Structural and allergenic properties of the Fatty Acid Binding protein from shrimp *Litopenaeus vannamei*

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Short title: FABP allergen in shrimp

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Abstract

Background: The shrimp *Litopenaeus vannamei* is an important source of food allergens but its allergenic repertoire is poorly characterized. Cross reactivity between crustacean and mites has been characterized, with tropomyosin, the most relevant allergen involved. The aim of this study was the structural and immunological characterization of an allergen belonging to the Fatty Acid Binding Protein (FABP) family from *L. vannamei* (LvFABP).

Methods: ELISA, skin prick test (SPT) and basophil activation assays were performed to determine IgE reactivity and allergenicity of LvFABP. LC-MS/MS and Circular Dichroism experiments were done for structural analysis. B-cell epitope mapping with overlapping peptides, and cross-inhibition studies using human sera were done to identify antigenic regions and cross-reactivity.

Results: The recombinant LvFABP showed IgE reactivity in 27% of allergic patients tested and showed allergenic activity when tested for basophil activation and SPT in shrimp sensitized patients. CD-spectroscopy of LvFABP revealed that the protein is folded with a secondary structure composed of mainly β-strands and a smaller fraction of α helices. This is consistent with molecular modelling results, which exhibit a typical β barrel fold with two α-helices and ten β-strands. Epitope mapping identified two IgE binding antigenic regions and inhibition assays found high cross reactivity between LvFABP and Blo t 13, mediated by the antigenic region involving amino acids 54 to 72.

Conclusions: Our results support LvFABP as an allergen with cross reactivity with the allergen Blo t 13. This new allergen could help to understand new mechanisms of sensitization to seafood such as shrimp.

Keywords: Allergen and epitopes, IgE, basophils, bioinformatics, food allergy.

INTRODUCTION

Food hypersensitivity reactions affect approximately 2.5% of the general population [1]. Shellfish allergy is a long lasting, life threating disorder affecting children and adults [2]. Among these, shrimps are the most predominant cause of allergic reactions and thus extensively studied. Several allergenic proteins in shrimp and others crustaceans have been detected, including arginine kinase [3], sarcoplasmic calcium binding protein [4], and myosin light chain [5]. Recently, hemocyanin was identified as an additional allergen in a freshwater shrimp as well as in other crustaceans [6] along with troponin C and triosephosphate isomerase [7]. Sensitization to shrimp and house dust mite (HDM) is correlated and these allergenic sources are important inducers of allergic reactions worldwide, mainly in regions with high intake of seafood and exposure to HDM such as tropical environments [8]. Tropomyosin is frequently involved in the cross reactivity between shrimp and HDM [9]. A 20 kDa allergenic component from shrimp extract also contributes to cross reactivity between shrimp and mites [10]. Identification of new allergens from shrimp could help to better understand its cross sensitization with HDM.

A nucleotide sequence coding for a FABP (GenBank: KF471026.1) was reported in the shrimp species *L. vannamei*. FABPs belong to the lipid binding protein superfamily, and are small proteins of approximately 15 kDa that play a crucial role in the transport of cytosolic long chain fatty acids and their metabolism [11]. This protein family shows an architecture consisting of 10 antiparallel β-strands forming two β-sheets surrounding an internal pocket or barrel structure and two short α-helices positioned at the end of the barrel [12,13]. Allergens from this protein family can accommodate different lipid ligands in this

pocket, such as: 11-([5-dimethylaminonaphthalene-1-sulfonyl amino]) undecanoic acid (DAUDA), dansyl-DL-a-aminocaprylic acid (DACA), cis-Parinaric acid (cPnA), and dehydroergosterol (DHE)) [14,15].

Allergens from Group 13 of HDM belong to the FABP family, e.g. Blo t 13 in *B. tropicalis*, Der f 13 of *D. farinae*, Der p 13 of *D. pteronyssinus,* Led d 13 of *Lepidoglyphus destructor* and Tyr p 13 of *Tyrophagus putrescentiae* [14-18]. Der p 13 uses TLR2 to promote HDM allergic response triggering the production of IL-8 and GM-CSF in respiratory epithelial cells through a MyD88, NF-kB, and MAPK-dependent signaling pathway [14]. In this process, lipid bound to Der p 13 seems to enhance the inflammatory process through innate immunity.

