

IgE binding epitope mapping with TL1A tagged peptides

Yuzhu Zhang^{a,*}, Shilpa R. Bhardwaj^a, Ana Vilches^a, Andrew Breksa^a, Shu-Chen Lyu^b, Sharon Chinthrajah^b, Kari C. Nadeau^b, Tengchuan Jin^{c,*}

^a US Department of Agriculture, Agricultural Research Service, Pacific West Area, Western Regional Research Center, 800 Buchanan Street, Albany, CA 94710, USA

^b Division of Pediatric Immunology, Allergy, and Rheumatology, Department of Pediatrics, Stanford University School of Medicine, 269 Campus Dr, Stanford, CA 94305, USA

^c Hefei National Laboratory for Physical Sciences at Microscale, Laboratory of Structural Immunology, CAS Key Laboratory of Innate Immunity and Chronic Disease, School of Life Sciences and Medical Center, University of Science and Technology of China, Hefei, Anhui 230027 China

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ABSTRACT

Linear IgE epitopes play essential roles in persistent allergies, including peanut and tree nut allergies. Using chemically synthesized peptides attached to membranes and microarray experiments is one approach for determining predominant epitopes that has seen success. However, the overall expense of this approach and the inherent challenges in scaling up the production and purification of synthetic peptides precludes the general application of this approach. To overcome this problem, we have constructed a plasmid vector for expressing peptides sandwiched between an N-terminal His-tag and a trimeric protein. The vector was used to make overlapping peptides derived from peanut allergens Ara h 2. All the peptides were successfully expressed and purified. The resulting peptides were applied to identify IgE binding epitopes of Ara h 2 using four sera samples from individuals with known peanut allergies. New and previously defined dominant IgE binding epitopes of Ara h 2 were identified. This system may be readily applied to produce agents for component- and epitope-resolved food allergy diagnosis.

1. Introduction

The incidence of individuals experiencing food allergies has increased over the past two decades (Gupta et al., 2011, 2019; Kumfer and Commins, 2019; Sicherer et al., 2003). Nine food groups are recognized as major allergen sources in the US. Food allergies affect not only the allergic patients and their families but also the agriculture and food industry and the public. Although extensive research has focused on the disease mechanisms and searching for effective treatments for allergies, there is no cure (Moore et al., 2017). With few exceptions, food allergens are proteins, and for each allergen source, only a small number of proteins cause most allergic reactions. What makes these food proteins allergens remains to be elucidated.

A food allergen triggers allergic reactions through its associations with immunoglobulin E (IgE) in individuals who were previously sensitized to the allergen. IgE binding epitopes of food allergens can be linear or conformational, and linear epitopes are regarded as the most responsible for severe, persistent allergies to peanuts and tree nuts (NIH Expert Panel, 2006). Defining and characterizing linear IgE

epitopes is essential to understanding the allergenicity of food allergens and developing methods for allergen mitigation or treatments. The strategy for defining linear IgE epitopes is testing the immunoreactivity of overlapping peptides with the IgE repertoires in patients' sera. Earlier studies approached goals with chemically synthesized peptides (15-mer, 10-mer) bound to cellulose membranes, and the dominant linear IgE epitopes of peanut allergen Ara h 1, Ara h 2, and Ara h 3 were determined (Burks et al., 1997; Rabjohn et al., 1999; Stanley et al., 1997) using selected patient sera. Whereas later studies took advantage of the microarray technology with a large number of patient sera (Shreffler et al., 2004). The results suggested that the number of epitopes recognized by the IgE pool in a patient's serum was a better indicator of the severity of the patient's allergy than which dominant epitopes the patient's serum recognized, suggesting the importance of IgE binding epitope mapping.

The implementation of the strategy, though, is impeded by the need to produce overlapping peptides scanning the whole sequence of an allergen. As a result, most food allergens' dominant linear IgE epitopes have not been determined. Chemical synthesis is currently the primary

* Corresponding authors.

E-mail addresses: Yuzhu.Zhang@usda.gov (Y. Zhang), jint@ustc.edu.cn (T. Jin).

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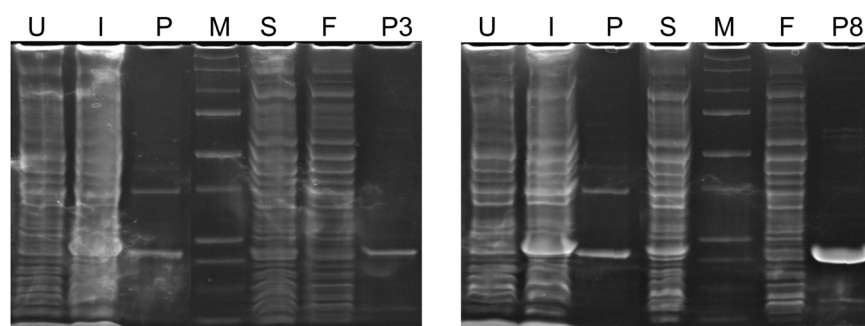


