Identification of Ribosomal Proteins as Cross-Reactive Allergens in a Case of Mushroom Food Allergy

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Although a variety of mushroom species are commonly consumed worldwide, mushrooms are a rare cause of IgE-mediated hypersensitivity reactions. One of the common characteristics of mushroom allergy is cross-reactivity among fungal species; however, mushroom allergens are poorly characterized [1–4]. Here, we have presented a Japanese case of immediate-type food allergy caused by four popular mushroom species in which ribosomal proteins were identified as cross-reactive mushroom allergens.

A 21-year-old Japanese man had a 7-year history of recurrent episodes of oral allergic symptoms (oral irritation, throat discomfort, and itching) and cough immediately after consuming meals containing each one of shiitake (*Lentinula edodes*), brown beech (*Hypsizygusmarmoreus*), king trumpet (*Pleurotuseryngii*), or hen-of-the-woods (*Grifolafrondosa*) mushrooms and the broth of shiitake mushrooms. These symptoms resolved spontaneously within 30–60 minutes of onset. He visited our

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hospital for further examination of mushroom allergy. He did not experience any food allergy symptoms after consuming meals without mushrooms. He had a history of asthma and atopic dermatitis from the age of 10–12 years. His total IgE level was 457.0 IU/mL, and the multi-panel IgE test (View Allergy 39®, Thermo Fisher Diagnostics K.K, Tokyo, Japan) revealed positivity for the following allergen-specific IgEs: Japanese cedar (index value: 15.63), Japanese cypress (8.76), Timothy (12.65), orchard grass (16.78), house dust (4.32), *Dermatophagoides pteronyssinus* (5.50), and shrimp (0.53). In this test, *Alternaria*- (0.48) and *Aspergillus*-specific IgEs (0.33) were detected at the suspected level(index value <0.50).A prick-to-prick test showed a wheal size of 8×7 mm with 10 mg/mL histamine (Torii Pharma, Tokyo, Japan), 1×1 mm with saline, 5×5 mm (2+) with raw *Ledodes*, 5×5 mm (2+) with broth of *Ledodes*, 8×9 mm (3+) with raw *H marmoreus*, 12×7 mm (3+) with raw *Gfrondosa*, 10×7 mm (3+) with raw *Flammulinavelutipes*, and0×0 mm (-) with raw *Auriculariaauricula-judae*.

To explore mushroom allergens, 5 g of edible parts of *Ledodes*, *H marmoreus*, *Peryngii*, and *Gfrondosa*were minced and homogenized with 1,000 μL of ice-cold phosphate-buffered saline (PBS). After centrifugation at 21,500×*g* for 10 min, the supernatant of each sample was collected as PBS-soluble protein. SDS-PAGE and immunoblotting were performed, as described previously [5], using40 μg of each

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PBS-soluble protein and 15% polyacrylamide gel. As negative controls, serum from healthy subject and blocking reagent (5% skim milk in Tris-buffered saline containing 0.1% Tween-20) were used as negative controls. Serum IgE antibodies were specifically reacted with a 15-kDa protein for *L edodes* (Supplementary Figure,A, lane L) and *P eryngii* (Supplementary Figure, A, lane P). To purify the 15-kDa allergen in PBS-soluble proteins of *L edodes*, the proteins were fractionated by precipitation with ammonium sulfate and dissolved in PBS as previously described[5]. Immunoblotting of each fraction revealed that the 15-kDa allergen precipitated with 30%–40% ammonium sulfate (Supplementary Figure, B, lane 40).

Next, to identify the 15-kDa allergens of *L edodes* (Supplementary Figure, B, lane 40) and *P eryngii* (Supplementary Figure, A, lane P), both protein bands were excised from the Coomassie brilliant blue-stained gel, and the mass spectra of these samples were obtained as previously described [5]. The generated mass lists were searched against the protein databases of *Ledodes*(txid5353) and *Peryngii* (txid5323) from NCBI (access date: 2020.12.19) using the database search software ProteinPilot™ (ver. 4.5; AB SCIEX LLC, Framingham, MA, USA). The15-kDa allergens of *Ledodes*and *Peryngii* were identified as ribosomal protein S8 (accession no. GAW05875.1) and ribosomal protein S15a (accession no. KAF9498209.1), respectively

