Distinct Lipid Transfer Proteins display different IgE-binding activities that are affected by fatty acid binding

To the Editor,

Nonspecific lipid transfer proteins (nLTPs) are relevant food allergens. They have a compact 3D structure, with a hydrophobic lipid-binding cavity. It seems that the complex with a ligand can affect protein allergenicity. Recently, we have shown that the binding of oleic acid (OLE) to the peach nLTP, Pru p 3, affects the conformation and IgE-binding activity of the allergen. We therefore investigated whether this observation can be extended to other homologous proteins. Thus, we analyzed nLTPs with different allergenic potential, Mal d 3 from apple (high), Cor a 8 from hazelnut (intermediate), and Hel a 3 from sunflower seed (low), and their interactions with 3 fatty acids: OLE, stearic (STE), and lauric (LAU) acids. We extracted natural nLTPs and produced recombinant Mal d 3 and Cor a 8 using Pichia pastoris. Ligand binding of nLTPs was assessed by ANS (1-anilinonaphthalene-8-sulfonic acid) displacement assay, measuring the decrease in ANS fluorescence. IgE reactivity of nLTPs, alone or bound to fatty acids, was tested by IgE-ELISA using sera from subjects sensitized to peach and/or hazelnut nLTP. For selected nLTPs/ligand complexes, molecular dynamic (MD) simulations were performed to analyze the impact of ligand binding on the protein conformation with a focus on specific IgE epitopes (details on methods performed to analyze the impact of ligand binding on the protein conformation with a focus on specific IgE epitopes (details on methods performed to analyze the impact of ligand binding on the protein conformation with a focus on specific IgE epitopes (details on methods performed to analyze the impact of ligand binding on the protein conformation with a focus on specific IgE epitopes (details on methods performed to analyze the impact of ligand binding on the protein conformation with a focus on specific IgE epitopes (details on methods performed to analyze the impact of ligand binding on the protein conformation with a focus on specific IgE epitopes (details on methods performed to analyze the impact of ligand binding on the protein conformation with a focus on specific IgE epitopes (details on methods performed to analyze the impact of ligand binding on the protein conformation with a focus on specific IgE epitopes (details on methods performed to analyze the impact of ligand binding on the protein conformation with a focus on specific IgE epitopes (details on methods performed to analyze the impact of ligand binding on the protein conformation with a focus on specific IgE epitopes (details on methods performed to analyze the impact of ligand binding on the protein conformation with a focus on specific IgE epitopes (details on methods performed to analyze the impact of ligand binding on the protein conformation with a focus on specific IgE epitopes (details on methods performed to analyze the impact of ligand binding on the protein conformation with a focus on specific IgE epitopes (details on methods performed to analyze the impact of ligand binding on the protein conformation with a focus on specific IgE epitopes (details on methods performed to analyze the impact of ligand binding on the protein conformation with a focus on specific IgE epitopes (details on methods performed to analyze the impact of ligand binding on the protein conformation with a focus on specific IgE epitopes (details on methods performed to analyze the impact of ligand binding on the protein conformation with a focus on specific IgE epitopes (details on methods performed to analyze the impact of ligand binding on the protein conformation with a focus on specific IgE epitopes (details on methods performed to analyze the impact of ligand binding on the protein conformation with a focus on specific IgE epitopes (details on methods performed to analyze the impact of ligand binding on the protein conformation with a focus on specific IgE epitopes (details on methods performed to analyze the impact of ligand binding on the protein conformation with a focus on specific IgE epitopes (details on methods performed to analyze the impact of ligand binding on the protein conformation with a focus on specific IgE epitopes (details on methods performed to analyze the impact of ligand binding on the protein conformation with a focus on specific IgE epitopes (details on methods performed to analyze the impact of ligand binding on the protein conformation with a focus on specific IgE epitopes). The different natural and recombinant nLTPs were purified and characterized (Figure S1). rMal d 3, rCor a 8, and nHel a 3 bound the ANS probe to varying extent (Figure 1A). All proteins showed the lowest preference for STE (Figure 1B-D) with a maximum fluorescence reduction of 17% for rMal d 3/STE (50 μmol/L). LAU induced a dose-dependent reduction of ANS binding to all proteins, reaching values at 50 μmol/L of 53%, 7%, and 26% for rMal d 3, rCor a 8, and nHel a 3, respectively. In general, OLE induced a more...
pronounced reduction of ANS fluorescence, equal to 59%, 23%, and 46% at 50 μmol/L for rMal d 3, rCor a 8, and nHel a 3, respectively.