Studies of B cell epitope mapping of FABP from *Schistosoma japonicum* using sera from immunized mice and ELISA with overlapping peptides [19], and the use of FABPs from invertebrates for developing vaccines against *Fasciolia hepatica* [20] suggest that this protein family has important immunological properties, and considering that shrimp is a common allergenic source, the allergenic role of its FABP deserves to be analyzed.

METHODS

Study population and serum samples

Serum samples were obtained from shrimp allergic patients and non-allergic individuals recruited at the Institute for Immunological Research in the context of a project aimed to study the immune response induced by FABP of different sources. All patients with

113 symptoms of shrimp allergy were skin prick tested with a battery of common standardized
114 allergens (*D. pteronyssinus, B. tropicalis*, Cockroach, dog and cat epithelia), and with a
115 shrimp extract prepared in the Institute. Test with wheals of 3 mm of diameter or greater
116 were considered positive. Thirty-six allergic patients (30 males, and 6 females, median age
117 32 years) and eighteen subjects without allergy history, negative SPT and negative IgE
118 levels to shrimp extract, were included as negative controls (Table 1). In addition, a serum
119 pool was prepared by mixing equal volumes of serum samples from four shrimp allergic
120 subjects. The study was approved by the research ethics committee of University of
121 Cartagena and written informed consent was obtained from all patients (approval:
122 10/06/2010; project code: 110752128386).
123

124 **Skin Prick test with LvFABP**

125 For SPT, a protein solution with a concentration of 25 ng/μL of LvFABP, 50%
126 glycerol, 0.4% phenol, was prepared, treated with LPS-Free Toxin eraser Kit (Genscript
127 Cat. No. L00338) and filtered using 0.22 μm filter. This protein concentration was chosen
128 after a dose titration with different concentration was done in five patients. A drop of
129 allergen solution was pricked on the forearm using a sterile lancet. Histamine (1 mg/mL)
130 and allergen diluent were included as controls. The test was considered positive if the mean
131 diameter of the wheal at 15 min was >3 mm larger than the negative control.
132

133 **Shrimp extract preparation**

A boiled shrimp allergenic extract was prepared following indications reported elsewhere [21] with modifications; ten grams of shrimp were boiled at 60°C in 100 mL of phosphate buffer saline (PBS) . Followed by homogenization at room temperature (RT). The protease inhibitor PMSF, (Merck 10837091001, Darmstadt, Germany) and sodium azide (Merck S2002, Darmstadt, Germany) were added to a final concentration of 0.01 mM and 0.5%, respectively. The mix was stirred for 4 h at 4°C, then centrifuged at 6500 r.p.m. The final solution was dialyzed overnight (ON) against deionized water using 3.5 kDa cut-off dialysis membrane (Spectra Pore. Sigma Aldrich D9277).

Recombinant proteins

The nucleotide sequence of FABP from *L. vannamei* (GenBank: KF471026.1) was inserted into the expression vector pET45b+ (Genscript. NJ, USA) and transformed into the *Escherichia coli* strain BL21 (DE3). The strain was grown in Luria–Bertani medium containing 100 mg/L ampicillin at 37 °C to an OD of 0.4 at 600 nm wavelength. Protein expression was induced by the addition of 1 mM isopropyl-β-D–thio-galacto-pyranoside, and the culture was incubated for 4 hours at 37°C. Cells were then harvested by centrifugation at 6500 r.p.m. at 4 °C for 15 min. The cell pellets were solubilized in 8 mol/L urea, 0.1 mol/L NaH_2PO_4, and 0.01mol/L Tris-HCl at pH 8.0, pulsed by ultrasound four times on ice and then incubated by continuous rotation for 3 h. Insoluble material was removed by centrifugation, and clear supernatant used for the protein purification. The supernatant was applied to nickel-nitrilotriacetic acid-Agarose (Qiagen. Hilden, Germany). The protein was eluted with native elution buffer (50 mM NaH_2PO_4, 0.5 M NaCl, and 250 mM imidazole at pH 8.0). Fractions containing eluted protein were

pooled and dialyzed against 50 mM NaH_2PO_4 and 0.5 M NaCl (pH 8.0). Blo t 13 was produced as indicated in reference [22]. Eluted protein was analyzed by SDS-PAGE stained with Coomassie Brilliant Blue R-250. The protein concentration was determined by Bradford assay.

