Identification of Ribosomal Proteins as Cross-Reactive Allergens in a Case of Mushroom 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 most common characteristics of mushroom allergy is cross-reactivity between fungal species; however, mushroom allergens are poorly characterized [1-4]. Here, we present a Japanese case of immediate-type food allergy caused by 4 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 of shiitake (*Lentinula edodes*), brown beech (*Hypsizygus marmoreus*), king trumpet (*Pleurotus eryngii*), or hen-of-the-woods (*Grifola frondosa*) mushrooms and the broth of shiitake mushrooms. These symptoms resolved spontaneously within 30-60 minutes of onset. He visited our 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 multipanel IgE test (View Allergy 39, Thermo Fisher Diagnostics K.K.) revealed positivity for the following allergen-specific IgEs: Japanese cedar (index value, 15.63), Japanese cypress (8.76), timothy grass (12.65), orchard grass (16.78), house dust (4.32), *Dermatophagoides pteronyssinus* (5.50), and shrimp (0.53). In this test, *Alternaria*-specific IgE (0.48) and *Aspergillus*-specific IgE (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), 1×1 mm with saline, 5×5 mm (2+) with raw *L edodes*, 5×5 mm (2+) with broth of *L edodes*, 8×9 mm (3+) with raw *H marmoreus*, 12×7 mm (3+) with raw *G frondosa*, 10×7 mm (3+) with raw *Flammulina velutipes*, and 0×0 mm (–) with raw *Auricularia auricula-judae*.

To explore mushroom allergens, 5 g of edible parts of *L edodes*, *H marmoreus*, *P eryngii*, and *G frondosa* were minced and homogenized with 1000 μL of ice-cold phosphate-buffered saline (PBS). After centrifugation at 21 500*g* for

Table. Amino Acid Sequence of Identified Mushroom Ribosomal Proteins

Origin		Amino acid sequences					
L edodes	1	MVRISVLNDC	LNNIVNAERR	GKRQVLVRPS	SKVVVKFLSV	MQRHGYIGEF	EIIDDHRAGK
P eryngii	1	MVRVSVLNDC	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 2 mushroom proteins.
Underlined characters: peptides identified by mass spectrometry and ProteinPilot analysis.

10 minutes, the supernatant of each sample was collected as PBS-soluble protein. SDS-PAGE and immunoblotting were performed as described previously [5] using 40 μg of each PBS-soluble protein and 15% polyacrylamide gel. Serum from healthy individuals 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 *L edodes* (txid5353) and *P eryngii* (txid5323) from the National Center for Biotechnology Information (access date: December 19, 2020) using the database search software ProteinPilot (ver. 4.5; AB SCIEX LLC). The 15-kDa allergens of *L edodes* and *P eryngii* were identified as ribosomal protein S8 (accession no. GAW05875.1) and ribosomal protein S15a (accession no. KAF9498209.1), respectively (Table). Additionally, we found that the primary structure of these 2 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 patient's serum was preincubated with fractionated *L edodes* proteins (Supplementary Figure, B, lane 40; 0, 1, 10 μg) for 2 hours at 37°C. As expected, IgE binding to the 15-kDa allergens for *L edodes* and *P eryngii* was inhibited by preincubation with fractionated *L edodes* proteins in a concentration-dependent manner (Supplementary Figure, C). These results suggest that ribosomal proteins from *L edodes* and *P eryngii* mushrooms are cross-reactive. In this case, we could not determine the cross-reactivity of *H marmoreus* and *G frondosa* because we did not obtain data on the specific IgE-binding to these proteins in the immunoblot analysis. Further testing is necessary to extract the allergens from these mushrooms.

Allergy to *L edodes* is very uncommon, and its allergens have not been identified. Ito et al [6] reported that IgE antibodies from a patient allergic to 3 different mushroom species (shiitake, shimeji, and maitake) reacted with the 15-kDa proteins for both raw and boiled *L edodes* extracts. Pravettoni et al [7] reported a patient with severe work-related asthma caused by *L edodes* packaging and confirmed IgE reactivity to *L edodes* proteins (15 kDa and 24 kDa). Thus, ribosomal protein S8 (15 kDa) may be a major allergen in *L edodes*. To our knowledge, *P eryngii* allergy has not been previously reported.

Ribosomal proteins of fungi such as *Aspergillus fumigatus* and *Alternaria alternata* are known mold allergens. These fungi mainly cause respiratory allergic diseases. Food allergies to mycoproteins or button mushroom (*Agaricus bisporus*) due to cross-reaction to molds have been reported [2-4]. In these reports, the patients' IgE reacted to acidic ribosomal protein P2 [2], manganese-dependent superoxide dismutase (MnSOD) [3], or porin family protein [4]. In the case we report, a low titer of specific IgE for *Alternaria* and *Aspergillus* was detected in serum using View Allergy 39; however, the patient was not sensitized to any of the mold allergen components in the ImmunoCAP ISAC test (Thermo Fisher Diagnostics), including Asp f 6 (MnSOD from *A fumigatus*). Furthermore, BLAST analysis revealed that ribosomal protein S8 (*L edodes*) and ribosomal protein S15a (*P eryngii*) are not homologous to any reported mold ribosomal protein allergens. In a study by Kayode et al [1], 4 patients with mushroom allergy had positive reactions to multiple mushroom species on the prick-to-prick test; however, only 1 patient had positive results for fungal aeroallergens in the skin prick test. The route of sensitization should be determined to establish the clinical relevance of mold sensitization and mushroom food allergy and reveal the pathogenesis of mushroom allergy due to ribosomal proteins.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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