Eighteen patients’ sera containing specific IgE to Pru p 3 and/or Cor a 8 (Table S1) were tested by direct IgE-ELISA applying rMal d 3, rCor a 8, and nHel a 3 (Figure S2). We used selected sera (n = 6 for rMal d 3 and rCor a 8 each) to test if the IgE binding to the proteins is influenced by the interaction with OLE and STE, chosen as representatives of unsaturated and saturated fatty acid, respectively. Due to its very low IgE reactivity, nHel a 3 was excluded from this analysis. Preincubation of rMal d 3 with OLE significantly increased IgE-binding (P < 0.05), whereas STE did not affect rMal d 3 IgE-binding properties (Figure 1E). Regarding rCor a 8, both fatty acids induced an increase in IgE-binding, but it was statistically significant only for OLE (P < 0.01; Figure 1F).

We performed computational calculations, detecting some differences in the cavity size of the apo nsLTPs: Mal d 3 had a larger pocket size (124 Å³) compared to both Cor a 8 and Hel a 3 (106 Å³ and 111 Å³, respectively). MD simulations were performed on Mal d

**FIGURE 1**  A-D. ANS displacement assay. (A) Fluorescence of ANS in the presence of different proteins. B, C, D. ANS fluorescence changes induced by incubating purified rMal d 3 (B), rCor a 8 (C), and nHel a 3 (D) with OLE, STE, or LAU at different molar ratios (protein:ligand 1:1 = 10 μmol/L ligand or 1:5 = 50 μmol/L ligand). (E-F) IgE-ELISA. Effect of ligand binding on the IgE reactivity of human sera to purified recombinant proteins: (E) rMal d 3 and (F) rCor a 8 alone (no ligand) or with ligands (OLE, STE). 6 serum samples. NHS: means of 3 samples.
3 and Cor a 8 in complexes with either OLE or STE. For both proteins, the inclusion of ligands significantly increased the cavity volume, affecting the protein conformation. As shown in Figure 2, both allergens underwent conformational changes upon OLE binding, especially displacing loop 3 (I59 to N63) of Mal d 3, which moved toward the allergen surface, and affecting the C-terminal region in both allergens, which is consistent with our previous findings. Notably, this C-terminal region has been identified in Pru p 3 as a major IgE epitope, thus explaining the increased IgE reactivity observed for the nsLTP/OLE complexes. Interestingly, we observed similar conformational changes for the Cor a 8/STE complex, although to a minor extent, which was paralleled by a slight, albeit not statistically significant increase in IgE reactivity. On the contrary, binding of STE to Mal d 3 did not induce any structural change in the allergen, but rather increased its stability, yet without changing the IgE-binding properties.

Considering differences in the allergenic potential of homologous proteins, we should take into account “the necessity of other matrix component to induce allergenic responses or differences and particularities in the epitope composition.” Here, we focused on both food matrix and epitopes. With regard to the latter, the epitope sequences identified in Pru p 3 are well conserved in Mal d 3, but less in the other two nsLTPs, especially in Hel a 3 (Figure S1A). This could explain its very low IgE-binding, as in most cases, Pru p 3 is the primary sensitizer.

Regarding the food matrix, we focused on how the interaction with fatty acids, commonly present in the food sources, could affect the allergenic properties of nsLTPs. Consistently with our previous observation for Pru p 3, all nsLTPs had the highest preference for OLE, whose binding induced major structural changes that affected IgE epitope orientation, and thus, their recognition by IgE, in both, Mal d 3 and Cor a 8.
Recently, the direct immunomodulatory effect of food-derived lipids has gained increasing interest. Tordesillas et al showed the adjuvant activity of the natural ligand of Pru p 3 in the allergen sensitization. Summarizing the recent findings, including our data, it still remains to be fully elucidated whether lipids alone or in complexes can activate key players of both, the innate and adaptive immune system within an allergic response.

