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Letter to the Editor

A case of millet allergy that developed into wheat-induced anaphylaxis by cross-reaction between millet and wheat antigens

Dear Editor,

Millet allergy causes asthmatic attacks by inhalation, or urticarial symptoms by oral ingestion of antigens in patients sensitized to millet.^{1–3} It may result in anaphylactic reactions in severe cases. In Japan, millet is commonly consumed as birdseed or as natural health food, but little is known about its cross-reactivity with other grains and natural course of the sensitivity. We, here, report a case of millet allergy, where a patient developed severe allergic reactions against wheat by cross-reaction with millet antigens. Immunoblotting confirmed cross-reactivity between millet and wheat antigens, and the sensitization decreased after avoiding exposure to the antigens. Procedures of skin testing, protein extraction and immunoblotting are provided in the [Supplementary Methods](#).

A 59-year-old Japanese woman visited us with a history of allergic symptoms induced by wheat intake. She had fed a budgerigar with birdseed containing millet in her room since the age of 43. She had suffered from asthma around the same period. At the age of 55, she developed serious urticaria and respiratory symptoms 30 min after eating a pancake and during a bicycle ride. She also repeatedly had difficulty in breathing after ingesting wheat in white sauce or hamburger without exercise. Because of the increase of anti- ω -5 gliadin-IgE, which implied the diagnosis of wheat allergy, she was advised to avoid eating wheat products. Nevertheless, she developed anaphylactic shock 20 min after eating noodle that was free of wheat but contained millet. IgE titers (ImmunoCAP™, Thermo Fisher Scientific/Phadia AB, Uppsala, Sweden) against several types of millet were high along with increased titers to gluten, ω -5 gliadin, and wheat ([Table 1](#)). Skin prick testing showed positive reactions to bread, wheat, and birdseed along with extensive reactions to Japanese millet and foxtail millet, the main constituents of the birdseed that this patient fed to her budgerigar. A diagnosis of millet- and wheat-induced anaphylaxis was made by these observations.

Western blotting with the patient's serum revealed IgE-binding to proteins of foxtail millet, Japanese millet and birdseed as well as fractionated wheat proteins ([Fig. 1a](#)). The bindings to wheat proteins were abolished by pre-treatment of the serum with lysates of foxtail millet, Japanese millet or birdseed ([Fig. 1b](#)). However, pre-treatment of the serum with wheat proteins did not achieve a significant reduction of IgE-binding to proteins of foxtail millet, Japanese millets and birdseed ([Fig. 1b](#)). This result of the competitive western blotting indicated that all IgE-binding determinants in wheat are present in millets, while millets had IgE determinants

other than those shared among wheat and millet antigens. Thus, the patient was considered to primarily sensitized by millet in birdseed, and a part of the patient's IgE antibodies against millet proteins was also cross-reactive to wheat antigens, leading to the development of wheat allergy. After diagnosis, she avoided exposure to birdseed dust and ingestion of grains and wheat products as much as possible. After 6 months following her first visit to us, her budgerigar died, and she completely stopped being exposed to birdseed and wheat. In this course, titers of specific IgE against wheat and grains have gradually decreased ([Table 1](#)).

In the literature, allergic sensitization to millet has been assumed to be induced by repeated inhalation of millet dust.^{1–3} Bird-keepers who daily inhale the dust of birdseed containing millets are at a high risk of developing millet allergy.³ Our patient fed a budgerigar in her room with birdseed containing Japanese millet and foxtail millet, and was daily exposed to airborne millet, possibly resulting in the respiratory sensitization to the millets. Several proteins of millet electrophoresed at 36, 60, 70 and/or 90 kDa have been reported as antigens for IgE of patients.^{1,3} In this study, serum IgE of the patient mainly bound to approximately 28, 36, 50, 56 and 60 kDa proteins of millet.

Cross-reactivity between millet and other grains such as barley, oats, rice and wheat has been reported in a certain population of patients with millet allergy,^{2–5} but not in others.^{1,3,6} Our patient showed the presence of specific IgE against several grains including millet and wheat in her serum. The result of competitive immunoblotting analysis suggests the primary sensitization to millet by birdseed containing several types of millet and the secondary cross-reactivity to wheat antigens in both water-soluble and insoluble fractions. The onset of asthma around the period when she

Table 1
Specific IgE titers against grains measured by the ImmunoCAP™ method.

