Four weeks after the omalizumab treatment, the percentages of 12 subjects who exhibited activation rates less than 10% in the BAT were 50% with HWP (0.1 μ g/mL), 100% with PBS (10 μ g/mL), 91.7% with EtOH (10 μ g/mL) and 50% with Alkali (10 μ g/mL). Eight weeks after the treatment, the percentages of the 12 subjects who showed activation rates less than 10% were 8.3%

Table 1
Characteristics of the subject.

Subject no.	Age (y)	Sex	Body weight (kg)	Total IgE level (IU/ml)	Specific IgE levels (kUa/L)		
					Wheat	Gluten	ω -5 gliadin
SHIM001	60	F	61	240	0.73	0.43	<0.10
SHIM002	42	F	45	164	0.24	0.25	<0.10
SHIM003	42	F	44	227	1.46	1.91	<0.10
SHIM004	40	F	48	57.2	0.22	0.36	<0.10
SHIM005	57	F	46	219	0.27	0.48	<0.10
HYOG001	69	F	51	192	0.1	0.11	0.65
HYOG002	49	F	53	340	0.36	0.46	<0.10
FUJI001	53	F	53	597	0.4	0.68	<0.10
FUJI002	47	F	49	69.5	0.23	0.35	<0.10
FUJI003	51	F	51	355	0.53	0.94	<0.10
OSAK001	50	F	53	952	0.39	0.55	<0.10
TOKY001	52	F	42	21	<0.10	<0.10	<0.10

[†] Specific IgE was determined using ImmunoCAP (CAP-FEIA, ThermoFischer Scientific, Uppsala, Sweden) of which cut-off values was below 0.35 (kUa/L).

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with HWP (0.1 $\mu\text{g/mL}$), 66.7% with PBS (10 $\mu\text{g/mL}$), 75% with EtOH (10 $\mu\text{g/mL}$) and 25% with Alkali (10 $\mu\text{g/mL}$). Twelve weeks after the treatment (end of the study), the percentages of the 12 subjects who showed activation rates less than 10% were 8.3% with HWP (0.1 $\mu\text{g/mL}$), 41.7% with PBS (10 $\mu\text{g/mL}$), 66.7% with EtOH (10 $\mu\text{g/mL}$) and 8.3% with Alkali (10 $\mu\text{g/mL}$). The activation rates were significantly reduced after 4 weeks of omalizumab treatment in HWP-, PBS-, and Alkali-induced BATs, but not in EtOH-induced BATs (Supplementary Table 1). Individual time courses of the activation rate in the BAT is shown in Figure 1a and Supplementary Table 2, and the representative results of FACS analysis is shown in Figure 1b. No adverse events were observed when administering omalizumab.

This study showed that short-term omalizumab treatment, the dose of which was fixed at 150 mg for 3 courses of administration,

significantly inhibited wheat allergen-induced basophil activation in patients with HWP-allergies, but the inhibition achieved with omalizumab treatment was reverted to the same levels as those before treatment after 12 weeks (Fig. 1a, and Supplementary Table 1, 2). This recovery of allergen-induced basophil activation during follow-up after omalizumab administration is similar to that in the patients with chronic spontaneous urticaria who were treated with/without omalizumab.⁹

In this study 3 preparation-covering all wheat proteins, PBS, EtOH, and Alkali, were used for the BAT in addition to HWP in order to see an effect of omalizumab-treatment on the allergic reaction to natural wheat proteins (Supplementary Fig. 2), because pathogenesis of HWP-allergy is caused by reaction to both HWP and natural wheat proteins.⁷ The inhibition in basophil activation against EtOH fraction was not significant. Generally, basophil activation against

