

Mimotope identification of dust mite allergen Der f 5 using phage-displayed random peptide libraries

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Received October 16, 2015; Accepted September 12, 2016

DOI: 10.3892/mmr.2016.5848

Abstract. Mimotope mapping enables the characterization of allergen epitopes for the development of diagnostic and therapeutic approaches. In the present study, a phage display peptide library was used for mimotope mapping based on the binding of antibodies against the recombinant group 5 allergen from the house dust mite *Dermatophagoides farinae* (Der f 5), an arthropod that causes indoor allergies worldwide. When three monoclonal anti-Der f 5 antibodies were used for biopanning, seven mimotopes were identified. Their common subsequence was '---[A][T]W[-S]H[HSFW][LM][PSKR][TLV][AST]-[DP][-L]'. When analyzed in combination with predicted discontinuous epitopes, amino acids P2, K3, K4, H5, F11, F13, L14, R72, T77, L79, R84, T39, F40, P44, T45 and K46 were identified as key residues in conformational epitopes of Der f 5. Therefore, the seven mimotopes or modification of the key amino acids may facilitate the development of blocking antibodies or epitope-specific immunotherapies for mite allergy.

Introduction

In Western industrialized nations, >25% of the population is affected by IgE-mediated allergic disorders (1); an epidemiological study has reported rising disease prevalence and increasing rates of allergen sensitization worldwide (2). Allergen-specific immunotherapy (ASIT), also referred to as desensitization, hyposensitization or specific immunotherapy, was introduced almost a century ago (3), and administers slowly increasing doses of a relevant antigen until a maintenance dosage is achieved or the patient is free of symptoms.

ASIT appears to induce the production of 'protective substances' that block the allergic reaction. The thermostable protective antibodies in serum from patients treated with ASIT are primarily IgG and are known as 'blocking antibodies' due to their ability to inhibit the interaction between the allergen and IgE (4,5). This phenomenon indicates that immune responses must be directed to the inhibitory antibody epitopes of the allergen or antigen. Thus, mapping of epitopes for a given allergen is useful for understanding the immune basis of ASIT, and for designing and developing novel allergen vaccines (5,6).

A mimotope is a macromolecule that mimics the structure of an epitope. It may cause an immune response similar to the one elicited by the epitope itself. A mimotope will be recognized by an antibody against the mimicked epitope. The binding portion of an antigen, or B-cell epitope, may be a short peptide from the protein sequence or a patch of atoms on the protein surface in the three-dimensional space. B-cell epitope prediction is useful to understand the immune basis of antibody-antigen recognition. Mimotopes that structurally mimic B-cell epitopes may be mapped using phage-displayed random peptide libraries. Mimotopes may then be used to develop novel diagnostics, therapeutics and vaccines (4).

The primary source of indoor allergens worldwide is the house dust mite, specifically *Dermatophagoides pteronyssinus* and *D. farinae* (7). In China, a cross-sectional survey of 6,304 patients suffering from asthma and/or rhinitis in 17 cities across mainland China revealed that 59.0 and 57.6% of participants had positive skin prick responses for *D. farinae* and *D. pteronyssinus*, respectively (8). Therefore, characterizing the allergens produced by these two species is relevant to mitigating allergic disease. Although 33 groups of house dust mite allergens have been identified to date (www.allergen.org/), the groups 1, 2, 4, 5 and 7 constitute the known primary and mid-potency allergens (9). Allergens of groups 1 and 2 constitute 50-60% of IgE binding to house dust mite extracts; the allergens of groups 4, 5 and 7 bind individually and together in proportion to the primary allergens, contributing >30% of the total titer (10). However, certain studies have revealed that group 5 allergens from *D. pteronyssinus* (Der p 5) are an important group of dust mite allergens in humans (11-13). Furthermore, recombinant Der p 5 peptide expressed in a pGEX vector system was demonstrated to

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Key words: conformational epitopes, house dust mite allergen, immunotherapy, mimotopes

have strong reactivity with serum IgE from >50% of asthma patients (14). Although group 1 and 2 allergens have been well characterized, as house dust mites are pervasive, it is important to have a good understanding of each allergen they produce. To increase understanding of domestic mite hypersensitivity, our laboratory cloned and expressed the dust mite allergen Der f 5 of *D. farinae* (15). The present study identified mimotopes of Der f 5 using phage-displayed random peptide libraries against monoclonal antibodies (mAbs) specific to house dust mite allergen Der f 5.