Identification of LvFABP by LC-MS/MS

To confirm its identity, the protein band after SDS-PAGE was digested enzymatically and the tryptic peptides were analyzed by LC-MS/MS. Amino acid sequence data was obtained for thirteen peptides, which were matched to ADK66280.1 (accession number) of FABP from *L. vannamei* using the MASCOT database. The proteomic service was provided by ICBR (University of Florida, Gainesville, Fl, USA).

Circular Dichroism

For circular dichroism spectroscopy, measurements were carried out at a concentration of 0.09 mg/mL in a 0.1 cm quartz cuvette while using a Jasco 1500 CD-spectrometer (Japan Spectroscopic Co., Tokyo, Japan). Far UV spectra were recorded in the range of 195 to 260 nm. The spectra were baseline corrected by buffer subtraction and then converted to mean residue ellipticities (θ) at the given wavelengths. The calculation of the secondary structure content was performed with Dichroweb [23].

B-cell epitope mapping

For epitope mapping of LvFABP, ten overlapping peptides representing the amino acids 1-20, 14-33, 27-46, 40-59, 53-72, 66-85, 79-98, 92-111, 105-124 and 118-136 of the sequence (Uniprot: E2IH93) were designed and their synthesis ordered to Genscript, USA. Each lyophilized peptide was reconstituted to 10 mg/mL in 2.5 % dimethyl sulphoxide and stored at -20ºC until use.

Epitope mapping was performed as indicated elsewhere [22,24] with modifications. One hundred µL of seven individual sera from shrimp allergic patients sensitized to LvFABP diluted 1:2, were pre-adsorbed with 150 µg/100 µL of each peptide. BSA and LvFABP (10 µg/100 µL) were used as negative and positive controls, respectively. Peptides showing a significant level of inhibition were then tested at different concentrations to observe the dose–response relationship. The degree of inhibition was expressed as cross-reactivity index (CRI). All experiments were performed in duplicate. In addition, peptide 5 was analyzed in an IgE cross-reactive assay: 150 µg/100 µL of peptide 5 were incubated with 100 µL of the serum pool diluted 1:4 for 5 h at RT. Then, wells coated with LvFABP or Blo t 13 were incubated with this mix ON at RT, and CRI determined.

Determination of IgE levels to LvFABP and Blo t 13, and cross-inhibition ELISA

The IgE reactivity to LvFABP was determined by ELISA. Microtiter plate wells (Immulon-4, Dynatech, Chantilly, VA, USA) were incubated with 0.5 µg/100 µL of LvFABP ON at RT. After three washes with PBS-Tween 20 (PBS-T), the wells were incubated with 100 µL of blocking buffer (PBS-T, 1% BSA, 0.02% sodium azide) for 3

200 hours at RT in a wet chamber. Then, they were incubated with 100 µL of serum samples
201 diluted 1:5 ON at RT, and after washing, the wells were incubated with 100 µL of alkaline
202 phosphatase conjugated anti-IgE antibody (Sigma A3525) diluted 1: 500. After the final
203 wash, 100 µL of paranitrophenyl phosphate was added, and the wells were incubated at RT
204 for 30 min. Absorbance at 405 nm wavelength was determined using a spectrophotometer
205 (Spectra MAX 250, Molecular Device, Sunnyvale, CA, USA). All experiments were
206 performed in duplicate. Sera from six non-allergic individuals were used to establish cut-
207 off. Positive IgE levels were defined as an optical density (OD) equal or greater than 0.12
208 (mean values from these samples plus two standard deviations). IgE reactivity to Blo t 13
209 was determined as in reference [22].

210 IgE cross-reactivity between LvFABP and Blo t 13 was analyzed by ELISA inhibition as
211 in references [22,24] with modifications; 100 µL of the serum pool was adsorbed with 100 µL
212 of increasing concentrations (0, 1, 10, 50, 75 and 100 µg/mL) of an inhibitor (LvFABP or
213 Blo t 13) for at 4 °C. Then, 100 µL of this mix was loaded into wells coated with the
214 relevant antigens and incubated at RT in a wet chamber. After three washes with PBS-T,
215 reaction was detected with anti-IgE antibodies (Sigma A3525) by ELISA, as described
216 above.
217