Fig. 1. Production of TL1A fused peptides. The SDS-PAGE analyses of the expression and purification of two Ara h 2 peptides are shown (left, P3; right P8). Lanes labeled U, I, P, and S represent uninduced, induced, pellet, and supernatant, respectively. F denotes the flowthrough of the metal chelating chromatography. The nickel column purified peptide-TL1A fusions were loaded to the last well of the gel. A pre-stained protein ladder (Thermo Fisher, catalog BP36031) containing protein molecular weight standards of 11, 17, 26, 34, 43, 50, 72, 95, 130, 170 kDa were used as references.

method of peptide production. It is relatively expensive, and there are inherent challenges in scaling up the production. In addition, purification of the peptide product can be problematic, and epitope mapping requires highly pure peptides to prevent false positives. Spot synthesis is another way of producing overlapping peptides. However, in-house production or outsourcing costs are both high and out of the range of many labs. Spot synthesis can falter with challenging sequences, and purification is practically impossible. Systems for recombinantly expressing peptides have also been developed, with the *E. coli* system being the first choice. However, the expressed peptide may be unstable due to microbial proteases in the expression system, and the production yield is unpredictable. Thus, different expression systems have been designed to solve various problems. Such as fusing peptides with partner proteins to enhance the peptide yield and solubility (Lamer et al., 2022; Zenin et al., 2022) and using a self-aggregating tag to induce the formation of aggregates of the target peptide with a fusion protein (Yang et al., 2022). Several studies also utilized recombinantly expressing tandem peptide repeats for peptide production (Nagaraj et al., 2019).

Nevertheless, mapping IgE binding epitopes requires large numbers of peptides, and none of the expression strategies has been reported to be suitable for such applications. We sought to develop a strategy to produce peptides using an *E. coli* system with the help of a TL1A expression tag. TL1A was expressed without a tag and yielded high protein levels in both the soluble and the inclusion partitions (Jin et al., 2007b). For IgE binding epitope mapping, it would be regarded as successful when a TL1A fused peptide is expressed either in the soluble partition or the insoluble partition because the peptide would be used to assess the linear IgE epitopes, not conformational epitopes. Here, we report an expression system for producing peptides sandwiched between a His-Tag and TL1A and its application to mapping IgE binding epitopes of peanut allergen Ara h 2.

2. Materials and methods

2.1. Plasmid construction

All DNA oligo were custom synthesized by IDT DNA Technologies (Coralville, IA). A forward primer (ccaaatGGATCCagagcagcgagataag) and a reverse primer (ccaaatCTCGAGctatagtaagaaggctccaaaga) was used to amplify the coding sequence for TL1A using pDEST-TL1A (Jin et al., 2007b) as a template. The PCR product was digested with *Bam*H I and *Xho* I and cloned into the pTMS1 vector (Zhang and Fan, 2017) to make pNH-TL1A for an unrelated study. pNH-TL1A was digested with *Pml* I and *Xho* I, and the released sequence coding for TL1A with a His-tag was inserted into pTGS1 (an in-house vector similar to pTMS1 with different cloning sites) prepared with the same enzymes to make pNHH-TL1A. pNHH-TL1A expresses TL1A with a long His-tag with 12 histidine residues, and the sequence coding the tag has a *Pml* I site. The sequence of the cloning sites or pNHH-TL1A is shown in the supplemental information (supplemental Fig. S1).

Nineteen pairs of DNA oligos were synthesized to make 16-residue peptides covering the entire sequence of the peanut allergen Ara h 2

(supplemental Table S1). Adjacent peptides have 9-residue overlaps. Each oligo pair were mixed at 10 μ M concentrations and heated to 96 °C for 2 min using a PCR machine. The PCR machine was then turned off, and the samples were kept in the PCR machine for half an hour. The annealed oligo pairs were ligated with pNHH-TL1A prepared with *Pml* I and *Bam*H I, and the ligation product was transformed into *dH5 α E. coli*. The transformation products were spread on LB plates containing kanamycin. Colonies were picked to inoculate 5 mL cultures for DNA miniprep using QIAprep Spin Miniprep Kit (QIAGEN Valencia, CA), following the manufacturer's protocols. DNA sequencing (performed by Elim Biopharm, Hayward, CA) was used to confirm the presence of the correct coding sequences. Nineteen plasmids were obtained and named pNH-H2Px-TL1A, where x is a number from 1 to 19, indicating the numbering of the peptide derived from peanut allergen Ara h 2.