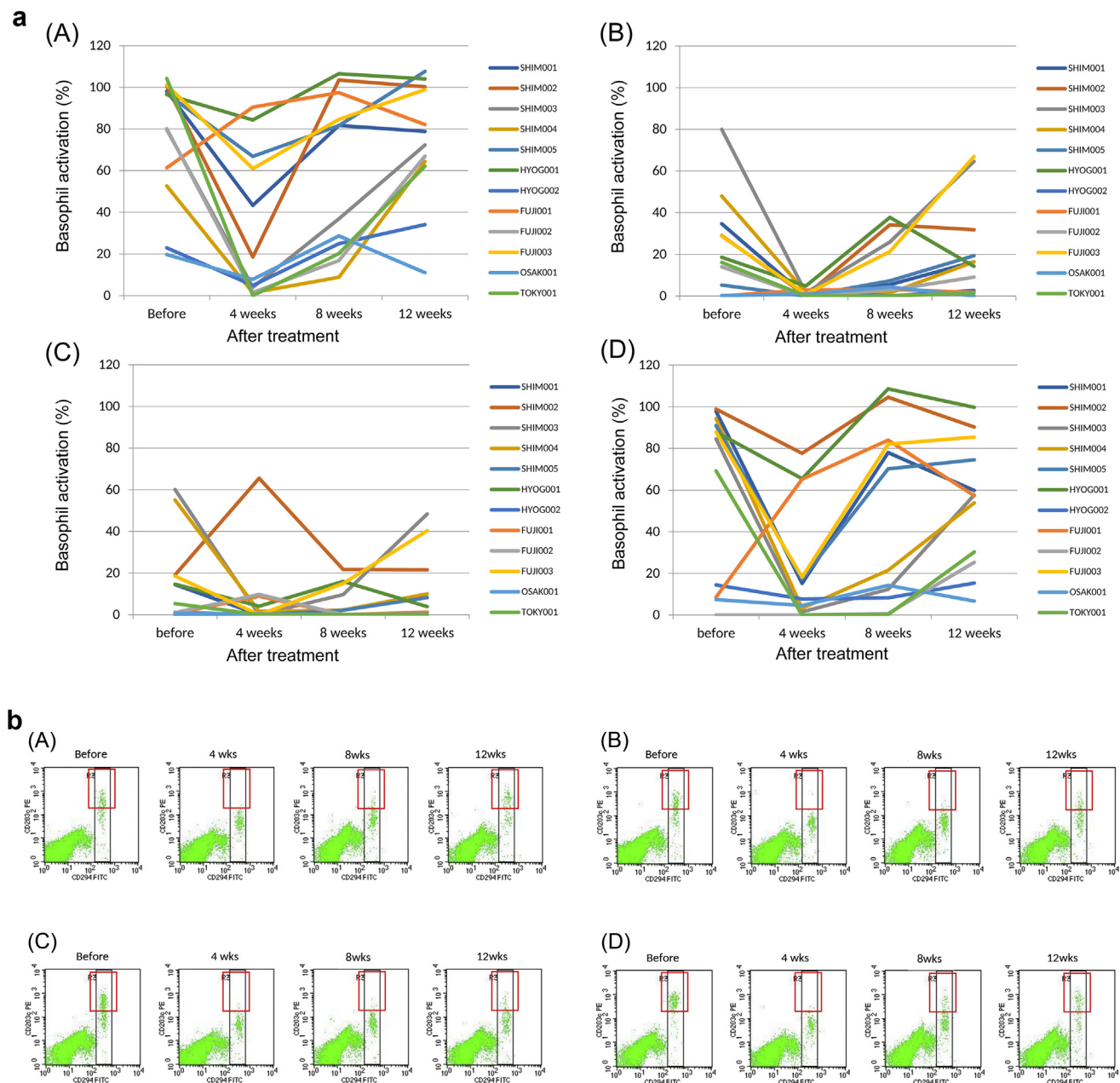


Fig. 1. a. Results of wheat allergen-induced BAT. Basophil activation (%) was calculated as the formula: allergen-induced fluorescence intensity – negative control/anti-IgE-induced fluorescence intensity – negative control. (A) HWP 0.1 $\mu\text{g/mL}$, (B) PBS 10 $\mu\text{g/mL}$, (C) EtOH 10 $\mu\text{g/mL}$, and (D) Alkali 10 $\mu\text{g/mL}$. b. Representative results of FACS analysis (dot plots). Short-term omalizumab treatment significantly inhibited wheat allergen-induced basophil activation in patient (SHIM004), but the inhibition achieved with omalizumab treatment was reverted to the same levels as those before treatment after 12 weeks. (A) HWP 0.1 $\mu\text{g/mL}$, (B) PBS 10 $\mu\text{g/mL}$, (C) EtOH 10 $\mu\text{g/mL}$, and (D) Alkali 10 $\mu\text{g/mL}$.

EtOH fraction was lower than those against other fractions before omalizumab treatment. It is considered that HWP-specific IgE less react to EtOH fraction due to structural difference of each protein fractions. In addition, as seen in SHIM002, there were several patients whose activation rates against EtOH fraction increased 4 weeks after omalizumab treatment (Fig. 1a). However, in this study we did not clarify the reason why the basophil activation against EtOH highly increased 4 weeks after omalizumab treatment in SHIM002. One possible explanation is that optimal dose of EtOH shifted to higher concentration after 4 week-omalizumab treatment by decreasing FcεRI expression compared to that before treatment (from less than 1 µg/ml to 10 µg/ml).