Materials and methods

Prokaryotic expression, purification and renaturation of pET28a (+)-Der f 5. The plasmid pET28a (+)-Der f 5 was constructed as described previously (15). pET28a (+)-Der f 5 (5 ng) was used to transform BL21 (DE3) competent *E. coli* cells (Agilent Technologies, Inc., Santa Clara, CA, USA). The BL21 *E. coli* cells expressing pET28a (+)-Der f 5 were cultured at 37°C overnight on lysogeny broth (LB) plates containing 50 µg/ml kanamycin. Expression was induced with isopropyl β-D-1-thiogalactopyranoside (IPTG) as previously described (15). Recombinant Der f 5 (rDer f 5) was isolated, purified, re-natured and verified by SDS-PAGE and western blotting as described previously (16). The purified recombinant fusion protein was analyzed using a 4800 matrix-assisted laser desorption/ionization time of flight (MALDI-TOF/TOF) mass spectrometer (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA), as described previously (17). The spectra generated were mass-calibrated using known standards and the peaks de-isotoped (17). The masses obtained were searched using MASCOT (SwissProt Database; Matrix Science, Ltd., London, UK) and a 50 ppm mass tolerance window. Significant matches from Peptide Mass Fingerprinting were confirmed by tandem mass spectrometry (MS/MS) using the search criteria described and an MS/MS-tolerance window of 0.5 Da (17).

Preparation of mAbs against rDer f 5. Conventional hybridoma technology was used to prepare mAbs against recombinant protein rDer f 5. The animal experiments were approved by the Institutional Animal Care and Application Committee of the Yancheng Health Vocational & Technical College (approval no. 20121018).

Female BALB/c mice (n=6; age, 6-8 weeks) were purchased from the Animal Testing Center of Nanjing Medical University (Nanjing, China). Mice were maintained at room temperature (23±2°C) at a relative humidity of 40-70% under a 12-h light/dark cycle, with *ad libitum* access to food and water. rDer f 5 (100 µg) was mixed with Freund's Complete Adjuvant (Sigma-Aldrich; Merck Millipore, Darmstadt, Germany) for the first immunization and administered via subcutaneous injection. Further injections of 100 µg rDer f 5 were administered once every 2-3 weeks. Following 4 injections, blood samples were collected via the tail vein. An indirect ELISA was used to determine the titer of antiserum with the recombinant allergen rDer f 5 as the coating antigen and horseradish peroxidase (HRP)-conjugated rabbit anti-mouse IgG [catalog no. ab97046; Abcam Trading (Shanghai) Company Ltd.,

Shanghai, China] as the secondary antibody. When the titer became >1:10,000, 1 mouse was selected for cell fusion.

Cell fusion was conducted with myeloma cells and spleen cells at a ratio of 1:20. The mixed cells were placed into a 50 ml centrifuge tube, diluted with Dulbecco's modified Eagle's medium (DMEM; Invitrogen; Thermo Fisher Scientific, Inc.), and centrifuged at 168 x g for 5 min at 4°C. The supernatant was discarded and the cell pellet homogenized. Polyethylene glycol (PEG; 0.8 ml, 50%) was added slowly for 90 sec, followed by 20-30 ml DMEM. The fused cells were placed into a 37°C water bath for 10 min, centrifuged at 168 x g for 5 min at 4°C, and the supernatant discarded. DMEM containing hypoxanthine, aminopterin and thymidine (Sigma-Aldrich; Merck Millipore) was added to the cell pellet. The fused cells were seeded into a 96-well plate (100 µl/well) and placed into a 5% CO₂ incubator. After 4 days, the plate was assessed and the cloning efficiency of hybridoma cells was >50% with a small quantity of cell debris; cells were healthy. The screening and analysis was performed after 10 days.