The oligo pair for peptide 10 was also cloned into pDim1CH to make pDim1-H2P10 using the same strategy applied to the constructs with pNHH-TL1A. pDim1CH has the same vector backbone as pNHH-TL1A but expresses human dim1 and has cloning sites 3' to the coding sequence of dim1 (supplemental Fig. S2). The correct sequence coding peptide 10 of Ara h 2 fused to the C-terminal of human dim1 was also confirmed by DNA sequencing.

2.2. Peptide production

The plasmids were transformed into BL21 Bacteria (BL21 StarTM (DE3), Thermo Fisher Scientific, Waltham, MA) for protein expression. For each peptide, a single colony was picked and used to inoculate a 50 mL BL culture containing kanamycin (50 μ g/mL). After incubating in a shaker at 37 °C overnight, 25 mL of the culture was used to start (1 L) larger cultures. When the OD at 600 nm of the larger cultures reached about 1, the shaker temperature was set to 18 °C, and the cultures were allowed to grow for one hour while the temperature settled down. The production of the peptides was induced by adding IPTG (Isopropyl β -D-1-thiogalactopyranoside) (GoldBio, St Louis, MO) to a final concentration of 1 mM. The cultures were harvested after 16 h the next day by spinning at 3900 g and collecting the pellets. The pellets were resuspended in 30 mL of buffer HB (20 mM imidazole, pH 8.0, 500 mM NaCl) and stored in 50 mL falcon tubes at – 20 °C.

2.3. Peptide purification

A frozen cell suspension was thawed by placing the 50 mL tube in a beaker containing room temperature water. The thawed cell suspension was transferred to a 100 mL plastic beaker, and 35 mL of buffer HB containing 1 mM benzimidazole was added. The bacteria were sonicated for 3 min in the beaker surrounded by ice and water. The lysate was cleared by centrifugation at 20,000g for 40 min at 18 °C. The His-tag protein was purified using an AKTA FPLC system (GE Healthcare, Piscataway, NJ) equipped with a 5-mL HisTrap FF Crude column (GE Healthcare). The column was equilibrated with 25 mL of Buffer HB, loaded with the supernatant, washed with 25 mL of buffer HB, and further washed with 25 mL of buffer HW (45 mM Imidazole, pH 8.0, 500

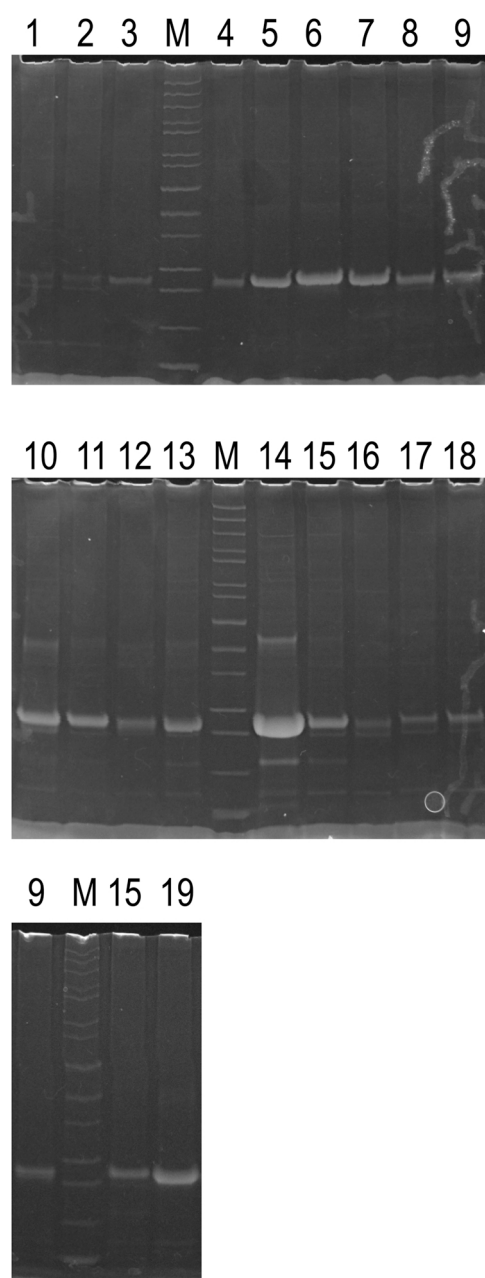


Fig. 2. Comparison of the soluble partitions of Ara h 2 peptide fusions. Equal volumes of nickel column purified peptide fusions H2P1-TL1A to H2P19-TL1A were loaded to different wells of gradient SDS gels. The gel lanes are labeled on top of the gel. Lane 1 was loaded with H2P1-TL1A, lane 2, H2P2-TL1A, and so on. Lane M was loaded with the EZ-Run Rec Protein ladder (Fisher, BP36021), which contained protein standards with molecular weights of 200, 150, 120, 100, 85, 70, 60, 50, 40, 30, 25, 20, 15, and 10 kDa.

mM NaCl). The protein was eluted with buffer HE (300 mM Imidazole, pH 8.0, 500 mM NaCl).

