Li et al. World Allergy Organization Journal (2024) 17:100922
http://doi.org/10.1016/j.waojou.2024.100922

WORLD ALLERGY ORGANIZATION JOURNAL

Open Access

Pru p 3-specific IgE affinity is crucial in severe peach-allergy patients

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ABSTRACT

Background: Peach allergy is common food allergen. Allergen components-specific antibodies of different isotypes in peach-allergy patients are poorly studied. Factors other than Pru p 3-sIgE levels may be related to severe symptoms.

Objective: To evaluated peach component-specific-IgE, IgG1, and IgG4 characteristics in individuals with and without peach allergy, and Pru p 3-sIgE affinity in patients with different clinical symptoms.

Methods: Fifteen healthy controls and 32 peach-allergy patients were enrolled. sIgE, sIgG1, and sIgG4 to 5 *Escherichia coli*-expressed peach-allergen components were determined by enzyme-linked immunosorbent assays. Pru p 3-sIgE affinity was measured in Pru p 3-sIgE-positive patients, using immunoadsorbance.

Results: Patients were divided into oral allergy syndrome (OAS) and peach-induced anaphylaxis (PIA) groups. Serum Pru p 1-, Pru p 2-, Pru p 3-, Pru p 4-, and Pru p 7-sIgG1s were detected. Pru p 1- and Pru p 2-sIgG1 levels were higher in healthy controls, but Pru p 3-sIgG1 levels were significantly higher in peach-allergy patients. Pru p 1-, Pru p 3-, and Pru p 4-sIgG4-positivity was significantly greater among patients than among controls. Pru p 3 was the predominant allergen in peach-allergy patients. Allergen-sIgG1 and sIgG4 were similar between OAS and PIA patients. Pru p 3-sIgE levels were significantly higher in PIA patients, but Pru p 3-sIgE-positivity was similar in both groups. In Pru p 3-sIgE-positive patients, Pru p 3-sIgE affinity was significantly higher in PIA than OAS patients.

Conclusions: Allergen-sIgG1 was associated with allergen exposure. Both Pru p 3-sIgE levels and affinity are key factors in severe peach-allergy patients.

Keywords: Affinity, Oral allergy syndrome, Peach allergy, Peach-induced anaphylaxis, Pru p 3-specific IgE

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http://doi.org/10.1016/j.waojou.2024.100922
Received 12 January 2024; Received in revised from 14 May 2024; Accepted 24 May 2024
Online publication date xxx

INTRODUCTION

Fruits and vegetables, particularly peaches, are common causes of food-induced anaphylaxis in China.[1,2] Non-specific lipid transfer protein (nLTP), named Pru p 3, is the major peach allergen. Its standard folding structure is comprised by 4 α-helices, 4 conserved disulfide bridges, and a non-structured C-terminal coil. Hence, it has resistance to pyro-hydrolysis and enzymolysis.[3] Many studies have suggested that severe systemic clinical symptoms are usually associated with patients sensitized to nLTP.[4] In addition to Pru p 3, there are other peach components, namely, Pru p 1, Pru p 2, Pru p 4, and Pru p 7, which have similar structural and protein features as Pru p 3 and are associated with anaphylaxis.[5]

Unlike IgE antibodies, the role of IgG antibodies in allergic diseases is controversial.[6] Some studies have suggested that specific (s) IgG1 antibodies are associated with allergen exposure.[7,8] Moreover, sIgG4 as a blocking antibody were increased after allergen-specific immunotherapy and inhibits allergen binding to IgE.[9] Despite this, allergen-specific antibodies of other isotypes have less frequently been investigated in patients with peach allergy.

Although many studies have suggested that Pru p 3-sIgE levels were significantly higher in patients with anaphylaxis than in those with oral allergy symptoms,[2,10,11] clinical reactions such as mild oral symptoms or urticaria are always present in patients with higher Pru p 3-sIgE levels. It has been suggested that the sensitivity of histamine release is closely related to the affinity of IgE for its antigen.[12] Thus, irrespective of whether there are other factors that influence the clinical symptoms in patients with peach allergy, it is assumed that there is a higher binding affinity of Pru p 3-sIgE in patients with severe symptoms.