One day prior to testing, an ELISA plate was coated with 5 µg/ml antigen (Der f 5 prokaryotic expression product) at 100 µl/well, with PBS (pH 7.4) as the coating buffer and HRP-conjugated rabbit anti-mouse IgG as the secondary antibody. The following day, 100 µl supernatant from the fused cells was added to each well. The positive wells were defined as sample well optical density (OD) value/negative well OD value ≥2.1. A single channel pipette was used to pick positive wells detected on the whole plate, to perform a confirmatory assessment; cells in the confirmed positive wells were subsequently subcloned.

For subcloning, cells in the positive wells were spread and counted. DMEM medium (4 ml total) was placed in centrifuge tubes. Then 100 µl cell suspension was placed into each tube, spread evenly, with 1 ml remaining in each tube. Additional DMEM was added to make a 4 ml total volume, spread evenly, with 100 µl remaining at the bottom of each tube. DMEM (5 ml) was added to the centrifuge tube, mixed and dropped into the first three rows of a 96-well plate, one drop per well, with 1.8-2 ml remaining at the bottom of the tube. A further 5 ml DMEM was added to the tube, mixed and dropped into the middle three rows of the 96-well plate, one drop per well, with 1.5-1.8 ml remaining at the bottom of the tube. A further 2.8-3 ml DMEM was added to the tube, mixed and dropped into the last two rows of the 96-well plate, one drop per well. Cells were observed under a light microscope 7-10 days later, to detect the wells with growing clones. Monoclonal wells were marked. Positive monoclonal cells were selected for subcloning. When the positive rate had reached 100%, monoclonal wells were selected for large-scale culturing.

An intraperitoneal injection of 0.5 ml liquid paraffin was administered to each mouse (n=6; age, 6-8 weeks). Between days 7 and 30 following injection of liquid paraffin, the pretreated mice were intraperitoneally injected with 1x10⁶ hybridoma cells. Between days 7 and 10 following injection of hybridoma cells, a syringe needle was used to remove as much liquid as possible. The mice were sacrificed by cervical dislocation.

The collected ascitic fluid was centrifuged, and the supernatant collected and purified. Protein A Sepharose (GE

Healthcare Life Sciences, Chalfont, UK) was used to pack the column. The ascitic fluid was diluted 1:10 with PBS and slowly loaded onto the column. Phosphate buffer was used to wash the column to the minimum value that could be detected with an ultraviolet detector. Glycine elution buffer was used to elute the purified antibody, which was dialyzed immediately at 4°C overnight. The purity, concentration and titer of the antibody were determined the following day.

Western blotting was used to verify the specificity of the mAbs. Recombinant protein (1 and 10 ng) was loaded onto 12% SDS-PAGE gels and transferred to nitrocellulose membranes (Tiangen Biotech Co., Ltd., Beijing, China) following electrophoresis. Membranes were incubated with 5% skim milk powder (50 g/l) for 1 h at room temperature. mAbs (1:1,000) were applied to the membranes overnight at 4°C. Membranes were washed three times, 10 min each with PBS containing 0.1% Tween-20 (PBST). The rabbit anti-mouse IgG-HRP (1:1,000) was applied and incubated at room temperature for 1 h. Membranes were washed with PBST three times for 15 min each and mAb binding was visualized using 1 ml TrueBlue peroxidase substrate (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD, USA) for 1 min.

Using bacteriophage library to screen Der f 5 mimic epitope. A Ph.D.TM-12 Phage Display Peptide Library (New England BioLabs, Inc., Ipswich, MA, USA) of random dodecapeptides fused to a minor coat protein (pIII) of M13 phage was used. The kit-28 gIII sequencing primer was 5'-HOGTATGGG ATTTTGCTAAACAAC-3', 100 pmol, 1 pmol/μl; the -96 gIII sequencing primer was 5'-HOCCCTCATAGTTAGCGT AACG-3', 100 pmol, 1 pmol/μl.