Antigen	Antigen specific IgE titers (UA/mL)			
	0 mo	+6 mo	+10 mo	+13 mo
Gluten	2.80	2.37	1.65	1.27
ω -5 gliadin	2.61	2.09	1.46	1.10
Wheat	27.9	2.82	2.77	2.03
Rye	62.9	N.D.	N.D.	N.D.
Barley	58.8	N.D.	N.D.	N.D.
Oats	≥ 100	30.8	27.8	25.6
Common millet	≥ 100	36.9	28.4	N.D.
Foxtail millet	≥ 100	58.6	48.2	46.6
Japanese millet	≥ 100	37.0	33.0	N.D.

mo, months; N.D., not done.

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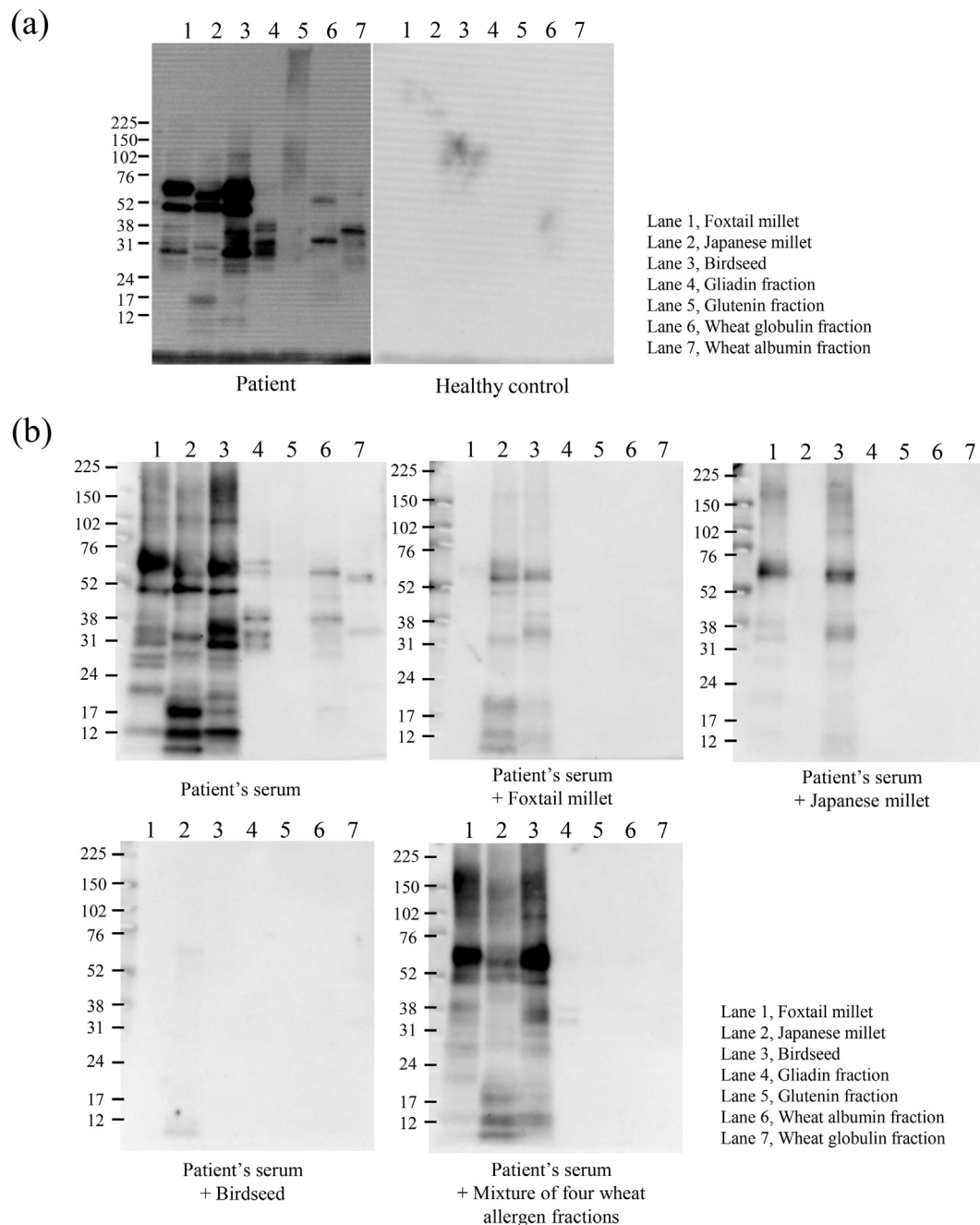