In this study, we aimed to evaluate the characteristics of peach allergen component-sIgE, -sIgG1, and sIgG4 in individuals with and without peach allergy, as well as the affinity of Pru p 3-sIgE for its antigen in patients with different clinical symptoms. To this end, we expressed 5 peach allergen components by using an *Escherichia coli* expression system, and established affinity measurements based on immunoadsorbent techniques.

METHODS

Peach allergic patient sera

Sera were obtained from 32 patients who had been diagnosed with peach allergy by an experienced allergist and 15 healthy control individuals. Healthy control individuals underwent routine allergen screening and medical history inquiries, confirming that they were non-atopic subjects. Patients were enrolled in an outpatient allergy department of the Peking Union Medical College Hospital from May 2018 to October 2019. The criteria for selection were as follows: (a) a convincing clinical history of allergic reactions after ingestion of peach within the 12 months prior to sera sampling; (b) specific-IgE levels to peach exceeding 0.35 KUA/L as determined by the ImmunoCAP system (ThermoFisher Scientific, Uppsala, Sweden); (c) naïve to allergen-specific immunotherapy. Based on the clinical manifestations, patients were categorized into 2 groups: oral allergy syndrome (OAS) and peach-induced anaphylaxis (PIA), where anaphylaxis was defined based on guidelines from the National Institute of Allergy and Infectious Diseases Demographic. Clinical data of these patients are shown in Table 1.

The study was reviewed and approved by the Ethical Committee of Peking Union Medical College Hospital. Written informed consent was obtained from each participant, or their parent or guardian.

Production of recombinant peach allergens in *E. coli*

Five recombinant allergens (Pru p 1, Pru p 2, Pru p 3, Pru p 4, and Pru p 7) were performed by the method of Sabrina et al.[13] with some modifications. In brief, nucleotide sequences of allergens were synthesized based on GenBank with the following accession numbers: DQ251187 (Pru p 1), EU424259 (Pru p 2), AJ277163 (Pru p 3), AJ491881 (Pru p 4), and XM72223776 (Pru p 7) after codon optimization. The synthetic nucleotide sequences of Pru p 1, Pru p 2 and Pru p 4 were inserted into pET-21a (BGI Geneland,

Jiangsu, China). The Pru p 3 and Pru p 7 genes were ligated into pCZN 1 vector (Biotyscience, Nanjing, China) using NdeI and *Xba*I sites. The ligated plasmids encoding peach allergens were transformed into *E. coli* BL21 (TransGen, Beijing, China) and Arctic-Express (Biotyscience, Nanjing, China). For the expression of allergens, *E. coli* were cultured in LB medium containing 50 µg/ml ampicillin. Then, protein expression was induced by adding isopropyl thio-β-D-galactoside at a final concentration of 0.5 mM. *E. coli* were cultured overnight at 20 °C and were collected by centrifugation. Proteins were extracted in phosphate-buffered saline (PBS) by dialysis and purified by Ni-chelating affinity chromatography on a 1-ml HisTrap FF 5 column (GE Healthcare, Chicago, IL, USA). After washing with 20 mM imidazole in Tris-HCl buffer (20 mM Tris, 0.15 M NaCl, pH 8.0), fractions were collected during a 20-ml linear gradient elution to 250 mM imidazole in the same buffer. Fractions containing purified proteins were pooled, dialyzed against PBS, and stored at −80 °C until further use.

Electrophoresis and immunoblotting

Recombination proteins were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) using 12% gels under reducing conditions and Coomassie Brilliant blue G-230 (Beyotime Biotechnology, Haimen, China) was used for staining. For immunoblotting, proteins were electrotransferred onto polyvinylidene difluoride membranes after SDS-PAGE. Then, washed and blocked membranes were incubated with a serum pool from peach-allergy patients (1:10 dilutions), and subsequently with horseradish peroxidase-conjugated mouse anti-human IgE monoclonal antibody. IgE-binding bands were detected by enhanced chemiluminescence.

Determination of recombinant allergen-specific IgE, IgG1, and IgG4

sIgE, sIgG1, and sIgG4 to 5 peach allergens were assessed by enzyme-linked immunosorbent assay (ELISA). Briefly, 96-well microtiter plates were coated with 2 µg/ml allergens in 0.05 M $NaHCO_3$ buffer (pH 9.6) at 4°C overnight. Blocking was performed with Tris-buffer containing 0.5% Tween-20 (TBST) with 1% bovine serum albumin (BSA), for 1 h at room temperature, and incubated with 100 µl serum that had been diluted 1:10 in TBST with 1% BSA, overnight. Horseradish peroxidase-conjugated anti-human IgE (ab99806), IgG1 (ab99774), and IgG4 (ab99823) were added. Tetramethylbenzidine was used for color development. Optical density was read at 450 nm. Samples with absorbance above 1.2 × the mean value of the blank control were considered positive.