218 **Reactivity with anti-Blo t 13 monoclonal antibodies**

219 The reactivity of anti-Blo t 13 monoclonal antibodies (mAbs) 5G3, 5H11 and 6D6 [25]
220 against LvFABP was analyzed by ELISA. Microtiter plate wells (Immulon-4, Dynatech,
221 Chantilly, VA, USA) were incubated with 0.5 µg/100 µL of LvFABP, Blo t 13 or Lit v 1 at

4°C and ON. After several washes with PBS-T, the wells were blocked with 100 µL of PBS-T, 1% BSA, 0.02% sodium azide for 3 hours, at RT in a wet chamber and incubated with 1 µg/100 µL of mAb for 2 hours at RT, after several washes 100 µL of alkaline phosphatase-conjugated anti mouse-IgG (SIGMA A3562), diluted 1: 10,000 was added and incubated for 2 hours at RT. After the final wash, a colorimetric reaction was developed by incubating with paranitrophenyl phosphate for 30 min at RT. Absorbance at 405 nm wavelength was determined using spectrophotometer (Spectra MAX 250, Molecular Device, Sunnyvale, CA, USA). Results were expressed in O.D.

Basophil Activation Test

Peripheral blood was collected from four shrimp allergic patients and one non allergic subject. Basophil activation was measured by flow cytometry using the Allergenicity Kit (Beckman Coulter, Inc. CA, USA). One hundred µL of blood was incubated with LvFABP at three concentrations (0.1, 1 and 10 µg/ml), for 15 minutes at 37ºC. Basophils were gated based on the expression of CRTH2 marker and activation was assessed by detection of the activation marker CD203c using phycoerythrin-conjugated CD203c mAb 97A6 in a Dakocytomation cytometer (Beckman Coulter, Inc. CA, USA). Data was analyzed using Summit 4.3 software (Beckman coulter, Inc CA, USA). Allergen-induced upregulation of CD203c was calculated using mean fluorescence intensities (MFI) obtained with stimulated (MFI stim) and unstimulated (MFI control) cells and expressed as the Stimulation Index (SI), (SI = MFI stim /MFI control). SI of $\geq$ 2.0 was considered indicative of a positive response.

Structural modelling and bioinformatics analysis

The amino acid sequences of LvFABP (Uniprot: E2IH93), Blo t 13 (Q17284), Der p 13 (E0A8N8), Der f 13 (A1KXH1), Lep d 13 (Q9U5P1), Aca s 13 (B0KZJ5), Tyr p 13 (Q66RP5) were retrieved from UNIPROT database (http://www.uniprot.org): Homology-based modeling of LvFABP structure was done using SWISS-MODEL [26] and the structure of Der f 13 (PDB: 2A0A) as a template. Models were refined in Deep-View (energy minimization and rotamer replacements). Their quality was evaluated by several tools [27].

For a structural comparison, Root Mean Square Deviation (RMSD) of both protein structures was calculated by a structural alignment using UCSF Chimera software package. (http://www.rbvi.ucsf.edu/chimera) [28]. The 3D model of LvFABP was used to map the conserved regions on this protein family using the program ConSurf, which scores the amino acids according to their degree of conservation [29,30]. All parameters were set up as default.

Statistical analysis

The comparison of antibody levels was done using the non-parametric Mann Whitney U test. All statistical analyses were two-tailed, and the significance level was set at $p < 0.05$. Analyses were performed using IBM Statistics SPSS v20 (IBM Corp) and GraphPad Prism.

RESULTS

Recombinant LvFABP was purified to homogeneity and its identity confirmed by LC-MS/MS

The purified shrimp FABP appeared as a single band with a mass of 15 kDa in SDS-PAGE (Fig 1A). Twenty-six peptides were matched in LC-MS/MS analysis with the sequence reported for this FABP in the UniProt database (Uniprot: E2IH93) (Table 2).

The CD spectroscopic analysis (Figure 1B) showed that recombinant LvFABP has β-strand and α-helical structural content. The shape of the CD-spectrum with a minimum at about 217 nm was indicative of mostly folded protein and the secondary structure calculation from the spectral data yielded 40% β-strands and 11% α-helices, consistent with the generated homology model of LvFABP.