2.4. Purification of peanut allergen Ara h 2

Ara h 2 was purified from raw peanut by buffer extraction and ammonium sulfate precipitation (Masityama et al., 2014) followed by gel filtration with a Superdex 200 preparative column (GE Healthcare).

2.5. SDS PAGE analyses

Purified peptide-TL1A samples were mixed 1:1 with an SDS sample

buffer (50 mM Tris-HCl, pH 6.8, 2% SDS, 0.1% bromophenol blue, 10% glycerol) containing 100 mM β -mercaptoethanol and incubated at 96 °C for 10 min. SDS-PAGE was carried out using gradient polyacrylamide gels (4–20%) with a HEPES-Tris running buffer (100 mM HEPES, 100 mM Tris, pH 8.0, 3 mM SDS). Gels were stained as previously described (Zhang et al., 2014) and documented with an ImageQuant LAS 400 Imager (GE Healthcare). ImageJ (Abramoff et al., 2004) was used to estimate the concentration of purified Ara h 2 peptide-TL1A fusions.

2.6. Spotting the peptides and controls onto membranes

For dot blot, Ara h 2, peptide 10 fused with dim1, and the peptide-TL1A fusions were prepared with 8 M urea to about the same concentration based on the ImageJ results. Two hundred nanoliters of the samples were spotted on nitrocellulose membranes using an NT8 Drop Setter (Formulatrix, Bedford, MA). The membranes were let dry at room temperature overnight for at least 16 h.

2.7. Peptide recognition by serum IgE

Serums 1 and 2 (with de-identified patient codes 5422 and 6202, respectively) were collected from patients with a history of IgE-mediated pecan and peanut allergies and with a positive oral food challenge to peanut and pecan. The patients were enrolled in the food allergy study at Stanford University under institutional review board approval with informed consent. Serums 3 and 4 were acquired from PlasmaLab International (Everett, WA), and the samples were collected from patients with peanut allergies. The sample IDs are 32342-DG and 29984-JD, respectively. Four sera samples collected from patients with milk allergies (with sample IDs 25284 SC, 24235 M, 27508 K, 24317 BB) were also acquired from PlasmaLabs and used as negative controls.

IgE binding of the peptides was assessed by dot blot. The membranes with sample spots were washed for 5 min with TBST (25 mM Tris, pH 7.4, 137 mM NaCl, 2.7 mM KCl, 0.1% Tween 20) three times. The spotted membranes were blocked with 10% nonfat dry milk in TBST for 1 h at room temperature. In the meantime, individual sera were diluted 25 times with TBST containing 1% nonfat milk. Blocked membranes were incubated with a particular serum (at 1:25 dilution with TBST) for 1 h at room temperature. The membranes were washed with TBST for 5 min, repeated three times, followed by incubating with a secondary antibody for 1 h at room temperature. The secondary antibody was horseradish peroxidase (HRP) labeled goat antibody against human IgE (SeraCare, Milford, MA), and it was diluted 5000 times with TBST containing 1% nonfat milk. The membranes were washed with TBST for 5 min, repeated three times, and the SuperSignal West Femto Substrate (Thermo Fisher) was used for detection using the ImageQuant LAS4000 imaging system in chemiluminescence mode. As a positive control, a membrane was blotted similarly but probed using a mouse monoclonal antibody against His-tag (QIAGEN) as the primary antibody, and an HRP conjugated goat anti-mouse antibody was used as the secondary antibody.

3. Results and discussion

3.1. Peptide expression and purification

TL1A is a tumor necrosis factor (TNF) superfamily member. In previous studies of the TL1A cytokine, we found that it can be recombinantly produced in bacteria (Jin et al., 2007a, 2007b). When the bacteria were lysed with freezing and sonication in aqueous solutions, high levels of TL1A were present in both the soluble and insoluble partitions (data not shown). In light of the known possible effects of fusion tags in expressing exogenous proteins in bacteria and the need for the production of overlapping peptides covering the sequences of various food allergens, we considered using TL1A to aid the expression of peptides.

