

Research article

Screening of B-cell epitopes of Der-p1 and Der-p2 major aeroallergens by computational approach for designing immunotherapeutics

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ABSTRACT

Introduction and Aim: Allergic diseases are IgE-mediated hypersensitivity reactions affecting approximately 30% of the general population globally. *Dermatophagoides pteronyssinus* (Der-p) is the most prevalent house dust mite (HDM) species consisting of 23 mite allergen groups. Among these, group 1 and 2 are major allergenic proteins, which causes allergic asthma in 80% of sensitized individuals, with elevated IgE titres in the serum. This study involves *in silico* analysis of potential B-cell epitopes of group 1 and group 2 of Der-p, which can be utilized in designing immunotherapeutic vaccines.

Materials and Methods: Allergen sequences obtained from the database- International Union of Immunological Societies (IUIS), for predicting of B-cell epitopes. The physiochemical properties and secondary structures of the obtained sequence were evaluated. The sequences were further subjected to determining antigenicity, surface accessibility, and prediction of linear and discontinuous B-cell epitope by utilizing IEDB tools.

Results: The linear and discontinuous B-cell epitopes of Der-p1 and Der-p2 aeroallergen were predicted. Further, Der-p1 and Der-p2 showed 6 linear epitopes each respectively. Conformational epitopes predicted were 123 of Der-p1 and 72 of Der-p2 respectively, by the ElliPro tool. Based on the structure, antigenicity, and surface accessibility, only 10% of Der-p1 and Der-p2 which binds to B-cell epitopes are linear and the majority are discontinuous.

Conclusion: The linear and conformational epitopes of Der-p1 and Der-p2 are predicted using *in silico* tools. These identified epitopes might be useful for developing epitope-based immunotherapeutics for HDM allergy.

Keywords: Allergic asthma; house dust mite; B-cell epitopes; *In silico* predictions; IEDB tools.

INTRODUCTION

Allergy, a non-communicable disease, is one of the most common immune disorders worldwide. Allergy-mediated immune response affects approximately 30% of the general population globally. In the last decade, the clinical report suggests that there is a significant increase in the prevalence of IgE-mediated allergy disorders namely allergic asthma, atopic dermatitis, and allergic rhinitis (1). House dust mites (HDM) are known to cause allergic respiratory disorders in sensitised individuals. HDMs are the primary source of indoor aeroallergens majorly affecting world population, and clinical reports suggests that it causes atopic disorders and are a major risk factor for allergic asthma (2). *Dermatophagoides pteronyssinus* (Der p), *Dermatophagoides farinae* (Der f), and *Blomia tropicalis* (Blo t) are the most prevalent HDM species known for causing allergic asthma (3). However, *Dermatophagoides pteronyssinus* is the most common and major species, and a total of 23 mite allergens are currently identified, among these, group one- and group two allergens shows allergen specific-IgE in

sensitized individuals suffering due to allergic asthma (Der-p1 and Der-p2) (4, 5).

Currently, the only technique for treating HDM allergy is allergen-specific immunotherapy (AIT). AIT is the most often used in clinical practice to provide patients with increasing concentrations of crude allergen extracts. However, there are significant drawbacks for using crude extracts due to cross-reactivity to other allergens, undefined endotoxin levels, or bacterial/fungal contamination (6, 7). Today, engineering peptides with defined epitopes provide new perspectives on the analysis and synthesis of allergens, hypoallergenic substances, and allergy shots. There are characterised allergens with multiple epitopes, which are used as allergy vaccines. Further, design of immunodominant epitopes interacting with B-cell and T-cell receptors are of high importance as they will be applicable in the early diagnosis of allergy and for allergen-specific immunotherapy approaches in clinic (8, 9).

B-cell epitopes are defined as antigenic determinants that have been identified by specific immunoglobulins resulting in the heightened immune response. This interaction is the center of adaptive immunity, which

suggests being mainly accountable for immunological memory as well as antigen-specific responses. The ability to recognize these binding regions in the sequence or structure of an antigen will be essential for the development of chemically synthesized vaccines, diagnostic procedures, and immunotherapeutics (10). There are two types of B-cell epitopes: 1) epitopes that are linear (continuous), which are made up of a linear sequence of residues, and 2) conformational (discontinuous) epitopes, which are mainly made up of residues that are not in the basic sequence of amino acid but are managed to bring together by the folded protein complexes. However, it has been demonstrated that many conformational epitopes in primary protein sequence comprises of several groups of linear epitopes. In this study, the objective will be to screen for potential B-cell epitopes, which might be utilized in designing specific immunotherapeutic vaccines (11).