The immunotube (MaxiSorpTM; Nalge Nunc International; Thermo Fisher Scientific, Inc.) was coated overnight at 4°C with 10 μg mAb diluted to 1 ml with TBS. The immunotube was blocked with 5 ml 1% bovine serum albumin (BSA; Sigma-Aldrich; Merck Millipore) at 37°C for 2 h and washed three times with TBS containing 0.1% Tween-20 (TBST). Subsequently, 3 ml phage sample (containing 0.5% BSA and 0.1% Tween-20) was added at 37°C for 2 h, with mixing every 30 min. The immunotube was again washed with TBST 10 times to remove the unbound phage. Bound phage was washed with 1 ml glycine-HCl (pH 2.1) for 5 min and 160 μl Tris neutralization solution was added. Elution was repeated and eluents combined. The eluted phage sample (10 μl) was used for gradient dilution. ER2738 competent *E. coli* cells provided with the Ph.D.-12 Phage Display Peptide Library were infected during mid-log phase, coated onto an LB/IPTG/Xgal Top-Agar plate and inverted in a 37°C incubator. Cells were counted the following day. The single phage plaque was used for subsequent experiments. The remaining 2.3 ml eluted phage samples were used to infect 50 ml ER2738 during early-log phase. Cells were oscillated and cultured at 37°C for 4.5 h to collect phage supernatants. These samples were used for the next round of screening following precipitation and concentration.

The phage was diluted with LB medium at a 10:1 ratio. The amplified phage monoclones were diluted to 1.0×10^8 - 1.0×10^{11} , and the unamplified were diluted to 1.0×10^1 - 1.0×10^4 . Phage monoclones (10 μl) at various dilutions were selected and added to 190 μl ER2738 bacterial solution of mid-log phase

(A600=1.0). Samples were mixed and poured into a preheated culture tube at 45°C, with an agar top layer. Culture tubes were gently shaken, and the solution was poured onto an LB plate containing IPTG and Xgal. The plate was inverted at 37°C overnight.

Monoclones were selected from LB/IPTG/Xgal plates following the third round of screening and seeded onto a 96-well deep well plate, with 500 μl LB (diluted at 1:100 with *E. coli* ER2738) in each well. Samples were cultured at 37°C for 4.5 h and centrifuged at 42 x *g* for 5 min at 4°C. Supernatant was collected to perform ELISA. Briefly, plates were coated with 200 ng/well mAb and incubated overnight at 4°C. Following blocking with 5% skim milk at 37°C for 1 h, the amplified enriched phage clones were added and incubated at 37°C for 1 h. Plates were washed and HRP-labeled anti-M13 phage IgG, provided with the Ph.D.-12 Phage Display Peptide Library, diluted 1:5,000 was added at 100 μl/well, and incubated at 37°C for 30 min. Color was developed with a tetramethylbenzidine (Kirkegaard & Perry Laboratories, Inc.) chromogenic substrate system. The OD450 value was determined following termination of the reaction.

Phages with greater OD values were selected and single-stranded DNA was extracted using sodium iodide according to the kit manufacturer's protocol. Sequencing with primer-96 gIII, 5'-CCC TCA TAG TTA GCG TAA CG-3' was performed. The obtained DNA sequences were inverted, converted and translated into amino acid sequences, which were used for analysis.

Results

Expression and purification of pET28a (+)-Der f 5. BL21 *E. coli* cells expressing the pET28a (+)-Der f 5 plasmid were used to isolate recombinant Der f 5. SDS-PAGE revealed specific bands at 15-20 kDa, estimated to be the fusion protein of Der f 5 and the vector pET28a (+). Recombinant protein was purified by affinity chromatography, resulting in a purity of 90% and a concentration of 1 mg/ml (Fig. 1A). MALDI-TOF/TOF revealed a peptide mass fingerprint consistent with the structure of Der f 5 (Fig. 1B).

Preparation and identification of mAbs. Mice were immunized with the pET-28a (+)-Der f 5 prokaryotic expression product. The titers of antisera for the recombinant protein were determined via indirect ELISA and the mouse with the greatest titer was selected for cell fusion. The spleen cells and Sp2/0 myeloma cells of the immunized mice were used to perform PEG fusion. Indirect ELISA determined the antibody secretion status in the cell culture supernatant. The positive clones were cultured according to the limiting dilution method, to obtain 3 continuously secreting specific anti-Der f 5 hybridoma cell lines, named 3G9, 6B8 and 10D6. The cell lines were prepared into ascitic fluid, and ELISA was used to determine the titer (Fig. 2A).