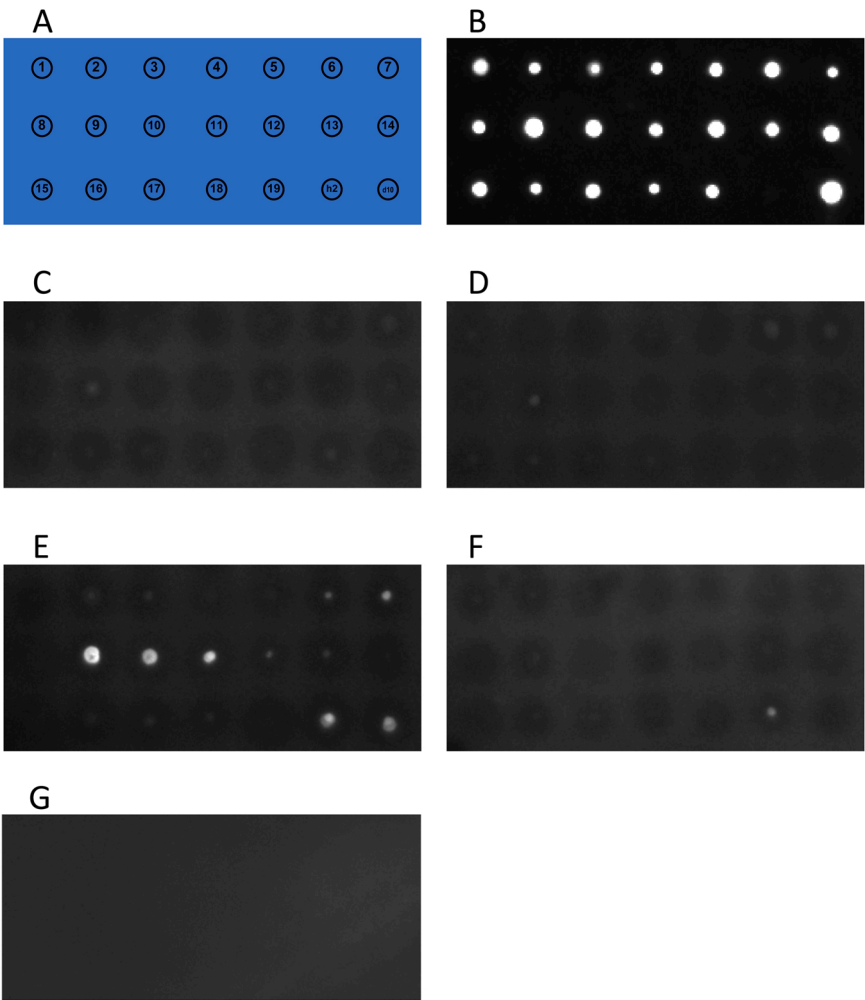


Fig. 3. Mapping linear IgE binding epitopes with peptide fusions. Areas of membrane spotted with the same pattern of peptide fusions and controls as shown in A) were used in dot blot experiments. B), The membrane was probed with an anti-His-tag antibody to assess the relative amount of peptide spotted. C-F), Membranes probed with serum 1, 2, 3, and 4, respectively. G), A representative of negative controls with sera of patients with milk allergy (see text).

Table 1
Recognized of Ara h 2 and its peptide by IgEs In individual serum.

	Ara h 2	Peptide																		
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Serum 1	+							+		+			+		+					
Serum 2	+	+					+	+		+						+	+			
Serum 3	+		+	+			+	+		+	+	+	+	+			+	+	+	
Serum 4	+									+										

To facilitate the expression of TL1A fused to a series of peptides, we generated the pNHH-TL1A vector that includes *Pml* I and *Bam*H I restriction sites and a His-tag (Fig. S1). The vector was prepared with *Pml* I and *Bam*H I digestion and used to construct expression plasmids for peptide expression. For a particular peptide, a DNA oligo pair for the peptide could be annealed to form a short double-stranded DNA with a blunt end and a *Bam*H I sticky end and ligated with the prepared vector without further modification.

As an example of the application of this method, we constructed plasmids to express 19 overlapping peptides covering the protein sequence of peanut allergen Ara h 2. The plasmids were named pNH-H2Pn-TL1A, where n is a number counting from 1 to 19 and represent the peptide number in the series. Expression of the peptide-TL1A fusions was carried out using the BL21 bacteria, and all 19 peptide fusions were successfully expressed and purified from the soluble partition using

nickel columns. The purified products were named H2Pn-TL1A, where n is the numbering of the peptide in the series. They will be referred to as peptides 1–19 (P1 to P19) hereafter. Among the peptides, numbers P1, P2, P3, P16, P17, and P18 had similar levels in the soluble partition when the bacteria were lysed by freeze-thaw and sonication. The expression and purification of P3 are shown in the left panel of Fig. 1. The rest of the peptides in the series had higher levels in the soluble partition. As a representative, the expression and purification of P8 are shown in the right panel of Fig. 1. Similarly, the His-tag at the C-terminal of the dim1-peptide10 was utilized to purify the dim1-peptide fusion. The purified product will be referred to as D10 hereafter.