METHODOLOGY

Sequence retrieval

The major two allergen sequences Der-p1 and Der-p2 of *Dermatophagoides pteronyssinus* were retrieved from the IUIS database, UniProt, and the database-structural database of allergenic proteins (12). The allergens were chosen based on the most prevalent allergen groups (group 1 and group 2) and the sequence data's availability.

Analysis of the physiochemical properties of allergens

Allergens' physiochemical properties which include isoelectric point, molar extinction coefficient, and molecular weight were analysed by Pepstats- (https://www.ebi.ac.uk/Tools/seqstats/emboss_pepstat/s/) (13).

Evaluating secondary structures of Der p 1 and Der p 2

PSIPRED V3.3- (<http://bioinf.cs.ucl.ac.uk/psipred/>) was used to anticipate the structural features of major Der-p1 and Der-p2 aeroallergens. Protein secondary structure analysis using PSIPRED is a popular and advanced technique. PSIPRED is an efficient and convenient secondary structure prediction system that analyses PSI-BLAST output using two feed-forward computational models. (Position-Specific Iterated – BLAST) (14).

Homology modelling and structure validation

Using the SWISS-MODEL software and the protein's FASTA sequence, the three-dimensional structure was predicted (<https://swissmodel.expasy.org/>). The input sequence template was found by utilising the Swiss model template repository (15). The created protein structure models were then evaluated for structural validity. The accuracy of the derived models was

checked using a Ramachandran plot generated by Procheck.

B-cell epitope prediction

The component of a vaccination that engages utilising the receptor of B-cell is known as a B-cell epitope. Since choosing the protein sequence having immunogenic property is a requirement for designing a peptide vaccine based on epitopes, the surface accessibility and antigenicity of the target epitopes was examined using divergent B-cell prediction algorithms.

Linear and discontinuous B-cell epitope predictions

BepiPred from the immune epitope database (IEDB) was used to predict linear B-cell epitopes (16), with a 0.35 default threshold value. The epitopes that have values below this cut-off were all preferred. Ellipro, which calculates an ellipsoid-based protrusion index approach, projects linear and discontinuous B-cell epitopes. Three algorithms form the foundation of the Ellipro process. (1) At first, the protein is thought to resemble an ellipsoid. (2) Following that, the protrusion index (PI) of residue is obtained, and (3) to anticipate the epitopes, the adjacent clustering algorithm of residue is then performed based on the calculated PI (17).

Surface accessibility predictions

The immune epitope database's Emini surface accessibility prediction tool was used to predict the peptides' surface accessible epitopes included in a sequence, with a 1.000 default threshold value (11). Then, for further investigation, we chose all peptides with lower values than this limit.

Epitopes antigenicity predictions

The protein antigenic sites were identified using the Kolaskar and Tongaonkar antigenicity methods. We applied 1.028 as the default threshold value (11). All antigenic peptides with values below this cut-off were chosen.

RESULTS

Physiochemical properties and evaluation of secondary structure

The general physiochemical properties of the major allergens Der-p1 and Der-p2 were selected in the current study (as compiled in Table 1). The molecular weight of the Der-p1 and Der-p2 allergen are approximately 25kDa and 15kDa, respectively. The secondary structure prediction by the PSIPRED V3.3 tool shows that Der-p1 consists of the transmembrane helix, α -helix, and β -strands (figure 1a) whereas Der-p2 majorly of β -strands and transmembrane helix (Fig. 1b).

Table 1: Physiochemical properties of Der-p1 and Der-p2 allergen

Properties/Allergen	Der-p1	Der-p2
Molecular weight	24968.82 (25kDa)	14106.32 (14kDa)
Residues	222	129
Residue weight	112.472	109.351
Isoelectric Point	5.5878	7.2779