Measurement of Pru p 3-specific IgE antibody affinity

To determine the affinity of Pru p 3-sIgE, we used a protocol based on ELISA with some modifications. In brief, serial double-dilutions of Pru p 3 were used to coat a 96-well plate in duplicate. Thereafter, the same procedure as mentioned above was followed. The affinity was defined as the EC_{50} (median effective concentration). To eliminate interference of Pru p 3-sIgG, serum was passed over protein G to separate IgG from IgE. The total IgG and IgE were determined with an AU5800 system (Beckman Coulter Inc, Indianapolis, IN, USA) and the ImmunoCAP system (ThermoFisher Scientific), respectively.

	Controls (n = 15)	OAS group (n = 19)	PIA group (n = 13)
Age, mean (range), years	27.47 (21–46)	26.21 (10–52)	19.00 (8–35)
Male, n (%)	6 (40%)	7 (36.8%)	5 (38.5%)
Mugwort pollen allergy, n (%)	0 (0%)	19(100%)	13 (100%)
Birch pollen allergy, n (%)	0 (0%)	7 (36.8%)	5 (38.5%)
Peach-sIgE, KUA/L, median, (range)	ND	4.75 (0.46–31.8)	13.7 (1.21–55.1)
Total IgE, KU/L, median, (range)	36.5 (6.9–666)	372 (65.6–1881)	236 (63.6–1811)

Table 1. Clinical and demographic characteristics of individuals. *OAS: Oral allergy syndrome; PIA: peach-induced anaphylaxis.*

Statistical analysis

The data analysis was performed using SPSS 23 (IBM Inc., Armonk, NY, USA) and Prism8.0 (GraphPad, La Jolla, CA, USA). Pearson's chi-squared test was used to compare frequency data. The Mann–Whitney *U* test was used to compare Ig levels of allergens. The EC_{50} was determined by Log(agonist) vs. response–variable slope (4 parameters) equation in Prism. $P < 0.05$ was considered as statistically significant.

RESULTS

Clinical characteristics

Thirty-two peach-allergy patients (12 males; mean age, 23.28 ± 10.56 years) and 15 CONTROLS individuals (6 males; mean age, 27.47 ± 6.03 years) were enrolled in this study (Table 1). According to their clinical manifestations, the peach-allergy patients were categorized into 2 groups. All the peach-allergy patients had pollen allergy. There was no significantly difference between the groups in total IgE (median, 372 KU/L; range, 65.6–1881 KU/L; median, 236 KU/L; range, 636–1811 KU/L, respectively) and peach-sIgE (median, 4.75 KUA/L; range, 0.46–31.8 KUA/L; median, 13.7 KUA/L; range, 1.21–55.1 KUA/L, respectively).

Allergen-specific IgG1, IgG4, and IgE in peach-allergy patients and controls

The characteristics of the allergen-specific IgG1, IgG4, and IgE between peach-allergy patients and controls groups. The specific IgG1 of 5 peach allergen components were above the positivity threshold value, which was defined as 1.2 × the mean absorbance of the blank control. For allergen-sIgG4, the positivity rate for the major peach allergens (Pru p 1, Pru p 3, and Pru p 4) were significantly higher in peach-allergy patients than in the controls group. The absorbance of allergen-sIgE was under the positivity threshold value in all non-allergic individuals. In allergic patients, at least 1 peach allergen component-sIgE exceeded the