Fig. 1. (a) Immunoblotting with the patient's serum detected by horse radish peroxidase (HRP)-conjugated goat anti-human IgE. Lysates of foxtail millet, Japanese millet and birdseed, and four fractionated wheat allergen solutions were loaded for SDS-PAGE, and immunoblotting was performed with sera of the patient and a healthy control. The patient revealed IgE binding mainly to 28, 35, 50, 56 and 60 kDa-proteins in foxtail millet, Japanese millet and birdseed. As for wheat proteins, the serum IgE bound to 28, 31, 37 and 38 kDa-proteins in gliadin fraction, as well as 31 and 52 kDa in globulin, and 37 kDa in albumin fractions. No IgE-binding was detected with serum of a healthy control. (b) Competitive immunoblotting with the patient's serum pre-treated with several grain- and the birdseed-extracts and wheat allergens. The patient's serum was pre-incubated with a lysate of foxtail millet, Japanese millets, birdseed, or the mixture of four wheat protein fractions, and then subjected to immunoblotting. The bindings of the patient's IgE to fractionated wheat proteins were abolished by the pre-treatments with lysates of foxtail millet, Japanese millets or birdseed. On the other hand, pre-treatment with the mixture of four wheat protein fractions did not achieve significant abolishment of IgE-binding to proteins of foxtail millet, Japanese millets and birdseed, except partial inhibition observed at ~12, 17, 28 and 37 kDa-bands. Note: Lane 6 and 7 are mixed up in panel (a) and (b).

started to feed a bird implies the early sensitization to millet. It is noteworthy that our patient developed the first severe allergic reaction against wheat while being unaware of millet allergy. Thus, patients with wheat allergy should be suspected of relevant allergy to millet, and hypersensitivity must be investigated through careful history taking and, if applicable, subsequent examinations.

There are few reports on the prognosis of millet allergy. While Hemmers *et al.* mentioned long-lasting retention of the

sensitization to millet in several patients,² our case showed that avoiding exposure to birdseed and grains resulted in a decrease of IgE titers to grains including millet and wheat. Takahama *et al.* also reported that the avoidance of millet decreased IgE against millet after 3 years in a woman with millet allergy.⁶ Further, Inomata *et al.* reported a case of bird-egg syndrome, where avoiding exposure to the antigens normalized IgE titers to bird feather/feces, egg and chicken meat, resulting in the successful induction of

tolerance to egg and chicken meat in 7 years.⁷ In a report on the prognosis of patients with wheat allergy sensitized to hydrolyzed wheat protein (HWP) in facial soap, the hypersensitivity against HWP decreased in clinical reactivity and histamine release assay after discontinuation of soap use.⁸ These facts suggest that, in a certain type of food allergies, involving sensitization by respiratory or percutaneous exposure of antigens, the avoidance of causative antigens may reduce the sensitivity and lead to the development of tolerance. Collectively, this case presented that millet allergy in a bird-keeper was initially recognized by a symptom of an allergic reaction against wheat, and the hypersensitivity against millet and the cross-reactivity against wheat chronologically decreased over time of the avoidance of causative antigens.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.alit.2019.12.011>.

Conflict of interest

The authors have no conflict of interest to declare.