Following purification, the ascitic fluid (3 ml) was collected with concentrations of 0.7 mg/ml 3G9, 0.4 mg/ml 6B8 and 0.67 mg/ml 10D6. SDS-PAGE revealed that the purity of the visible antibody following purification was >85% (Fig. 2B). The recombinant protein (rDer f 5) was probed with mAbs

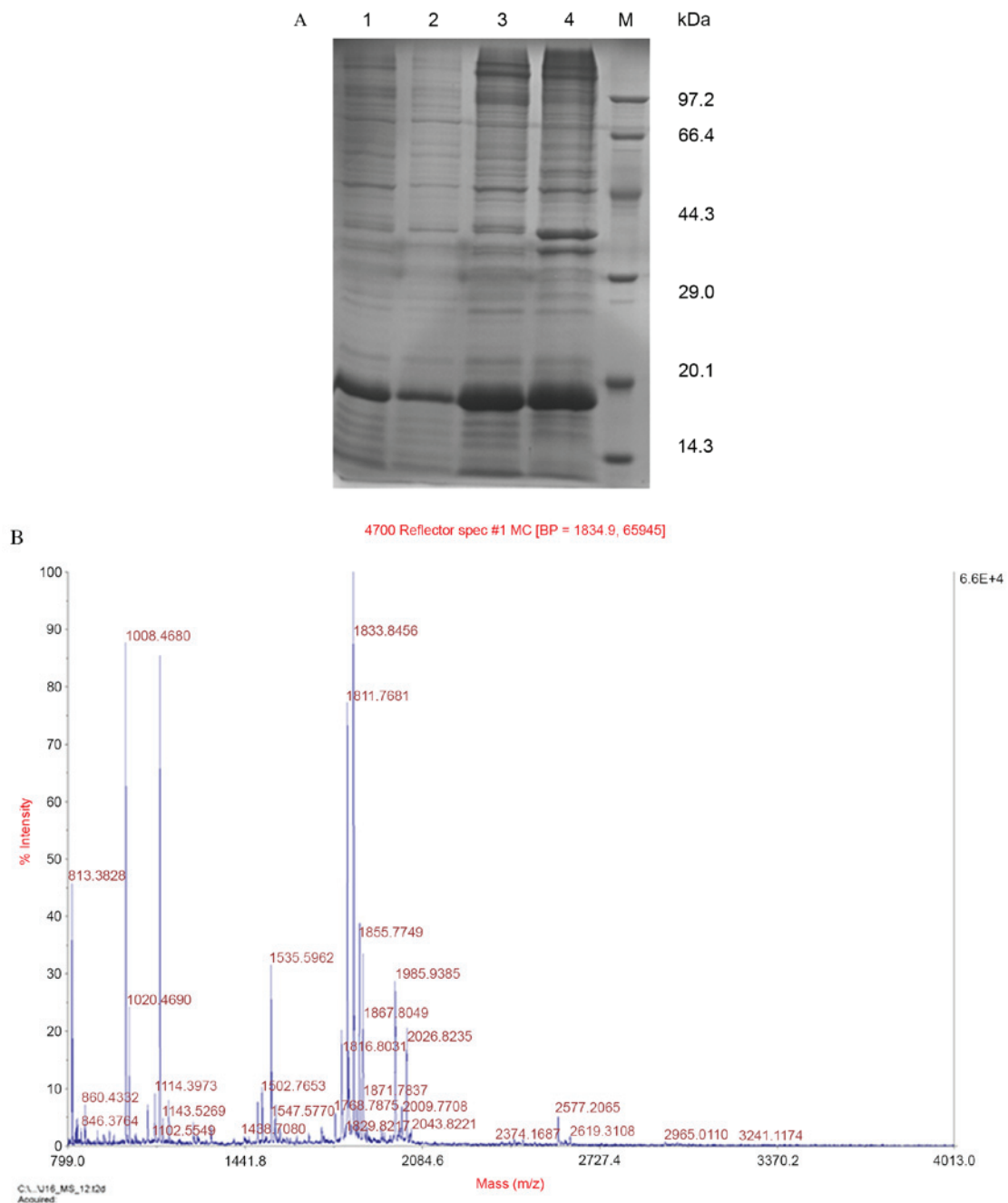


Figure 1. Expression and purification of recombinant protein Der f 5. (A) SDS-PAGE analysis of the protein expressed from the pET28a (+)-Der f 5 recombinant plasmid in BL21 *E. coli* cells. The lane contents are as follows: M, protein marker; 1, supernatant of cells containing pET28a (+)-Der f 5; 2, flow-through from BL21 *E. coli* cells containing pET28a (+)-Der f 5 following Ni-chelating affinity chromatography; 3, washing of BL21 *E. coli* cells containing pET28a (+)-Der f 5 following Ni-chelating affinity chromatography; 4, eluent of BL21 *E. coli* cells containing pET28a (+)-Der f 5 following Ni-chelating affinity chromatography. Specific bands at 15-20 kDa were estimated to be the fusion protein of Der f 5 and the vector pET28a (+). (B) The first-order structure of the purified recombinant fusion protein from BL21 *E. coli* cells by matrix-assisted laser desorption/ionization time of flight mass spectrometry. The peptide mass fingerprint was consistent with the structure of Der f 5.

3G9, 6B8 and 10D6 by western blotting; all three mAbs bound to the recombinant protein (Fig. 2C).