In conclusion, this multidisciplinary study analyzed for the first time the ligand-binding capacities of Mal d 3, Cor a 8, and Hel a 3 and confirmed that these individual homologous allergens display different, higher and lower, IgE-binding activities, due to differences in their epitope structure. Furthermore, the volume and structural properties of the ligand-binding cavity of individual nsLTPs are critical for their ligand-binding activities. Upon ligand binding, the accessibility of these epitopes changed. Taken together our data provide molecular evidence how ligand binding to nsLTP affects IgE-binding activity.

ACKNOWLEDGMENTS
This work was funded by the Austrian Science Fund (FWF): DK W1248 to PD and PH, and SFB F4603 to KHS and SG. This research was also supported in part by the Marie-Curie project CARAMEL within the 7th European Community Framework Programme (Grant n 626572 to RAi) and by PL-Grid Infrastructure for TB. Computations were performed at the Academic Computer Centre Cyfronet AGH.

CONFLICT OF INTEREST
RAi has received funding from the Marie-Curie project CARAMEL within the 7th European Community Framework Programme. KHS, PD, SG, and PH have been supported by the Austrian Science Fund (FWF). The remaining authors report no conflicts of interest.

Keywords
fatty acid binding, food allergens, IgE epitope, nonspecific lipid transfer proteins, protein structure

ORCID
Roberta Aina 1 https://orcid.org/0000-0001-6624-6351
Pawel Dubiela 1 https://orcid.org/0000-0001-8537-4404
Sabine Geiselhart 1 https://orcid.org/0000-0002-4749-3485
Merima Bublin 1 https://orcid.org/0000-0002-7191-9431
Maurizio Bruschi 1 https://orcid.org/0000-0002-5709-818X
Christian Radauer 1 https://orcid.org/0000-0001-9920-4449
Christoph Nagl 1 https://orcid.org/0000-0001-5342-2563
Piotr Humeniuk 1 https://orcid.org/0000-0002-8277-1700
Riccardo Asero 3 https://orcid.org/0000-0002-8277-1700
Charlotte Gotthard Mortz 4 https://orcid.org/0000-0001-8710-0829

1Department of Pathophysiology and Allergy Research, Medical University of Vienna, Vienna, Austria
2Department of Earth and Environmental Science, University of Milano Bicocca, Milan, Italy
3Ambulatorio di Allergologia, Clinica San Carlo, Paderno Dugnano (MI), Italy
4Department of Dermatology and Allergy Center, Odense Research Center for Anaphylaxis (ORCA), Odense University Hospital, Odense, Denmark
5Department of Dermatology, University Hospital St. Poelten, Karl Landsteiner University of Health Sciences, St. Pölten, Austria
6Karl Landsteiner Institute of Dermatological Research, Karl Landsteiner Gesellschaft, St. Pölten, Austria
7Jerzy Haber Institute of Catalysis and Surface Chemistry, Polish Academy of Sciences, Krakow, Poland

Correspondence
Karin Hoffmann-Sommergruber, Department of Pathophysiology and Allergy Research, Medical University of Vienna, Vienna, Austria.
Email: karin.hoffmann@muv.ac.at

REFERENCES
1. Salminen TA, Blomqvist K, Edqvist J. Lipid transfer proteins: classification, nomenclature, structure, and function. Planta. 2016;244:971-997.
2. Scheurer S, Schülke S. Interaction of non-specific Lipid-Transfer Proteins with plant-derived lipids and its impact on allergic sensitization. Front Immunol. 2018;9:1389.
3. Dubiela P, Aina R, Polak D, et al. Enhanced Pru p 3 IgE-binding activity by selective free fatty acid-interaction. J Allergy Clin Immunol. 2017;140(1728-1731):e10.
4. Asero R. In patients with LTP syndrome food-specific IgE show a predictable hierarchical order. Eur Ann Allergy Clin Immunol. 2014;46:142-146. Erratum in: Eur Ann Allergy Clin Immunol. 2014;46:239.
All items of the food allergy quality of life questionnaires are relevant for peanut-allergic patients