Allergenic activity of LvFABP was confirmed by ELISA, SPT and basophil activation test

Ten sera of thirty-six (27.7%) showed positive IgE reactivity to LvFABP and twenty-five sera were IgE positive to Blo t 13 (69.4%), IgE antibody levels to LvFABP were lower than to Blo t 13 ($p = 0.001$), (Fig. 2A). One serum reacted exclusively to LvFABP, thirteen reacted exclusively to Blo t 13 and nine sera reacted to both LvFABP and Blo t 13 (Fig 2B). Thirteen serum samples were reacting to neither of the two proteins.

289 Basophil activation using LvFABP showed that samples from three patients were activated
290 (Fig 2C). The sample from the non-allergic subject was not activated. SPT with LvFABP
291 was performed in fifteen allergic patients, of which only five (33.3%) showed positive
292 results (Table 3).

293

294 **LvFABP and Blo t 13 are highly cross-reactive**

295 In the cross-inhibition ELISA, the IgE-reactivity of the serum pool to LvFABP was
296 inhibited up to 83% by Blo t 13 at the highest concentration of inhibitor (Fig 3A), and
297 the IgE reactivity to Blo t 13 was inhibited up to 62% by LvFABP (Fig. 3B). ELISA with
298 the monoclonal antibody specific for Blot 13 (5G3) showed high reactivity against
299 LvFABP, but not to Lit v 1, which is the structurally unrelated allergen Tropomyosin. No
300 reaction was found with the control anti Der p 2 mAb (4C1), (Fig 3C).

301 **An epitope located in a region with high homology explains IgE cross reactivity**
302 **between LvFABP and Blo t 13**

303 Epitope mapping identified two IgE-binding regions in LvFABP (Fig 3D). In one
304 region, four sera showed IgE reactivity, as indicated by CRI values from 38 to 62%, against
305 peptides 4, 5 and 6, which cover amino acid residues 40 to 85. In the other region, three
306 sera showed IgE reactivity, as indicated by CRI values from 30 to 70%, against peptides 9
307 and 10, which cover residues 107 to 136. When the reactivity with peptides 4, 5 and 9 was
308 analyzed using different peptide concentrations, a dose–response relationship was observed
309 in the inhibition, supporting the findings (Fig 3E). In order to determine if peptide 5 is
310 involved in IgE cross reactivity between LvFABP and Blo t 13, we performed an inhibition

assay with peptide 5, observing a 58% of CRI with Blo t 13. In this assay, the CRI with LvFABP was 70% when Blo t 13 was used as inhibitor (Fig 3F). FABPs from shrimp and HDM share 46% of identity in their amino acid sequences. The higher grade of conservation is in the residues 54 to 72 (Figure 4).

Molecular modelling supports the identified IgE cross-reactive region

LvFABP exhibited the typical fold of the FABP family with two α helices and ten β strands forming a β-barrel in the 3D model (Fig 5A), which is in concordance with results of the CD analysis. The positions of equivalent α carbons in the two molecules can be aligned with a RMSD of 0.8 Å (Figure 5B). IgE-binding regions are located in the strands βD, βE and βH, βI (Figures 5C and D). Local RMSD between the β strands from LvFABP and Blo t 13 showed a value of 0.6 Å, suggesting high structural homology in the antigenic regions. Additionally, a ConSurf analysis revealed that the region located on βD and βE strands is most conserved among FABP family members (Figures 5E and 5F).

DISCUSSION

The characterization of the complete set of allergenic components from shrimp remains to be done. For the species *L. vannamei*, four allergens have been characterized: Lit v 1, Lit v 2 [3], Lit v 3 [5] and Lit v 4 [4]. Here, we report the characterization of the FABP allergen from this species, showing 27% of serum IgE reactivity in a group of shrimp allergic patients. This is the lowest frequency of IgE reactivity reported for a shrimp allergen. HDM allergens belonging to the FABP family have a low frequency of IgE reactivity [14, 16-18], with exception of Blo t 13, for which a frequency of 57% has been

found in a Cuban allergic population [31]. SPT and basophil activation confirmed the allergenic activity of LvFABP. In the basophil degranulation assays 3 of the 4 samples from shrimp allergic patients induced degranulation at a concentration of 10 µg / mL of LvFABP. The differences in allergen specific IgE repertoire among shrimp allergic patients, which can affect the IgE cross-linking on basophil membrane, could explain in part of this result [32]. Basophil activation results in combination with serum IgE reactivity and SPT, indicate that this recombinant protein has key properties of an allergen. However, assays with a larger number of patients must be done to evaluate its clinical importance.