Screening Der f 5 mimic epitopes in the phage library. The mAbs against recombinant Der f 5 were used to screen the random peptide library. Following three rounds of strict screening, the recovery rate of 6B8 had increased from 2.1×10^{-7} to 3.7×10^{-5} . Following four rounds of screening, the recovery rate of 3G9 had increased from 1.2×10^{-6} to 3.5×10^{-4} . Following four rounds of screening, the recovery rate of 10D6

had increased from 8.3×10^{-7} to 3.1×10^{-6} . The results indicated that the specific phage clones had been enriched to varying degrees.

Following three cycles of screening, the supernatant of antibody 6B8 was used for ELISA; 15 positive clones were obtained. The sequencing results for these clones were consistent, all 'CLATWHSMRCSS' (Table I). The polypeptide screening of mAbs 3G9 and 10D6 performed following the third screening cycle revealed relatively weaker positive clone signals or a reduced positive rate. Therefore, a fourth cycle of

Table I. Amino acid sequences of positive clones.

Monoclonal antibody	Random peptide sequences	Frequency	OD values in ELISA	Formula	Theoretical pI	GRAVY
6B8	CLATWHSMRCS	15	0.895~1.140	$C_{56}H_{88}N_{18}O_{17}S_3$	8.08	0.067
3G9	NQSFLPLDFPFR	7	0.194-0.258	$C_{71}H_{101}N_{17}O_{18}$	5.84	-0.250
	CLATWHSMRCS	4	0.450-0.709	$C_{56}H_{88}N_{18}O_{17}S_3$	8.08	0.067
	GAPVNLIHYLST	1	0.469	$C_{59}H_{93}N_{15}O_{17}$	6.74	0.550
10D6	HWSYSHKVTPLW	4	0.171-0.406	$C_{75}H_{101}N_{19}O_{17}$	8.61	-0.775
	QTSVWWLATAPD	7	0.405-0.799	$C_{64}H_{91}N_{15}O_{19}$	3.80	-0.083
	ATWSHLLSSAGL	5	0.348-0.524	$C_{56}H_{83}N_{17}O_{17}$	6.96	0.033
	FSHHMPMRDLA	1	1.147	$C_{64}H_{100}N_{22}O_{16}S_2$	9.61	-0.758

The theoretical pI and GRAVY were calculated by ProtParam Tools (www.expasy.org). OD, optical density; pI, isoelectric point; GRAVY, grand average of hydropathicity.