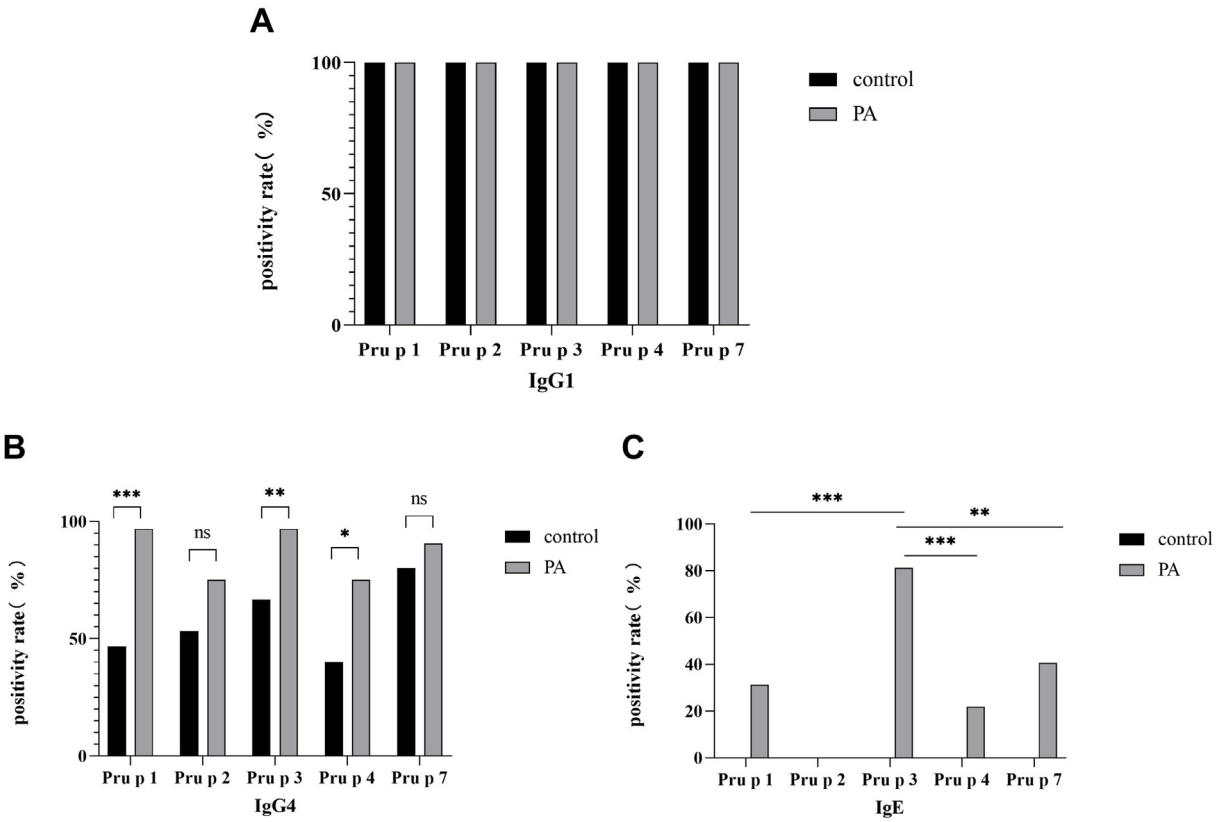

Fig. 1 The positivity rate of peach allergen-specific IgG1, -IgG4, and -IgE between peach-allergy patients and healthy controls. PA: peach-allergy patients. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

positivity threshold. The positivity rate of Pru p 3-sIgE (81.3%) was significantly higher than that of other component-sIgE (Fig. 1).

We analyzed sIgG1 and sIgG4 levels to allergen components between peach-allergy patients and the controls group. The Pru p 1- (OD450 median, 1.80, quartiles, 1.33–2.80; median, 2.97, quartiles, 1.66–3.12, respectively) and Pru p 2-sIgG1 (OD450 median, 1.68, quartiles, 1.15–2.70; median, 2.70, quartiles, 1.95–3.07, respectively) levels were significantly lower in peach-allergy patients than in the controls group, but the Pru p 3-sIgG1 level was significantly higher in peach-allergy patients (OD450 median, 3.65. quartiles, 3.14–3.80). The levels of sIgG4 to 5 allergen components were higher in peach-allergy patients (Fig. 2).

Levels and affinity of Pru p 3-sIgE in Pru p 3-sIgE-positive patients

In allergic patients, there was no difference in allergen-sIgG1 and sIgG4 levels between the OAS and PIA groups. The absorbance of Pru p 3-sIgE exceeded the positivity threshold in all individuals in the PIA group, but only in 68% of the OAS group. The levels of Pru p 3-sIgE were significantly higher in the PIA (OD450 median, 1.77; quartiles, 0.51–3.66) than in the OAS (OD450 median, 0.35; quartiles, 0.08–1.15) group. However, levels of Pru p 3-sIgE levels in the Pru p 3-sIgE-positive individuals in both groups were similar (Fig. 3).