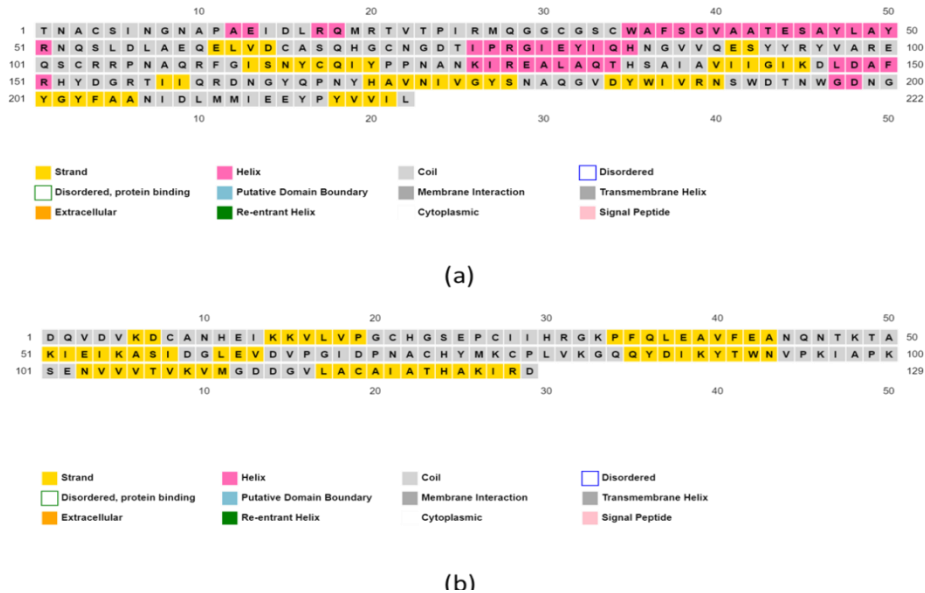


Fig. 1: Evaluation of secondary structures of (a) Der-p1 and (b) Der-p2 by PSIPRED V3.3

Homology modelling and structure validation

The protein sequences were obtained from the allergen nomenclature database. The sequence of Der-p1 (GenBank nucleotide- FM177224) and Der-p2 (GenBank nucleotide- FM177223) were modelled using SWISS-MODEL (<https://swissmodel.expasy.org/>). The modelled structures are depicted in figure 2. These structures were further validated using PROCHECK and Ramachandran plots as shown in figure 3. The modelled Der-p1 structure showed 88.7% of residues in the preferred region and 10.8% of residues in the permitted region of the Ramachandran plot. Further, the modelled Der-p2

structure showed 93.7% of residues in favoured regions and 5.4% in the allowed region.

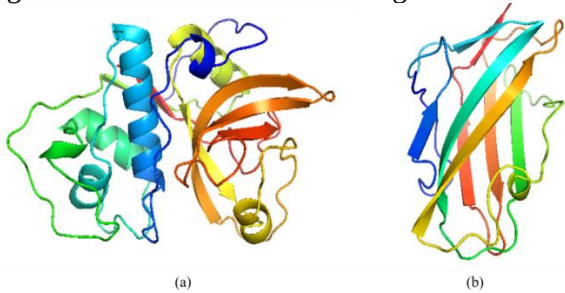


Fig 2: Homology modelling of allergen sequence using SWISS-MODEL (a) Der-p1 and (b) Der-p2

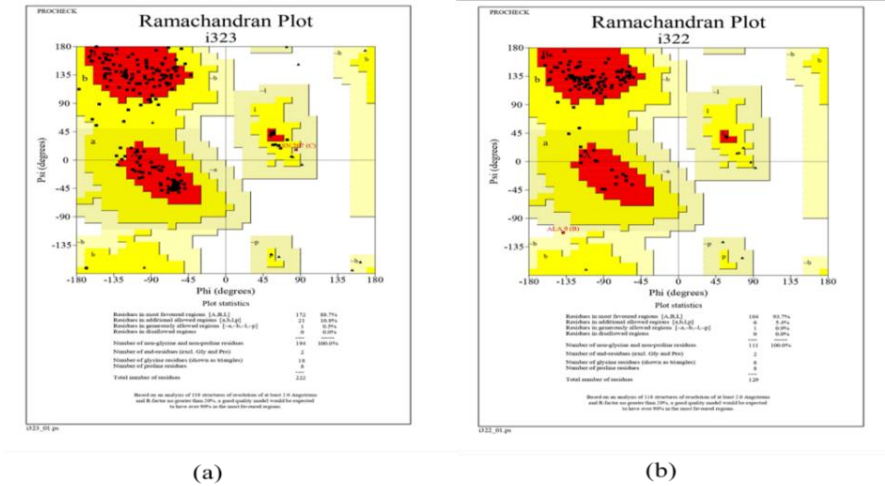


Fig.3: The RAMPAGE generated the Ramachandran plot. The Ramachandran plot calculates the allowed regions for backbone dihedral angles ψ vs ϕ amino acid residues in protein (a) Der-p1 and (b) Der-p2.

B-cell epitope prediction

The protein sequence of Der-p1 and Der-p 2 were examined by IEDB’s BepiPred Linear epitope prediction, Kolaskar and Tongaonkar antigenicity and Emini surface accessibility methods, to determine the specific areas of the proteins which are present on the surface and being immunogenic and further can interact with the B-cell receptor.