To the Editor,

Peanut allergy and the management of peanut allergy may have a considerable impact on health-related quality of life (HRQL). HRQL can be measured by the Food Allergy Quality of Life Questionnaire-Adult Form, Food Allergy Quality of Life Questionnaire-Teenager Form and Food Allergy Quality of Life Questionnaire-Child Form (FAQLQ-AF, FAQLQ-TF and FAQLQ-CF).1-3 The FAQLQs were developed to measure the impact of food allergy on HRQL irrespective of the causative food allergen.1-6 The FAQLQs were shown to be valid and responsive instruments.1-6 At present, it is unknown whether all items contained in these instruments are relevant for peanut-allergic patients and whether intervention studies on peanut allergy would be better served by the existing FAQLQs in their entirety or by a peanut-specific selection of FAQLQ items. This study aims to identify whether all items of the FAQLQ-CF, FAQLQ-TF and FAQLQ-AF are considered important by peanut-allergic patients and, consequently, whether the FAQLQs are valid in a subgroup of peanut-allergic patients.

The FAQLQ-AF, FAQLQ-TF and FAQLQ-CF are self-reported instruments for measuring the impact of food allergy on HRQL in adults (≥18 years of age), adolescents (13-17 years of age) and children (8-12 years of age), respectively.1-3 The FAQLQ-AF contains 29 items, the FAQLQ-TF contains 23 items, and the FAQLQ-CF contains 24 items. Each item is scored on a 7-point scale and ranges from 1 (minimal impairment in HRQL) to 7 (maximal impairment in HRQL). The FAQLQs have been shown to be valid, reliable and responsive instruments.1-7

In order to ascertain whether HRQL items of the FAQLQ-CF, FAQLQ-TF and FAQLQ-AF were suitable to peanut-allergic patients, some retrospective analysis was performed using databases generated during the development and validation of the FAQLQs.

Firstly, all patients with a physician-diagnosed peanut allergy were selected from the development database to identify FAQLQ items that were reported most frequently and had the highest impact scores (ie, overall importance) for peanut-allergic patients (ranging from 1.0 "not" to 5.0 "extremely").1-3 Additionally, the overall importance of each FAQLQ item was compared and correlated (Pearson correlation coefficient) between peanut-allergic patients and non-peanut-allergic patients and tested for significance (P < 0.05).

Secondly, the cross-sectional validity1-3 of the FAQLQs was determined in a peanut-allergic subgroup. Spearman correlation coefficients (ρ) were calculated between the FAQLQ mean scores and the Food Allergy Independent Measure (FAIM) mean score. The FAIM is an instrument that measures the patients perceived disease severity and has previously been used successfully in validating the FAQLQs.6 Moderate correlation was to be expected (0.40-0.60).1-4

Thirdly, the longitudinal validity4 of the FAQLQs was determined in a peanut-allergic subgroup. Correlations were calculated between the change in total FAQLQ and the change in total FAIM score of patients who underwent a double-blind placebo-controlled food challenge (DBPCFC) with peanut (longitudinal validity). HRQL was measured 1 month before (baseline) and 6 months after (follow-up) a DBPCFC.

Data were analysed with SPSS software for Windows. Table 1 shows the participant characteristics.

Overall importance analysis showed that all items exceeded the previously used cut-off level of >1.37 in the peanut-allergic subgroup.1-3 Figure 1 shows that peanut-allergic patients reported a higher overall impact than non-peanut-allergic patients (P < 0.05). Thus, all items may be considered to be important. The Pearson correlation coefficient between the overall importance of peanut-allergic patients and non-peanut-allergic patients was 0.70, P < 0.001 (FAQLQ-AF), 0.71, P < 0.001 (FAQLQ-TF) and 0.80, P < 0.001 (FAQLQ-CF).

Cross-sectional validity analysis showed that the total FAQLQ score correlated significantly with the total FAIM score in the...