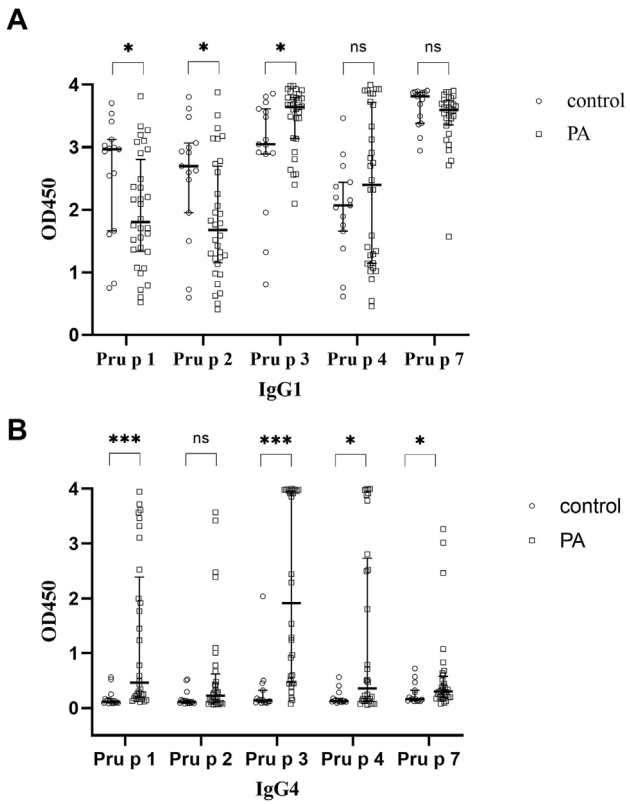

Fig. 2 The levels of peach allergen-specific IgG1 and -IgG4 between peach-allergy patients and a healthy control group. PA: peach-allergy patients. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

In our study, affinity was explored using EC_{50}, the concentration generating 50% of the individual maximum of OD450 absorbance in an ELISA assay. A higher value of EC_{50} indicates a lower affinity. We found a high concentration of Pru p 3-sIgG in the serum. Thus, to assess the effect of Pru p 3-IgG in determining affinity of Pru p 3-sIgE, we used protein G to separate IgG from IgE, which cannot bind to protein G. After elution, there was less than 10% IgG in IgE fraction. We found no difference in the affinity of Pru p 3-sIgE for its antigen after most of the IgG had been removed (Fig. 4A). We then coated reaction plates with different initial concentrations of Pru p 3 protein to determine the affinity of Pru p 3-sIgE. There was no difference between using 2 μg/ml and 10 μg/ml as initial concentration (Fig. 4B). Consequently, we coated the plates with 2 μg/ml Pru p 3 as initial concentration and used serum directly to measure the affinity of Pru p 3-IgE for its antigen. The affinity of Pru p 3-sIgE in OAS patients (EC_{50} median, 0.76 μg/ml, quartiles, 0.66–1.08 μg/ml) was significantly lower than that in PIA patients (EC_{50} median, 0.39 μg/ml, quartiles, 0.25–0.69 μg/ml) (Fig. 4D).

DISCUSSION

In this comparison of patients with peach allergy and control individuals, Pru p 1- and Pru p 2-sIgG1 levels were higher in the latter, but Pru p 3-sIgG1 levels were significantly higher in allergic patients. Pru p 1-, Pru p 3-, and Pru p 4-sIgG4-positivity was significantly greater among the peach-allergy patients than among controls. Allergen-sIgG1 and sIgG4 were similar between OAS and PIA patients. Moreover, Pru p 3-sIgE affinity was significantly higher in PIA than in OAS patients.