3.2. Peptide spotting

To assess the binding of the peptides with IgE molecules in patient

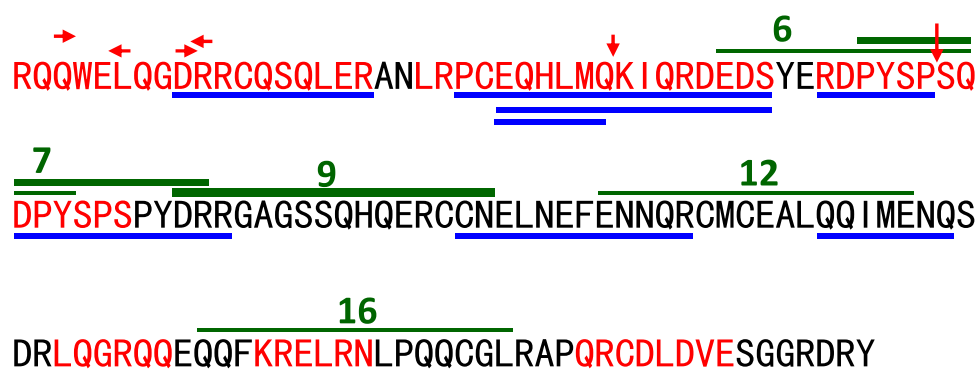


Fig. 4. Linear IgE binding epitopes of Ara h 2. Peptides with binding activity to IgE molecules in at least half of the sera are shown by green bars on top of the Ara h 2 sequence. These include peptides 6, 7, 9, 12, and 16. The thickness of the bar corresponds to the number of sera they bound. Previously identified Ara h 2 epitopes (Stanley et al., 1997) are also shown and are indicated by red letters in the sequence. The starting and ending residues of overlapping epitopes are shown by red arrows pointing to the right and left, respectively. Epitopes next to each other are separated by a red arrow pointing downward. Blue bars underneath the sequence indicate the positions of the linear epitopes previously identified in a study (Shreffler et al., 2005) using microarrays with

synthesized peptides of different lengths and different numbers of overlapping residues.

sera, equal volumes of purified peptides were loaded into separate wells of three homemade gradient gels. P9 and P15 were each in two gels to assess the relative concentrations of the purified peptides (Fig. 2). For each gel, the relative strength of the peptide bands in different lanes was analyzed with the ImageJ software. P9 and P15 were used to estimate the relative strength of the peptide bands in different gels.

Based on the ImageJ estimation of the relative concentrations of the purified peptides, final peptide samples were prepared in 4 M urea. The 19 overlapping peptides along with Ara h 2 (h2) and D10 were then spotted on nitrocellulose membranes. The protein concentrations of samples were about 27 μ M, and 200 nL of the solutions was spotted for each dot. The dots were spotted in a pattern similar to the well pattern of a 384-well plate. Each membrane contained 10 identical sub-patterns separated by dots of Coomassie brilliant blue R. Using the blue dots as guides, we cut the membrane into pieces, each having a dot pattern as shown in Fig. 3A.

3.3. IgE recognition of the peptides

To assess the suitability of the peptide fusions for determining IgE epitopes, the His tag of the peptide fusions was used to evaluate the relative amount of peptide spotted onto the membrane. An anti-His tag antibody was used to blot one of the membranes as a positive control, and the results are shown in Fig. 3B. All 19 TL1A fused Ara h 2 peptides and peptide 10 fused to human dim1 were detected by the anti-His tag antibody, while Ara h 2 purified from peanuts was not recognized by the antibody.

Serum IgE recognition of the peptides was demonstrated with four patient sera, as shown in Fig. 3C–F and summarized in Table 1. All four of the sera recognized the natural allergen, although the amount of IgE molecules bound differs considerably, as expected. Serum 3 had the highest level of Ara h 2 specific IgE antibodies. It also contained IgE molecules associated with more peptides than the other sera. All the sera recognized more than one peptide at the current experiment's sensitivity level. Fig. 3G shows that serum 24235 M did not associate with any peptide spots, nor did any of the other control sera (data not shown).

Ten distinct Ara h 2 IgE binding epitopes were determined to be dominant more than 30 years ago with peptides synthesized on derivatized cellulose membranes (Stanley et al., 1997). Whereas in a study with peptide microarrays (Shreffler et al., 2005), nine Ara h 2 dominant IgE binding epitopes were defined, with two of the epitopes residing in a longer one. These two studies had seven epitopes in common. With the TL1A fusions, five peptides were recognized by at least half of the sera. Three of these (P6, P7, and P16) encompassed previously identified epitopes, while the other two (P9, DRRGAGSSQH⁹QERCCN; and P12, ENNQRCMCEALQQIME), including a peptide recognized by all four sera, were not identified as Ara h 2 dominant IgE binding epitopes in the previous studies (Fig. 4). Possible reasons why the epitope contained in

P9 was not previously reported include differences in the serum sets used. It is also possible that the property of the peptides covering this part of Ara h 2 could cause issues in synthesizing the peptides on the membrane or printing microarrays. Unlike the method described here, where the His-tag is used as an internal positive control, previous spot synthesizing and microarray experiments had no positive controls.