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(Table). Additionally, we found that the primary structure of these two proteins was significantly similar to 110/127 (87%) amino acid identities using the basic local alignment search tool (BLAST).To evaluate the IgE cross-reactivity between *L edodes* and *P eryngii*, the serum of the patient was pre-incubated with fractionated *L edodes* proteins (Supplementary Figure, B, lane 40; 0, 1, 10 μg) for 2 h at 37°C. As expected, IgE binding to the 15-kDa allergens for *Ledodes* and *Peryngii* was inhibited by preincubation with fractionated *Ledodes* proteins in a concentration-dependent manner (Supplementary Figure, C). These results suggest that ribosomal proteins from *Ledodes* and *Peryngii* mushrooms have cross-reactivity. In this case, we could not determine the cross-reactivity of *H marmoreus* and *G frondosa* because we did not obtain the specific IgE-binding to these proteins in immunoblot analysis. Further considerations are necessary to extract the allergens from these mushrooms.

Allergy to*L edodes* has rarely been reported and it allergens have not been identified. Ito et al. reported that IgE antibodies from a patient with food allergy with three different mushroom species (shiitake, shimeji, and maitake) reacted with the 15-kDa proteins for both raw and boiled *Ledodes* extracts [6]. Pravettoni et al. reported a patient with severe work-related asthma caused by *Ledodes* packaging and confirmed IgE reactivity to *Ledodes* proteins (15 kDa and 24 kDa)[7]. Thus, ribosomal protein S8

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(15 kDa) maybe a major allergen in *Ledodes*. To our knowledge, *Peryngii* allergy has not been previously reported.

Ribosomal proteins of fungi, such as *Aspergillus fumigatus* and *Alternaria alternata*, are known as mold allergens. These fungi mainly cause respiratory allergic diseases. Food allergies to my coproteins or button mushroom (*Agaricusbisporus*) due to cross-reaction to mold shave been reported[2–4]. In these reports, the patients' IgE reacted to acidic ribosomal protein P2[2], manganese-dependent superoxide dismutase (MnSOD) [3],orporin family protein [4].In our patient, a low titer of specific IgE for *Alternaria* and *Aspergillus* was detected in the serumusing View Allergy 39®; however, the patient was not sensitized to any mold allergen components contained in ImmunoCAP ISAC™ test (Thermo Fisher Diagnostics), including Asp f 6 (MnSOD from *A. fumigatus*). Furthermore, BLAST analysis revealed that the ribosomal protein S8 (*Ledodes*) and ribosomal protein S15a (*Peryngii*) are not homologous to any reported mold ribosomal protein allergens. In a study by Kayode et al., four patients with mushroom allergy showed positive reactions to multiple mushroom species on the prick-to-prick test; however, only one patients showed positive results for fungal aeroallergens in the skin prick test [1]. The sensitization route should be determined to establish the clinical relevance of mold sensitization and mushroom food allergy and

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reveal the pathogenesis of mushroom allergy due toribosomal proteins.

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Conflict of interests

The authors have no conflicts of interest to declare.

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Tables

Table 1. Amino acid sequence of identified mushroom ribosomal proteins

Origin	Amino acid sequences					
L edodes	1MVRISVLNDC	LNNIVNAERR	GKRQVLVRPS	SKVVVKFLSV	MQRHGYIGEF	EIIDDHRAGK
P eryngii	1MVRVSVLNDC	LNNMVNAERR	GKRQVLVRPS	SKVVVKFLSV	MQRHGYIGEF	EIIDDHRSGK
L edodes	IVIQLNGRLN	KTGVISPRFN	VQVTQIESWV	NLLLPSRGFG	IIILTTSSGI	LDHEEARRKN
P eryngii	IVVQLNGRLN	KTGVISPRYN	IQANQIESWV	NLLLPARSFG	YIILTTSSGI	MDHEEARRKN
L edodes	VGAMFVAPRR	----------	----------	----------	NHDSHSLTAT	ELP 493
P eryngii	VGGKLLGYVY	130				

The name of each protein is as follows: *L edodes*, ribosomal protein S8 (accession no. GAW05875.1) and *P eryngii*, ribosomal protein S15a (accession no. KAF9498209.1).

Shaded characters: matched amino acid residues between two mushroom proteins.

Underlined characters: identified peptides by mass spectrometry and ProteinPilot™ analysis.

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