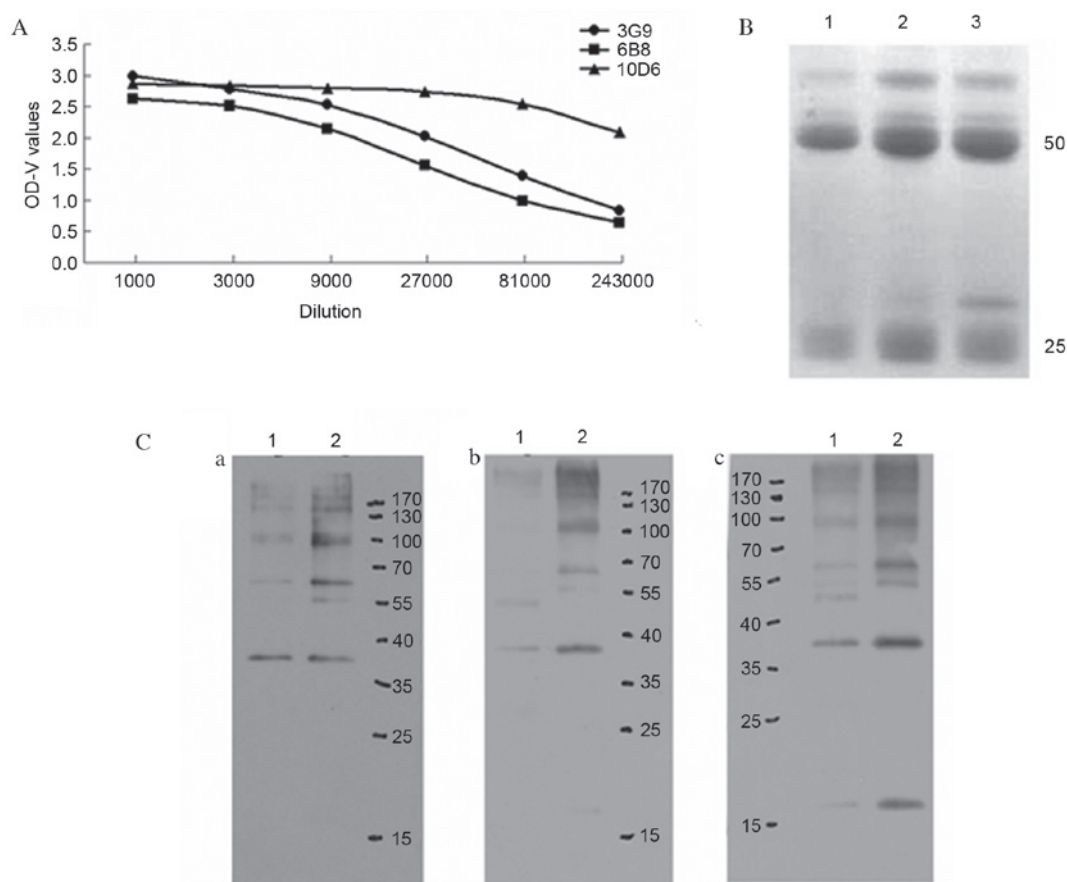


Figure 2. Anti-Der f 5 mAb titer, purity and specificity. (A) ELISA was used to detect titers of anti-Der f 5 mAb in hybridoma cell lines. (B) SDS-PAGE was performed to determine the purity of the rDer f 5 mAbs. The lane contents are as follows: 1, hybridoma cell line 3G9; 2, hybridoma cell line 6B8; 3, hybridoma cell line 10D6. The upper bands correspond to mAb heavy chain with a molecular weight of 50 kDa; the lower bands correspond to mAb light chain with a molecular weight of 25 kDa. (C) Identifying the specificity of rDer f 5 mAbs via western blotting, using mAbs from (a) 6B8, (b) 3G9 and (c) 10D6 hybridoma cell lines. The lane contents are as follows: 1, 1 ng recombinant protein; 2, 10 ng recombinant protein. mAb, monoclonal antibody.

screening was performed on these two clones. Following three cycles of screening, antibody 3G9 produced 7 positive clones, all 'NQSFLPLDFPFR' and antibody 10D6 produced 13 positive clones, with sequencing results as follows: 'HWSYSHKVTPLW' (1 positive clone), 'QTSVWWLATAPD' (7 positive clones) and 'ATWSHLLSSAGL' (5 positive clones; Table I).