In our study, all patients with peach allergy were also allergic to mugwort pollen, whereas only 37.5% patients were allergic to birch pollen. This

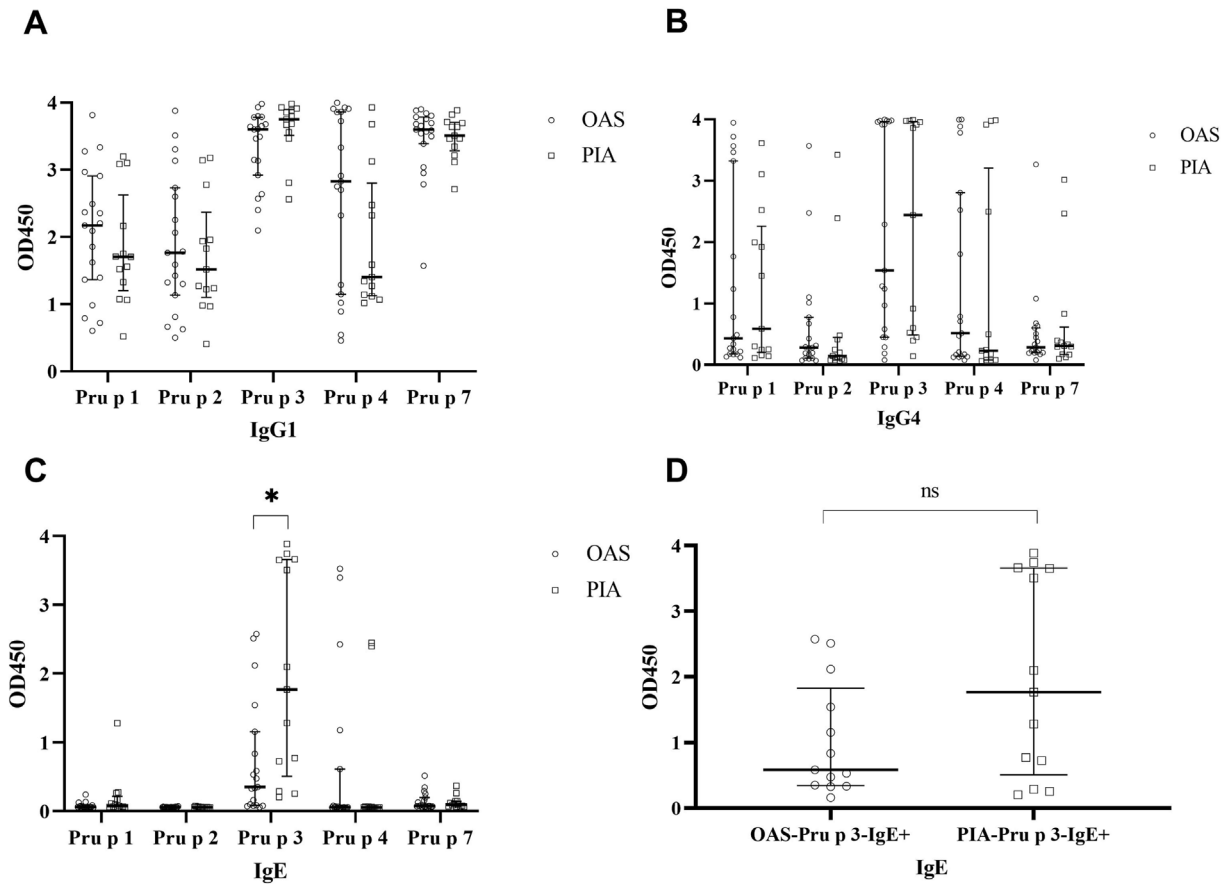

Fig. 3 The levels of peach allergen-specific IgE between peach-allergy patients with OAS and PIA. OAS: Oral allergy syndrome; PIA: peach-induced anaphylaxis. *, $p < 0.05$.

implies that mugwort pollen was a more important factor in patients with peach allergy, as reported previously in China.[2,11]

Measuring allergen-specific immunoglobulin based on crude allergen extraction can be confounded by the high non-specific background signal caused by human immunoglobulins, particularly IgGs. Knowing allergen components have provided a revolutionary advance in understanding clinical characteristic in food allergy.[14] Recombinant proteins produced by *E. coli* expression system are easy to handle and allows high protein production.[15] In our study, 5 peach allergen components were successfully expressed and purified.

