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ORIGINAL ARTICLE

Evaluation of serum IgE in peach-allergic patients with systemic reaction by using recombinant Pru p 7 (gibberellin-regulated protein)

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KEYWORDS
Component-resolved diagnosis (CRD);
Fruit allergy;
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Abstract
Background: Lipid transfer protein (LTP) is a major fruit allergen. It has, however, recently been revealed that the systemic reaction in peach-allergic patients is related not only to LTP (Pru p 3) but also to gibberellin-regulated protein (Pru p 7). We investigated recombinant Pru p 7 (rPru p 7) for its potential use in worldwide standardization for the diagnosis of peach allergy.
Methods: Natural Pru p 7 (nPru p 7) was purified from peach crude extract using a monoclonal antibody affinity column. Complementary DNA for Pru p 7 was cloned and expressed in *Escherichia coli* and *Pichia pastoris*. Serum immunoglobulin (Ig) E in peach-allergic patients was examined by enzyme-linked immunosorbent assay (ELISA) using nPru p 7 and rPru p 7 (*E. coli* product: erPru p 7 and *P. pastoris* product: prPru p 7).

Abbreviations: cDNA, complementary DNA; CRD, component-resolved diagnosis; DTT, dithiothreitol; ELISA, enzyme-linked immunosorbent assay; FDEIA, food-dependent exercise-induced anaphylaxis; GRP, gibberellin-regulated protein; Ig, immunoglobulin; LTP, lipid transfer protein; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; PFAS, Pollen Food Allergy Syndrome; OR, oral reaction; SR, systemic reaction; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.

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Peach;
Pichia pastoris;
Recombinant allergen

Results: Peach-allergic patients ($n = 27$) were diagnosed and categorized into oral reaction ($n = 10$) or systemic reaction ($n = 17$). The nPru p 7 positivity based on serum IgE levels was 52% in the systemic-reaction group and 0% in the oral-reaction group ($P < 0.05$). In the systemic-reaction group, there was no significant difference in reactivity between nPru p 7 and prPru p 7, but the reactivity of erPru p 7 was significantly lower than those of nPru p 7 and prPru p 7 ($P < 0.05$).
Conclusions: We found that prPru p 7 exhibited reactivity in ELISA comparable to that of nPru p 7 for the diagnosis of peach allergy with systemic reaction.

Introduction

Peach (*Prunus persica*) allergy is one of the most common allergies in the Mediterranean region and has been reported in Japan.[1–7] The number of reports of peach allergy concerning component-resolved diagnosis (CRD) has recently increased.[8–10] CRD may be useful for clinical-type classification.[6,11] Although Pru p 3 is known to be a popular plant allergen component, some cases of systemic reaction (SR) cannot be explained by Pru p 3.[6,12,13] Pru p 3 is typically present in the outer part of the peach, and it sometimes does not cause symptoms when the peeled peach is eaten.[14] Tuppo reported Pru p 7 as a new peach allergen in Europe.[15] In Japan, many peach-allergic patients with SRs have shown sensitivity to Pru p 7, although few patients have shown sensitivity to Pru p 3.[6,16] Therefore, the contribution of Pru p 7 to peach allergy should be revealed promptly and globally. However, it is very difficult to obtain Pru p 7 free from Pru p 3 because of their similar protein chemical properties.[15,17] Therefore, the development of recombinant Pru p 7 (rPru p 7) is an urgent issue for the advancement of Pru p 7 research and diagnosis.[7,18,19] For the expression of recombinant proteins, *P. pastoris* is widely used.[20–25] Pokaj found that *P. pastoris* is superior to *Escherichia coli* as an expression system for the production of large quantities of soluble, properly folded, and biologically active rCor a 8 [hazelnut lipid transfer protein (LTP)].[26]

In this study, we comparatively examined serum immunoglobulin (Ig) E levels in peach-allergic patients by enzyme-linked immunosorbent assay (ELISA) using natural Pru p 7 (nPru p 7) and rPru p 7 (*E. coli* product: erPru p 7 and *P. pastoris* product: prPru p 7) purified using monoclonal antibody (mAb) columns.

Methods

Study participants

A total of 84 participants with fruit allergies (including allergies except peaches) were enrolled in the Fruits Allergy Component Study Group (http://www.fruit-allergy.jp/) from June 2014 to December 2015. After enrolment, we applied to the ethical review boards of each facility and examined only those who were approved after obtaining written informed consent. Exclusion criteria included participants who had no symptoms when they ate peach or those whose symptoms were unclear or not reproducible. After obtaining informed consent, the participants answered the questionnaire and then underwent a skin-prick test with peach. This study was conducted according to the World Medical Association's Declaration of Helsinki. Ethical approval was obtained from the Fujita Health University Ethics Committee in July 2014 (reference number: 14-075) for all sites taking part in this study.

Definition of symptoms

We evaluated the following types of symptoms induced by the ingestion of peach according to the participant's questionnaire as follows: Oral reaction (OR): Itching or tingling sensations in the oral mucosa, palate, or throat that developed within 5–10 min after peach ingestion and localized symptoms with no SR.[27] SR: Symptoms presented in $\geq$2 organs after peach ingestion. Systemic symptoms included anaphylaxis and food-dependent exercise-induced anaphylaxis.

Preparation of peach crude extract

Peaches (*P. persica*, cultivar Asama–Hakutou strain) at the commercial ripening stage were obtained from a local store. Entire peach fruits (peel and pulp) were homogenized with an extraction solution (2 mM disodium ethylenediamine tetraacetate, 10 mM sodium N,N-diethyldithiocarbamate, 3 mM sodium azide, and 2% solid polyvinyl polypyrrolidone) at a 1:1 [w:v] ratio. After filtration through gauze, the homogenate was centrifuged at 10,000 $\times$ *g* for 15 min at 4 °C. A cation exchange resin (Toyopearl CM-650M; TOSO, Tokyo, Japan) was added to the supernatant and mixed overnight at 4 °C. The resin was collected by centrifugation, packed, and washed with 20 mM phosphate buffer at pH 5.0 in a column. The proteins absorbed to the resin were eluted as the crude extract with 0.5 M sodium chloride in the same buffer.

Production of mAbs and purification of natural antigens

The production of hybridomas producing mAbs specific to peach Pru p 7 or Pru p 3 and the purification of natu-

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