Linear and discontinuous B-cell epitopes

BepiPred linear epitope prediction was the first method used to identify B-cell epitopes, and the average peptide score was 0.321, with a minimum value of -0.008 and a maximum value of 1.381. All scores at / above the standard threshold value of 0.350 were considered to be probable linear epitopes, as depicted in Fig. 4 and Table 2. The allergy's early

stage reactions involve both linear and discontinuous B-cell epitopes, although discontinuous epitopes predominate. These linear and conformational epitopes of both Der-p1 and Der-p2 were predicted using ElliPro and presented in Figs. 5.1 and 5.2 and Table 3.

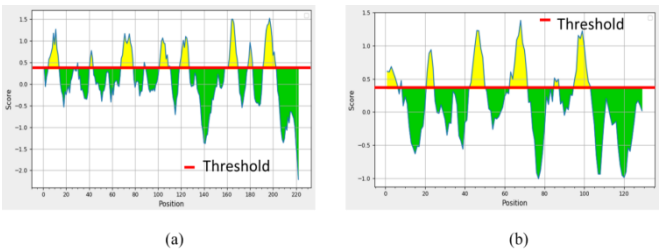


Fig.4: Based on BepiPred epitope prediction, yellow peaks are recommended as B-cell epitope, whereas green peaks are not a) Der-p1 and b) Der-p2

Table 2: The predicted linear B-cell epitopes. a) Der-p1 and b) Der-p2

No.	Chain	Start	End	Peptide	Total residues	Score
1	A	82	117	YIQHNGVVQESYYRYVAREQSCRPN QRFGISNYC	36	0.741
2	A	1	25	TNACSINGNAPAEIDLRQMRTVTPI	25	0.727
3	A	46	57	AYLAYRQQSLDL	12	0.68
4	A	119	136	IYPPNANKIREALAQTHS	18	0.65
5	A	153	161	YDGRTHIQR	9	0.585
6	A	193	200	DTNWGDNG	8	0.516

No.	Chain	Start	End	Peptide	Total residues	Score
1	A	30	34	HRGKP	5	0.743
2	A	93	103	NVPKIAPKSEN	11	0.694
3	A	58	67	IDGLEVDVPG	10	0.677
4	A	18	26	VPCHGSEP	9	0.657
5	A	70	85	PNACHYMKCPLVKGQQ	16	0.629
6	A	42	50	EANQNSKTA	9	0.6

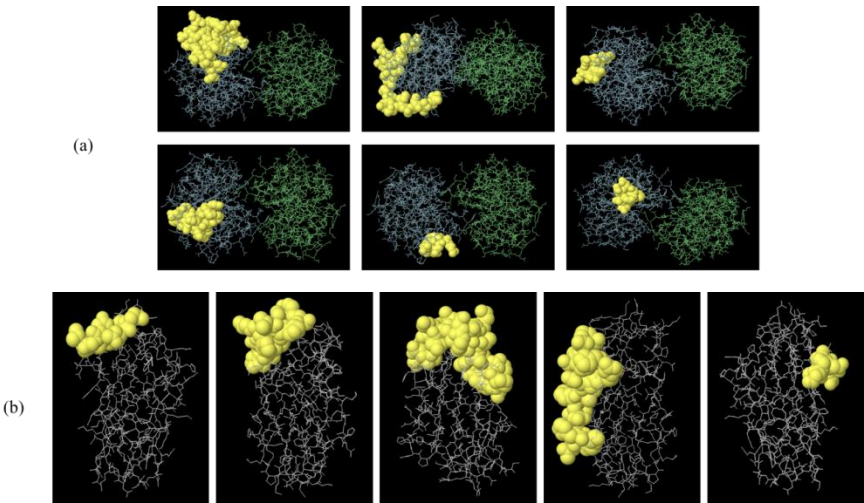


Fig. 5.1: The linear B-cell epitopes predicted by ElliPro. The yellow region on the surface shows the predicted epitopes. a) Der-p1 and b) Der-p2