The inhibition rates varied among the patients, as shown in Figure 1a. This variation was well associated with serum IgE levels at the starting point; strong inhibition was observed in the 3 subjects (SHIM004, FUJI002, and TOKY001) in whom serum IgE levels were less than 100 IU/ml, whereas an incomplete response was observed in the remaining subjects in whom serum IgE levels were greater than 100 IU/ml (Table 1). This finding indicates that the higher doses of omalizumab are necessary for subjects with higher serum levels of IgE to achieve complete inhibition for allergen-induced basophil activation. This finding is compatible with previous findings that the administration dose of omalizumab can be determined by the serum levels of IgE and body weight of patients with bronchial asthma (established according to the package insert).¹⁰ Our study has several limitations and is a pilot study with a small sample size, which lacks a placebo group and oral food challenge tests after omalizumab-treatment. However, this study provides the basic data for the next large-scale study involving food challenge test to investigate whether clinical allergic manifestations to wheat exposure remain or not after omalizumab administration.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.alit.2019.09.006>.

Conflict of interest

YC received lecture fees and grant support from Novartis. AY received lecture fees from Novartis. The rest of the authors have no conflict of interest.

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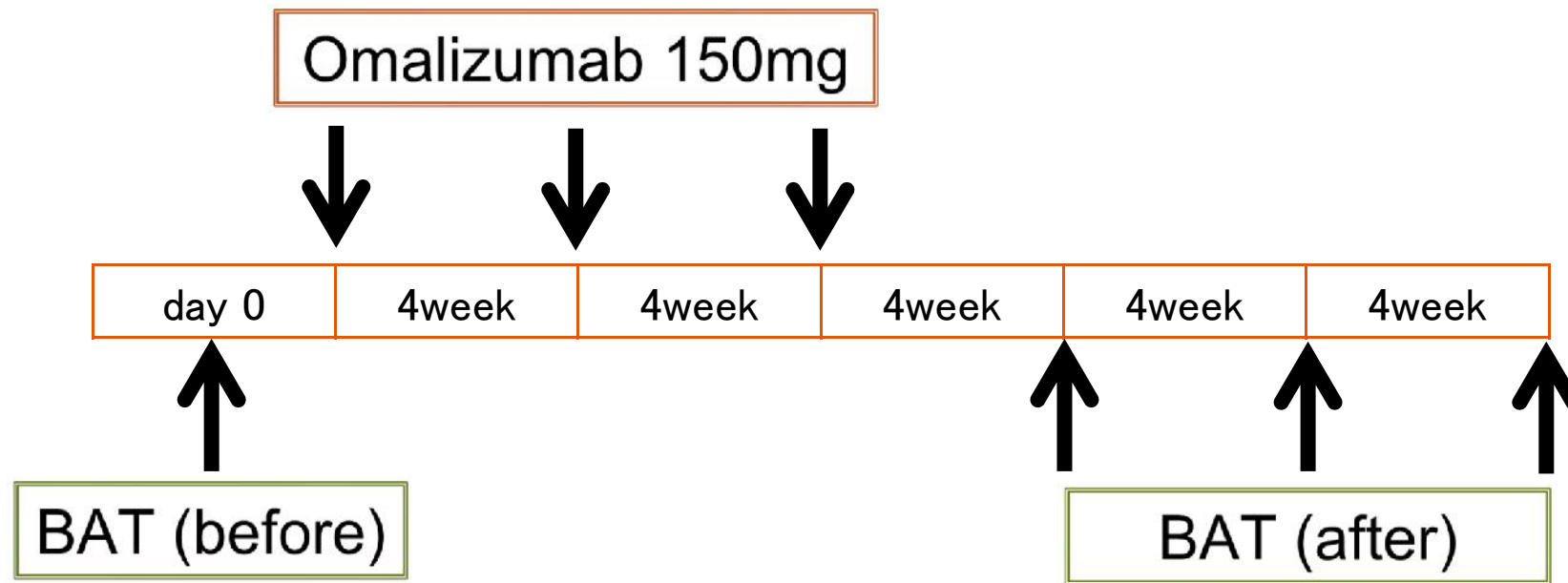
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Supplementary Methods. Preparation of wheat fractions.