Following four cycles of screening, there were 2 sequences of positive clones from antibody 3G9: 'CLATWHSMRCS' (4 positive clones) and 'GAPVNLIHYLST' (1 positive clone). In addition, there were 2 sequences from antibody 10D6: 'HWSYSHKVTPLW' (3 positive clones) and 'FSHHMPMRDLA' (1 positive clone). The 7 mimic

D01	— CLATW — HSMRCS —S—	12
D06	— ATWSHLSSA — GL—	12
D03	GAPVNLIHYLST ———	12
D04	— HWSYSHKVT —PLW	12
D07	— FSHHMPMRDLA	12
D02	— NQSFLPLDFPFR	12
D05	— QTSVW — WLATAPD —	12

Figure 3. Mimotope alignment, as determined using the ClustalW interface MIMOX. WH had the greatest frequency.

epitopes obtained in total from all 3 mAbs were entered into MIMOX (www.immunet.cn/mimox) for comparison; results are presented in Fig. 3. 'WH' had the greatest frequency, with its derived common subsequence '---[A][T]W[-S]H[HSFW][LM][PSKR][TLV][AST]-[DP] [-L]-'. Specifically, the frequency of amino acid W at the sixth position was 57%; the possibility of amino acid S at the seventh position was 43%; the possibility of amino acid H at the eighth position was 57%; the possibility of amino acid L at the tenth position was 57%; and the possibility of amino acid L at the sixteenth position was 43%. The Der f 5 amino acid sequence was entered into ElliPro (tools.immuneepitope.org/tools/ElliPro/iedb_input). This predicted that it may include 3 discontinuous epitopes, located at 1-14, 68-84 and 38-47. These results suggested that P2, K3, K4, H5, F11, F13, L14, R72, T77, L79, R84, T39, F40, P44, T45 and K46 were the key amino acids.

Discussion

With the increasing prevalence of allergic disorders resulting from house dust mite allergens, investigations are focused on characterizing allergens and their epitopes, for the development of novel and more effective specific immunotherapies. A relatively novel technique, phage display, creates a mimic of a natural epitope, referred to as a mimotope, to permit its characterization (18). Phage display libraries are based on random peptides that may be used to mimic the binding site for an antibody. In the present study, three mAbs were successfully raised against recombinant Der f 5: 6B8, 3G9 and 10D6. To locate the binding site of these mAbs, a phage surface capsid protein displayed 12-mer peptide library was used to search for sequences. Results revealed that the mAb 6B8 recognizes a sequence 'CLATWHSMRCS', the mAb 3G9 recognizes the three sequences 'NQSFLPLDFPFR', 'CLATWHSMRCS' and 'GAPVNLIHYLST', and the mAb 10D6 recognizes the four sequences 'HWSYSHKVTPLW', 'QTSVWWLATAPD', 'ATWSHLSSAGL' and 'FSHHMPMRDLA'. A phage surface capsid protein display 12-mer peptide library consists of $\sim 10^9$ electroporated sequences amplified once to yield ~ 100 copies of each sequence in 10 μ l of the supplied phage. The

peptides recognized by these three mAbs have certain residues in common with the sequences in Der f 5. Following alignment of the seven sequences recognized by these three mAbs, the amino acids 'WH' were revealed to have the greatest frequency. The common subsequence was deduced to be '---[A][T]W[-S]H[HSFW][LM][PSKR][TLV][AST]-[DP] [-L]-'. These seven mimotopes may serve as a treatment vaccine to be developed for immunotherapy.

Mimotope analysis-based methods may predict linear and conformational epitopes and have therefore become more widely used. Although algorithms have been suggested, identifying the exact localization of the interaction site mimicked by mimotopes remains an obstacle. The present study predicted the epitopes for Der f 5 allergen using ElliPro, an online tool that implements Thornton's method, together with a residue clustering algorithm, the MODELLER program and the Jmol viewer (19). Three discontinuous epitopes, located at residues 1-14, 68-84 and 38-47, were identified. The common subsequence deduced from the seven mimotopes, combined with the three discontinuous epitopes predicted by ElliPro, resulted in the prediction of key residues at P2, K3, K4, H5, F11, F13, L14, R72, T77, L79, R84, T39, F40, P44, T45 and K46. Therefore, modifying these key amino acids may be beneficial for epitope-specific immunotherapy.

Acknowledgements

The present study was supported by the National Sciences Foundation of China (grant nos. NSFC 30060166, NSFC81001330 and NSFC31272369).

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