When the microarray techniques were well developed and applied to mapping linear IgE binding epitopes of allergens, peptides with 1 or 2 overlapping amino acids were often used to print the microarrays to locate the epitopes more precisely. Earlier studies used peptides with larger overlapping regions to determine dominant linear epitopes. The present study developed an inexpensive method that more laboratories with limited budgets can adapt and demonstrated its application to mapping IgE binding epitopes of peanut allergen Ara h 2. Future development of the technique into a high throughput method will enable it to use a short offset of the overlapping peptides and locate the epitopes as precisely as the microarray technology.

Besides epitope mapping, the TL1A fused peptides can also be used to study the responses of mast cells and basophils when they are cross-linked by IgE antibodies. If the IgE antibody specific to an epitope is isolated/produced, mast cells (such as the LAD2 cells) can be loaded with the IgE. Because three copies of the same epitope are displayed on TL1A, the TL1A-peptide fusion can activate the cells. The same epitope fused with Dim1 can be used as a negative control because Dim1 is a monomeric protein in solution (Zhang et al., 2003). Thus, the Dim1-peptide cannot cross-link the IgE antibodies. Dim1 behaved similarly to TL1A regarding their expression levels in the soluble and insoluble partitions of the bacteria when lysed. Although we have not made a large number of Dim1-peptide fusions, we expect that Dim1 can also be used to produce peptides with different properties.

It is worth noting that using overlapping peptides to map IgE binding epitopes of food allergens only applies to linear epitope mapping. Various studies about determining conformational IgE binding epitopes of food allergens were reported in the literature. To our knowledge, however, these only apply to a limited number of targeted IgEs, and a method to map conformational epitopes on the surface of an allergen that bind the repertoire of IgE antibodies in a serum remains to be developed.

In summary, the results showed that TL1A could be used as a fusion partner to facilitate the production of peptides in *E. coli*. The method's utility was demonstrated with its application to expressing overlapping Ara h 2 peptides. In addition, the peptide fusions were present in both the soluble and the insoluble partitions (data not shown), indicating the method's potential utility in producing peptides with even more diverse properties. Information about IgE binding epitopes can be used for accurate and reproducible diagnoses of allergies (Suarez-Farinas et al., 2021). Epitope-specific IgE antibody profiling can be used to predict the probabilities of tolerating discrete doses of food protein for food

challenges (Suprun et al., 2022). However, the dominant IgE binding epitopes of most food allergens, and pollen allergens, have not been identified. The method of producing peptide TL1A fusions developed in this study offers another approach for researchers to obtain information about IgE binding epitopes of allergens for better diagnosis and prediction of the prognosis of allergies and for understanding better the allergenicity of proteins.

CRedit authorship contribution statement

Yuzhu Zhang: Conceptualization, Investigation, Methodology, Writing - original draft, Writing - review & editing. **Shilpa R. Bhardwaj:** Investigation. **Ana Vilches:** Investigation. **Andrew Breksa:** Writing - review & editing. **Shu-Chen Lyu:** Resources. **Sharon Chinthrajah:** Resources. **Kari C. Nadeau:** Resources, Writing - review & editing. **Tengchuan Jin:** Methodology, Writing - review & editing.

Data availability

Data will be made available on request.

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Conflicts of interest

The authors have no conflict of interest to declare. Mention of trade names or commercial products in this publication is solely to provide specific information and does not imply recommendation or endorsement by USDA. USDA is an equal opportunity provider and employer.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.molimm.2022.12.001.

References

- Abramoff, M., Magalhaes, P., S.Ram, 2004. Image processing with imageJ. *Biophoton. Int.* 11, 36–42.
- Burks, A.W., Shin, D., Cockrell, G., Stanley, J.S., Helm, R.M., Bannon, G.A., 1997. Mapping and mutational analysis of the IgE-binding epitopes on Ara h 1, a legume vicilin protein and a major allergen in peanut hypersensitivity. *Eur. J. Biochem. / FEBS* 245, 334–339.
- Gupta, R.S., Springston, E.E., Warrier, M.R., Smith, B., Kumar, R., Pongracic, J., Holl, J. L., 2011. The prevalence, severity, and distribution of childhood food allergy in the United States. *Pediatrics* 128, e9–e17.