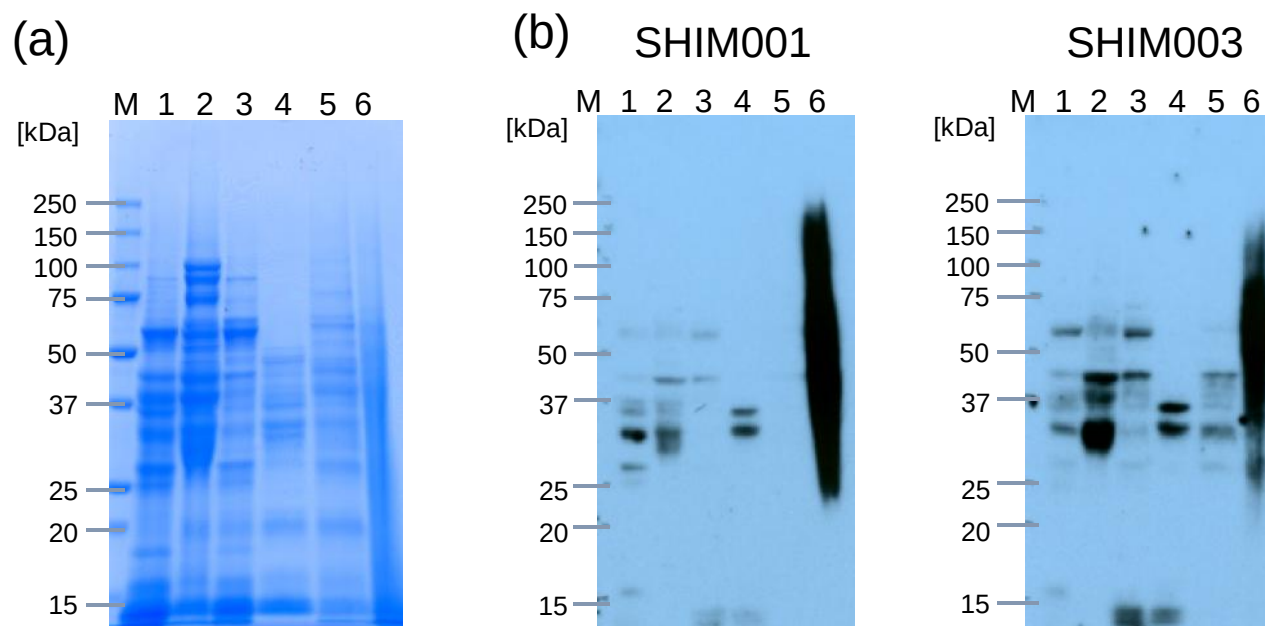
Commercial blend wheat flour (Camellia) was purchased from Nissin Flour Milling Inc., Kobe, Japan) for fractionation of wheat flour proteins. Wheat flour was mixed with 0.6 volumes of water with an arm kneader, and the dough was placed in water for 30 min. Water- soluble wheat proteins were extracted twice with two volumes of water with an arm kneader, and used for analysis (water-soluble wheat proteins). The residue was powdered with a tissue lyser (Qiagen, Tokyo, Japan) after lyophilization. The powdered water-insoluble wheat proteins were used for subsequent experiments (water-insoluble wheat proteins).

Four grams of wheat flour were ultrasonicated in 40 mL of PBS on ice for 15 minutes and stirred at 4°C overnight, then the supernatant was obtained after centrifugation at 20,000 g for 15 minutes (water/salt-soluble fraction; PBS fraction). After washing with PBS three times, the sediment was then dissolved in 40 mL of 70% ethanol (EtOH) with ultrasonification on ice for 15 minutes. The supernatant was obtained after centrifugation at 20,000 g for 15 minutes (water/salt insoluble, EtOH fraction). After washing with 70%EtOH three times, the sediment was further dis-solved in 40 mL of 2%Na₂CO₃/0.1N NaOH and the supernatant was obtained as an alkaline soluble fraction (alkali fraction). The fractions were dialyzed in PBS overnight and protein concentrations were determined with a densitometry, then diluted to 100 µg/ml in PBS and stored at -20 until use.



Supplementary Figure 1. Overall study design.

Omalizumab (150 mg) was injected subcutaneously at day 0, 4 weeks, 8 weeks, and CD203c-based basophil activation test (BAT) was performed day 0, and 4, 8 and 12 weeks after the omalizumab treatment.



Supplementary Figure 2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting analyses of wheat protein fractions and HWP.

(a) Gel stained with Coomassie Brilliant Blue; (b) Immunoglobulin E (IgE) immunoblotting with the patients' sera (2 HWP-allergy patients). M, marker proteins; Lane 1, water-soluble wheat proteins (10 μ g/lane); Lane 2, water-insoluble wheat proteins (10 μ g/lane); Lane 3, PBS (5 μ g/lane); Lane 4, EtOH (5 μ g/lane); Lane 5, Alkali (5 μ g/lane), Lane 6, HWP (10 μ g/lane).