IgE is typically found at extremely low levels in plasma, but it is the key factor responsible for allergic diseases.[16] Unlike IgE, the role of IgG in allergy has remained controversial. Several studies have shown that allergen-specific IgG as a blocking antibody can antagonize the actions of allergen-specific IgE after allergen-specific immunotherapy,[9,17-20] is only associated with allergen exposure, and is nonpathogenic.[7] However, allergen-specific IgG can also play a role in anaphylaxis.[21] In our study, sIgG1 to peach allergen components were detected in all patients and healthy controls, implying that subjects had been exposed to peach allergens. Looney et al.[22] found evidence that secondary isotype-switching of IgG1-expressing B cells is the primary source of IgE in humans, suggested that IgE is derived from antigen-experienced B cells. This may be the reason for the sIgG1 levels to Pru p 3, which had the highest positive rate of IgE, and which were significantly higher in patients with peach allergy than in controls in our study. In a recent study, Decuyper et al[10] found that Pru p 3-sIgG4 levels were associated with anaphylaxis in patients with peach allergy from Barcelona. However, there was no such correlation in our study: Pru p 3-sIgG4 levels were similar in our OAS and PIA groups. Furthermore, we found that the levels

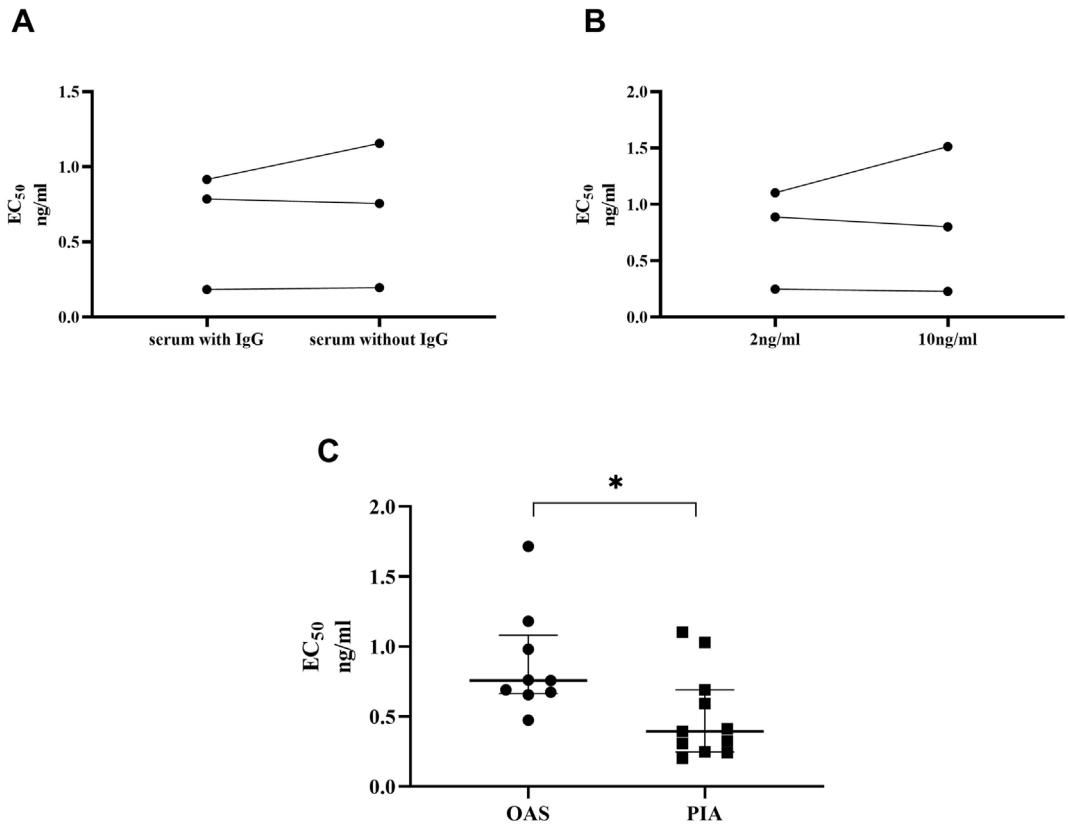

Fig. 4 The affinity of Pru p 3-specific IgE in peach-allergy patients. A. The effect of Pru p 3-IgG; B. the effect of different initial coating concentrations; C. The affinity of Pru p 3-specific IgE in peach-allergy patients with OAS and PIA. OAS: Oral allergy syndrome; PIA: peach-induced anaphylaxis; EC_{50}, median effective concentration. *, $p < 0.05$.