- Gupta, R.S., Warren, C.M., Smith, B.M., Jiang, J.L., Blumenstock, J.A., Davis, M.M., Schleimer, R.P., Nadeau, K.C., 2019. Prevalence and severity of food allergies among US adults. *Jama Netw. Open* 2.
- Jin, T., Guo, F., Kim, S., Howard, A., Zhang, Y.Z., 2007a. X-ray crystal structure of TNF ligand family member TL1A at 2.1 Å. *Biochem. Biophys. Res. Commun.* 364, 1–6.
- Jin, T., Kim, S., Guo, F., Howard, A., Zhang, Y.Z., 2007b. Purification and crystallization of recombinant human TNF-like ligand TL1A. *Cytokine* 40, 115–122.
- Kumfer, A.M., Commins, S.P., 2019. Primary prevention of food allergy. *Curr. Allergy Asthma Rep.* 19, 7.
- Lamer, T., van Belkum, M.J., Wijewardane, A., Chiorean, S., Martin-Visscher, L.A., Vederas, J.C., 2022. SPI "sandwich": combined SUMO-Peptide-Intein expression system and isolation procedure for improved stability and yield of peptides. *Protein Sci.* 31, e4316.
- Masitama, K., Yamamoto, K., Ito, K., Kitagawa, E., Yamaki, K., 2014. Simplified methods for purification of peanut allergenic proteins: Ara h 1, Ara h 2, and Ara h 3. *Food Sci. Technol. Res.* 20, 875–881.
- Moore, L.E., Stewart, P.H., deShazo, R.D., 2017. Food allergy: what we know now. *Am. J. Med. Sci.* 353, 353–366.
- Nagaraj, S., Reddy, P.N., Ramlal, S., Paul, S., Peddayelachagiri, B., Parida, D.M., 2019. A novel tandem repeat cloning technique for creation of multiple short peptide repeats to differentiate closely related antigens. *J. Immunol. Methods* 469, 11–17.
- NIH Expert Panel, 2006. Food allergy: Report of the NIH Expert Panel on Food Allergy Research. NIH.
- Rabjohn, P., Helm, E.M., Stanley, J.S., West, C.M., Sampson, H.A., Burks, A.W., Bannon, G.A., 1999. Molecular cloning and epitope analysis of the peanut allergen Ara h 3. *J. Clin. Investig.* 103, 535–542.
- Shreffler, W.G., Beyer, K., Chu, T.H., Burks, A.W., Sampson, H.A., 2004. Microarray immunoassay: association of clinical history, in vitro IgE function, and heterogeneity of allergenic peanut epitopes. *J. Allergy Clin. Immunol.* 113, 776–782.
- Shreffler, W.G., Lencer, D.A., Bardina, L., Sampson, H.A., 2005. IgE and IgG4 epitope mapping by microarray immunoassay reveals the diversity of immune response to the peanut allergen, Ara h 2. *J. Allergy Clin. Immunol.* 116, 893–899.
- Sicherer, S.H., Munoz-Furlong, A., Sampson, H.A., 2003. Prevalence of peanut and tree nut allergy in the United States determined by means of a random digit dial telephone survey: a 5-year follow-up study. *J. Allergy Clin. Immunol.* 112, 1203–1207.
- Stanley, J.S., King, N., Burks, A.W., Huang, S.K., Sampson, H., Cockrell, G., Helm, R.M., West, C.M., Bannon, G.A., 1997. Identification and mutational analysis of the immunodominant IgE binding epitopes of the major peanut allergen Ara h 2. *Arch. Biochem. Biophys.* 342, 244–253.
- Suarez-Farinas, M., Suprun, M., Kearney, P., Getts, R., Grishina, G., Hayward, C., Luta, D., Porter, A., Witmer, M., du Toit, G., Lack, G., Chinthrajah, R.S., Galli, S.J., Nadeau, K., Sampson, H.A., 2021. Accurate and reproducible diagnosis of peanut allergy using epitope mapping. *Allergy* 76, 3789–3797.
- Suprun, M., Kearney, P., Hayward, C., Butler, H., Getts, R., Sicherer, S.H., Turner, P.J., Campbell, D.E., Sampson, H.A., 2022. Predicting probability of tolerating discrete amounts of peanut protein in allergic children using epitope-specific IgE antibody profiling. *Allergy*.
- Yang, X., Lin, Z., Jing, Y., 2022. Cleavable self-aggregating tags (cSAT) for therapeutic peptide expression and purification. *Methods Mol. Biol.* 2406, 131–143.
- Zenin, V., Yurkova, M., Tsedilin, A., Fedorov, A., 2022. Enfuvidite biosynthesis in thermostable chaperone-based fusion. *Biotechnol. Rep.* 35, e00734.
- Zhang, Y., Fan, Y., 2017. A mutant sumo facilitates quick plasmid construction for expressing proteins with native n-termini after tag removal. *Mol. Biotechnol.* 59, 159–167.
- Zhang, Y., Du, W.X., Fregevu, C., Kothary, M.H., Harden, L., McHugh, T.H., 2014. Expression, purification, and characterization of almond (*Prunus dulcis*) Allergen Pru du 4. *J. Agric. Food Chem.* 62, 12695–12700.
- Zhang, Y.Z., Cheng, H., Gould, K.L., Golemis, E.A., Roder, H., 2003. Structure, stability, and function of hDim1 investigated by NMR, circular dichroism, and mutational analysis. *Biochemistry* 42, 9609–9618.