LvFABP and Blo t 13 have a high degree of cross reactivity. The monoclonal antibody 5G3 reacted with LvFABP, indicating that this allergen and Blo t 13 share an antigenic region, which can explain part of IgE reactivity as indicated by the inhibition assay using peptide five (figure 3F). The 5G3 mAb binds to an epitope located in a region spanning residues 54 to 72 of Blo t 13 located in strands βD and βE [22], and our sequence alignment revealed that this sequence is highly conserved between LvFABP and the FABP from HDM. ConSurf analysis supports that this region is involved in the cross reactivity between shrimp and *B. tropicalis* FABP. Additionally, it could be implicated in cross reactivity with FABP from different allergenic sources such as insects and others crustaceans [33].

CD analysis and homology modelling indicate that LvFABP shares a similar fold to allergens from HDM such as Blo t 13, Der f 13, and Der p13. Blo t 13 has the capacity to

bind different fatty acids in its pocket [15], which was also demonstrated for Der p 13 [14]. The binding to hydrophobic ligands is selective in these allergens and despite structural similarities, their precise binding propensities differ [14, 15]. The lipid binding capacity of LvFABP remains to be analyzed. However, due to the high structural homology of these proteins and the result our bioinformatics analysis, we hypothesize that a similar lipid binding capacity can be assumed for LvFABP. This function seems to be pertinent to its allergenic potential. The capacity to bind hydrophobic ligands is crucial for the induction of a TLR2-associated allergic response by Der p 13 [14] and this biochemical property could also be involved in the allergic response induced by LvFABP, which should be explored with the appropriate functional assays.

Based on the frequency of IgE reactivity, LvFABP seems to have an irrelevant role in term of IgE mediated response. However, other mechanisms could participate in the induction of Th2 immune response. Here we show that it has allergenic activity and could be clinically important. In addition, its high IgE cross-reactivity with very common environmental allergens such as those from HDM, make LvFABP one of the food allergens to keep in mind when co-sensitization to shrimp and HDM exists, as occurs with the common pan-allergen tropomyosin [34,35]. We propose to name this allergen as Lit v 7.

In conclusion, we report the allergenic properties of LvFABP, as detected by serum IgE reactivity, SPT and basophils activation test. We identified potential IgE-binding

regions in this molecule that could explain its high cross reactivity with members of the group 13 HDM allergens.

CONFLICT OF INTEREST

The authors have declared that no competing interest exist.

AUTHOR CONTRIBUTIONS

Author Contributions: Conceptualization, PL and MM; Formal analysis, MM, MD, WJ, KW, LC and PL; Investigation, MM, DM, ZJ, WJ; Funding acquisition, PL and WK; Project administration, PL; Resources, CL, WK and PL; Writing – original draft, MM; All the authors contributed to the review, editing and approved de manuscript.

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FIGURE LEGENDS

Fig 1. Expression and purification of recombinant LvFABP. A. SDS-PAGE stained with Coomassie blue. MW: Molecular weight marker, L: Lysate, and F1: eluted protein. Arrow indicates protein expressed. B. Circular Dichroism spectrum of LvFABP.

Fig 2. Antibody reactivity and allergenicity in shrimp allergic population. A: Levels of IgE antibodies to LvFABP and Blo t 13. B: Venn diagram showing numbers of patients with IgE reactivity to LvFABP, Blo t 13 or both allergens. C: At the concentration of 10 μg/mL LvFABP induced activation of basophils in samples from three shrimp allergic patients, whereas in sample from one allergic patient and the control was not.

Fig 3. IgE Cross reactivity and epitope mapping analysis. A: Blo t 13 inhibited IgE reactivity to LvFABP up to 83%. B: LvFABP inhibited IgE reactivity to Blo t 13 up to 62%. C: Reactivity of anti Blo t 13 monoclonal antibodies (5G3, 5H11 and 6D6) and anti Der p 1 mAb 4C1. D: Cross-reactivity index of synthetic peptides and LvFABP. Diluted sera were preadsorbed with each overlapping peptide, BSA or LvFABP and reacted with plate wells coated with LvFABP. E: Dose–response curves, inhibition of IgE binding to LvFABP. Peptides 5 and 9 showed 65 and 57% of maximum inhibition, respectively. F: IgE reactivity inhibition to FABP with peptide 5 and Blo t 13. CRI of 40 and 54% were produced by peptide 5 with LvFABP and Blo t 13, respectively.