of sIgG4 were typically elevated in peach allergic patients compared to controls. T follicular helper (Tfh) cells is critical for the generation of antibody responses and a heterogeneous population, and Tfh2 subgroup has been shown to promote class switching to IgE or IgG4. The observed elevation of sIgG4 levels in peach allergic patients may therefore reflect the activity of Tfh2 cells in promoting the production of IgG4 antibodies as part of the allergic immune response.[23]

All patients with peach allergy were allergic to at least 1 allergen component expressed in our study. Pru p 3 is reported to be the most important allergen in patients with peach allergy,[24-27] because the positivity rate for Pru p 3-sIgE was higher and the levels of Pru p 3-sIgE were significant associated with severe reactions in patients with peach allergy. In recent research, Pru p 7 was found to cross-react with cypress pollen allergen, which can resist pepsin and heat degradation similar to Pru p 3.[28] Sensitization to cypress pollen predicted severe reactions after ingestion of peach in Japanese individuals.[29,30] In our study, the Pru p 3-sIgE-positivity rate was significantly higher that to other components. Only 40.6% patients were positive for Pru p 7-sIgE and the levels of Pru p 7-sIgE were markedly lower and showed no relationship with severe allergic reactions. The Pru p 3-sIgE levels were significantly higher in patients with anaphylaxis than in those with OAS. This was consistent with the results of previous studies.[11] However, we found no difference between the groups in terms of Pru p 3-IgE-positivity individuals.

Affinity represents the strength of interaction between antigen and antibody. FcεRI is the high-affinity IgE receptor, which can bind to the IgE present in human blood at low titers and regulate the action of mast cells and basophils.[31] Some studies have shown that IgE affinity also affects binding to antigens in allergic diseases. IgE affinity was shown to influence the efficiency of histamine release,[12] and the results of the skin prick test.[32] Thus, we established a method that was based on ELISA to measure the affinity of Pru p 3-sIgE. No previous study has reported

measuring IgE affinity in a food allergy. We measured the affinity of Pru p 3-sIgE in patients with Pru p 3-sIgE-positivity and found that the EC_{50} of Pru p 3 was significantly lower in patients with PIA than in those with OAS. This implied that a higher affinity of Pru p 3-sIgE is more likely to lead to anaphylaxis in peach allergy. Tordesillas et al showed that the binding epitopes of Pru p 3 differed between OAS and PIA patients with peach allergy.[33,34] This may explain why Pru p 3-sIgE affinity was higher in patients with PIA in our study.

CONCLUSION

IgG1 was associated with allergen exposure and as the primary source of IgG4 and IgE. Pru p 3 was shown to be an important allergen in patients with peach allergy. In addition to the Pru p 3-sIgE level, the affinity of Pru p 3-sIgE was another key factor in peach-allergy patients with anaphylactic reactions to peach.

Abbreviations

OAS, oral allergy syndrome; PIA, peach-induced anaphylaxis; nLTP, Non-specific lipid transfer protein; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; ELISA: enzyme-linked immunosorbent assay; TBST, Tris-buffer containing 0.5% Tween-20; BSA, bovine serum albumin; EC_{50}, median effective concentration.

Funding

This work was supported by the National Program for Postdoctoral Researchers (GZC20230297), Chinese Academy of Medical Sciences Initiative for Innovative Medicine (CAMS-I2M), 2021-I2M-1–017, National Major Science and Technology Projects, 2019ZX09301131, National Natural Science Foundation of China (No. 82070033) and Peking Union Medical Foundation, China (Jia He).

Availability of data and materials

This manuscript has not been published or presented elsewhere in part or in entirety and is not under consideration by another journal. All study participants provided informed consent, and the study design was approved by the appropriate ethics review board.

Authors contributions

Jun-Da Li finished experiments, analyzed and interpreted the data and drafted the article.
Xilian Yi purified protein expression experiments in this study.
Kai Guan substantial acquisition of the cases and conception.
Jia Yin made substantial contributions to conception and design and gave final approval of the version to be published.

Ethics approval and consent to participate

All the patients have signed a written informed consent form before they were recruited. This study was approved by the ethics committee of Peking Union Medical College Hospital.

All authors' consent for publication

All the authors listed have approved the manuscript.

Declaration of competing interest

All of authors report no competing interests or financial disclosure.

Acknowledgement

We are appreciated all the patients and investigators who participated in this study.

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