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References

1. Rombold S, Ollert M, Sbornik M, Rakoski J, Darsow U, Ring J. Immediate-type respiratory allergy to millet-containing seed mixture of bird food. *World Allergy Organ J* 2008;**1**:135–7.
2. Hemmer W, Sesztak-Greinecker G, Wohrl S, Wantke F. Food allergy to millet and cross-reactivity with rice, corn and other cereals. *Allergol Int* 2017;**66**:490–2.
3. Bohle B, Hirt W, Nachbargauer P, Ebner H, Ebner C. Allergy to millet: another risk for atopic bird keepers. *Allergy* 2003;**58**:325–8.
4. Urisu A, Yamada K, Masuda S, Komada H, Wada E, Kondo Y, et al. 16-kilodalton rice protein is one of the major allergens in rice grain extract and responsible for cross-allergenicity between cereal grains in the Poaceae family. *Int Arch Allergy Appl Immunol* 1991;**96**:244–52.
5. Stuck BA, Blum A, Klimek L, Hormann K. Millet, a potentially life-threatening allergen. *Allergy* 2001;**56**:350.
6. Takahama H, Shimazu T. Common millet anaphylaxis: a case of a bird-keeper sensitized to millet via inhalation, who developed anaphylaxis after oral ingestion. *Clin Exp Dermatol* 2008;**33**:341–2.
7. Inomata N, Kawano K, Aihara M. Bird-egg syndrome induced by alpha-livetin sensitization in a budgerigar keeper: successful induction of tolerance by avoiding exposure to avians. *Allergol Int* 2019;**68**:282–4.
8. Hiragun M, Ishii K, Yanase Y, Hiragun T, Hide M. Remission rate of patients with wheat allergy sensitized to hydrolyzed wheat protein in facial soap. *Allergol Int* 2016;**65**:109–11.

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Supplementary Methods

Skin prick testing

The prick test for wheat and bread was performed by using commercial reagents (Torii Pharmaceutical Co., Ltd., Tokyo, Japan), while the skin reactivity in response to Japanese millet, foxtail millet, and birdseed obtained from a pet shop were examined by the prick-to-prick method.

Protein extraction

Proteins from foxtail millet, Japanese millet, and birdseed obtained from a pet shop were extracted from crushed and homogenized grain samples into RIPA buffer (Thermo Fisher Scientific, Waltham, MA, USA) by sonication. After centrifugation, the supernatants were collected and stored for the following experiments. Fractionated wheat proteins were prepared by purchasing commercially available purified wheat proteins of gliadin and glutenin (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan), and by fractionating wheat extract according to the solubility characteristics of albumin and globulin^{1, 2}. Briefly, the latter fractions of albumin and globulin were extracted by stirring 10 g wheat flour with 100 mL of 0.5 M NaCl. After centrifugation, the supernatant was subjected to the dialysis with distilled water. The dialyzed solution was centrifuged, and then the supernatant and the pellet were separately freeze-dried and collected as the albumin fraction and the globulin fraction, respectively. The protein concentrations were measured by BCA kit (Thermo Fisher Scientific, Waltham, MA, USA).

SDS-PAGE and immunoblotting

The denatured protein samples were loaded at 0.3 µg/lane into the 5-20% gradient SDS-PAGE gel (ATTO, Tokyo, Japan) and transferred to a polyvinylidene difluoride membrane (Millipore, Billerica, MA) using a semi-dry blotting system (ATTO, Tokyo, Japan). After blocking by Blocking one (Nakalai tesque, Inc., Kyoto, Japan) for 1 hour, the membrane was incubated with the patient's serum diluted to 1:100 in a sample buffer (TOYOBO, Tokyo, Japan) at 4 °C overnight. After washing, the membrane was incubated with horse radish peroxidase (HRP)-conjugated goat anti-human IgE antibody (KPL, MD, USA) at room temperature for 1 hr, followed by the detection of chemiluminescence. In the competitive assay of immunoblotting, the patient's serum

was pre-incubated with 30 µg/ml of each inhibitor protein sample.

1. Chinuki Y, Morita E. Wheat-dependent exercise-induced anaphylaxis sensitized with hydrolyzed wheat protein in soap. *Allergol Int* 2012; **61**: 529-37.
2. Sandiford CP, Tatham AS, Fido R, Welch JA, Jones MG, Tee RD, et al. Identification of the major water/salt insoluble wheat proteins involved in cereal hypersensitivity. *Clin Exp Allergy* 1997; **27**: 1120-9.