Fig 4. Sequence alignment. FABP from shrimp and HDM share a 46 % of identity in their amino acid sequences. The higher conserved region is located between residues 56 to 80 of Blo t 13 sequence.

Fig. 5. Structural analysis. A: Representation of 3D model of LvFABP showing typical fold (front view). B: Superposition of LvFABP structure (brown) and Blo t 13 (blue) with R.M.S.D. of 0.8 Å. C and D: Surface representation of the model showing the positions of two IgE binding epitopes identified by epitope mapping, back and front views, respectively. E and F: space fill representation of the model generated by ConSurf. E: back view, the violet colors with highest numbers 7-9 are more represented in the region equivalent to the beta E and beta D strands, indicating high conservation of this region. F: front view, this image shows a lower representation of the color numbers 7-9 in the region equivalent to epitope located in the beta I and beta H strands, indicating a comparatively lower grade of conservation.

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557 Table 1. Characteristic of the allergic patients used in this study.

Patient	Sex	Age (y)	IgE (OD)	reaction
CAM001	F	48	0,164	ANA, U
CAM002	M	21	0,134	GI
CAM007	M	22	3,48	ANA, AE
CAM008	M	28	0,623	AE
CAM010	M	21	0,118	I
CAM011	M	22	0,266	I
CAM012	M	14	0,124	I
CAM014	F	18	0,15	ANA, AE
CAM016	M	19	0,143	U, I
CAM017	M	19	0,157	GI, ANA, U
CAM018	M	18	3,51	U
CAM019	F	20	0,116	U
CAM020	M	32	0,156	ANA, AR, I
CAM023	F	51	0,195	AR, I
CAM024	M	54	0,187	I
CAM029	M	19	0,257	AR, ANA
CAM031	M	25	0,122	U, I
CAM032	F	32	0,053	ANA, AR, I
CAM037	M	17	0,083	AR
CAM038	M	25	0,805	I, GI
CAM043	F	19	0,183	GI
CAM048	M	22	0,102	I
CAM049	F	35	0,608	AR
CAM050	F	32	0,473	GI
CAM056	F	24	0,142	GI
CAM061	M	7	0,184	ANA

CAM062	F	19	0,708	I
CAM065	M	51	0,362	GI, I, U
CAM066	M	21	1,745	ANA, GI, I
CAM069	M	21	0,127	I, GI
CAM072	F	43	0,186	U, ANA
CAM073	M	11	0,138	I, AR
CAM074	F	21	0,111	AR
CAM077	F	18	0,315	I
CAM080	M	18	0,115	U
CAM082	F	25	0,231	AR

AR: allergic rhinitis, ANA: anaphylaxis, I: Itch, U: urticaria, GI: gastrointestinal symptoms.

Table 2. LC-MS/MS identified fraction of 15 KDa as FABP from *L. vannamei.*

Protein	Uniprot Acc.	Molecular weight (KDa)	Peptides matched	Percent coverage
Intracellular Fatty Acid Binding Protein (*L. vannamei*)	E2IH93	15	26	63
RNA binding Protein Hfq (Enterobacteria)	P0A6X3	11	5	44
ybdQ Gene product (*E. coli*)	P39177	16	4	39
Keratin 1 (*Homo sapiens*)	Q14533	66	4	7.3

Table 3. SPT results. Subjects with positive skin tests to shrimp extract were tested with recombinant LvFABP. Histamine and glycerol/Saline solution were used as positive and negative controls, respectively.

Patients	Sex	Age (Years)	Shrimp Extract	LvFABP	Histamine	Glycerol/Saline solution
			Wheal/Erythema (mm diameter)			
CAM008	M	28	6/25	3/10	7/20	0/0
CAM038	M	25	6/17	0/0	6/18	0/0
CAM043	F	19	16/26	4/4	5/24	0/0
CAM056	MF	22	16/26	5/10	6/10	0/0
CAM061	MF	24	9/21	0/0	6/26	0/0
CAM062	M	7	11/21	0/0	6/25	1/0
CAM065	F	19	3/10	0/5	5/29	0/0
CAM048	M	51	5/28	4/19	6/20	0/0
CAM066	M	21	5/13	0/0	5/27	0/0
CAM069	M	21	9/29	0/3	12/32	0/0
CAM072	F	43	7/27	0/0	8/28	2/0
CAM073	M	11	5/15	0/0	6/23	0/0
CAM074	F	21	8/28	2/6	8/46	0/0
CAM080	F	25	3/30	0/0	8/12	0/0
CAM082	F	25	4/28	3/20	7/35	0/0

FIGURE 1.

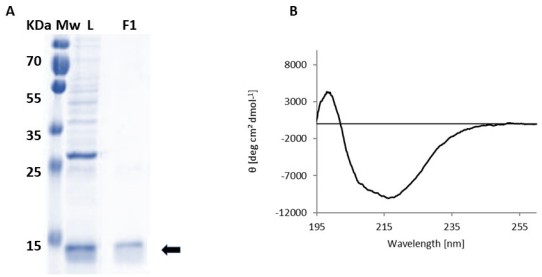
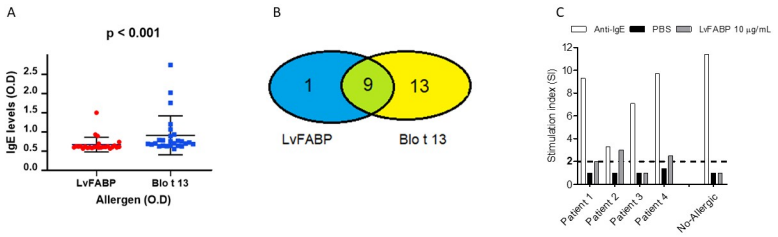


FIGURE 2.



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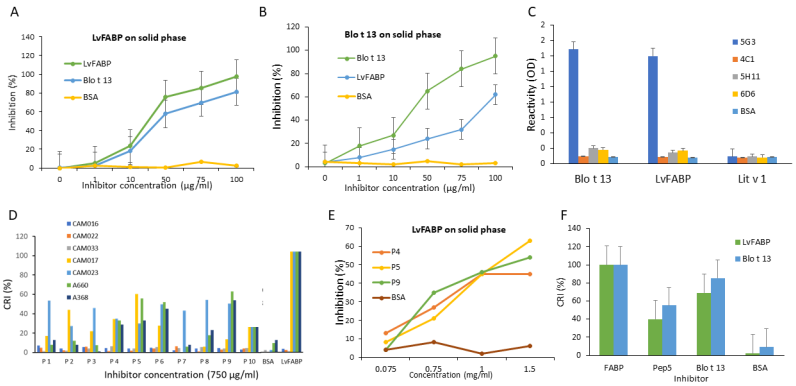
592 FIGURE 3.



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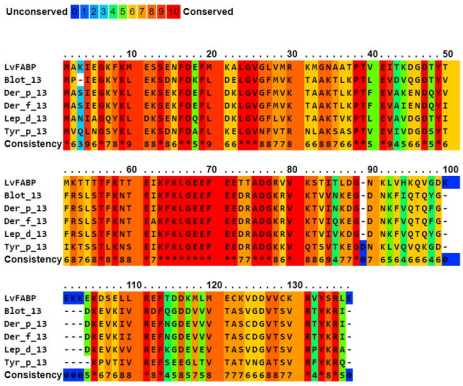
594 FIGURE 4.



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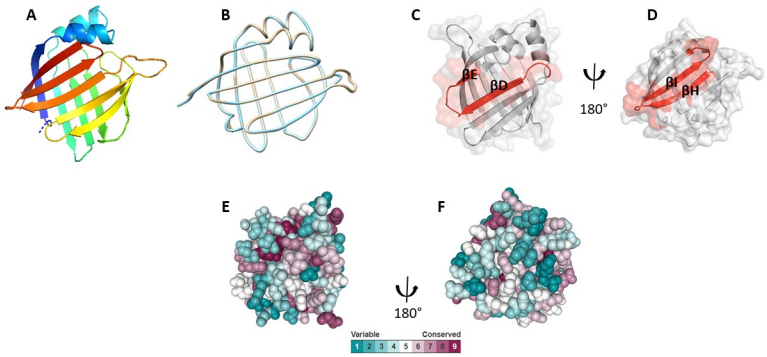
596 FIGURE 5.



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