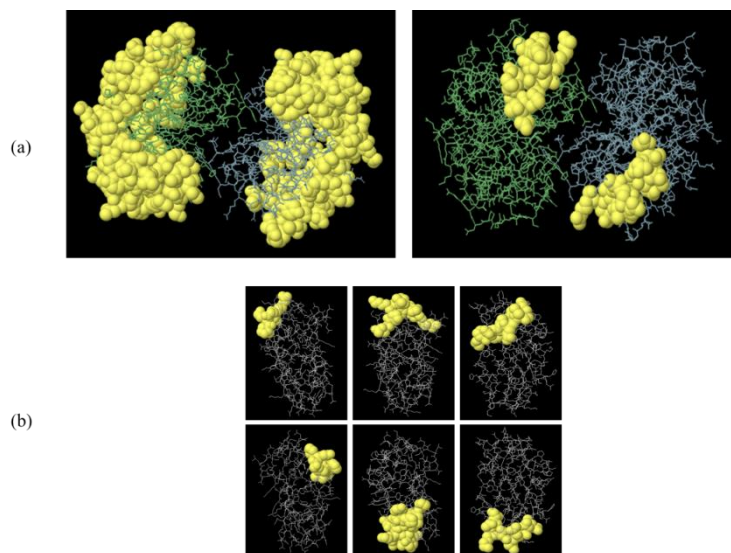


Fig. 5.2: Discontinuous B-cell epitopes predicted by ElliPro. The yellow region on the surface shows the predicted epitopes. a) Der-p1 and b) Der-p2

Table 3: ElliPro predicted discontinuous B-cell epitopes. a) Der-p1 and b) Der-p2

No.	Residues	Total residues	Score
1	A:K145, A:D146, A:L147, A:D148, A:A149, A:R151, A:H152, A:D162, A:N163, A:G164, A:Y165, A:Q166, A:P167, A:N168	14	0.826
2	A:T1, A:N2, A:A3, A:C4, A:S5, A:I6, A:N7, A:G8, A:N9, A:A10, A:P11, A:A12, A:E13, A:I119, A:Y120, A:P121, A:P122, A:N123, A:N125, A:K126, A:E129, A:S178, A:N179, A:A180, A:Q181, A:G182, A:V183, A:D184, A:A206, A:N207, A:I208, A:D209, A:E214, A:E215	34	0.705
3	A:G29, A:G30, A:C31, A:G32, A:D64, A:C65, A:S67, A:Q68, A:H69, A:G70, A:C71, A:H72, A:G73, A:D74, A:Y94, A:R95, A:Y96, A:V97, A:A98, A:R99, A:E100, A:Q101, A:S102, A:C103, A:R104, A:R105	26	0.647
4	A:D56, A:L57, A:Q84, A:H85, A:N86, A:V89, A:Q90, A:E91, A:S92, A:Y93, A:P106, A:N107, A:A108, A:Q109, A:R110, A:F111, A:G112, A:I113, A:S114, A:N115, A:L222	21	0.644
5	A:R51, A:Q53, A:S54, A:L55	4	0.623
6	A:I25, A:M27, A:Y153, A:D154, A:G155, A:R156, A:T157, A:I158, A:I159, A:Q160, A:R161, A:D193, A:T194, A:N195, A:W196, A:G197, A:D198, A:N199, A:G200	19	0.603
7	A:Q18, A:M19, A:T21, A:A49, A:Y50	5	0.559

No.	Residues	Total residues	Score
1	A:I58, A:D59, A:G60, A:L61, A:S101, A:E102, A:N103	7	0.767
2	A:H30, A:R31, A:G32, A:K33, A:P34, A:V94, A:P95, A:K96, A:I97, A:A98, A:P99, A:K100, A:D129	13	0.751
3	A:E42, A:A43, A:N44, A:Q45, A:N46, A:S47, A:K48, A:T49, A:A50, A:D69, A:P70, A:N71, A:C73, A:H74, A:Y75, A:M76, A:K77, A:C78, A:P79, A:L80, A:V81, A:K82, A:G83, A:Q84, A:Q85, A:G112, A:D113, A:N114, A:G115	29	0.645
4	A:D1, A:V3, A:D4, A:V5, A:K6, A:D7, A:C8, A:A9, A:N10, A:H11, A:E12, A:H22, A:G23, A:S24, A:E25, A:P26, A:H124	17	0.561
5	A:E62, A:V63, A:D64, A:V65, A:P66, A:G67	6	0.554

Emini surface accessibility prediction

Emini surface accessibility prediction was analysed by surface accessibility on average predictions of protein, with a threshold value of 1.000, with an optimum value of 4.734 and a bare minimum of 0.064, which signifies that all values that are equal to or higher than the standard threshold of 1.000 which predicts potential accessibility on the surface.

Kolaskar and Tongaonkar's antigenicity prediction

Antigenicity on average of Der-p1 and Der-p2 in Kolaskar and Tongaonkar's antigenicity predictions was 1.055, with a range of highest value of 1.251 and minimum value of 0.918. In this analysis, all values are equal or higher than the standard threshold of 1.055 are shown as antigenic determinants that could exist. The outcomes of predicted B-cell epitopes that were conserved and passed the Emini and Kolaskar surface accessibility and antigenicity respectively are represented in Figs. 6, 7 and Tables 4, 5 respectively.

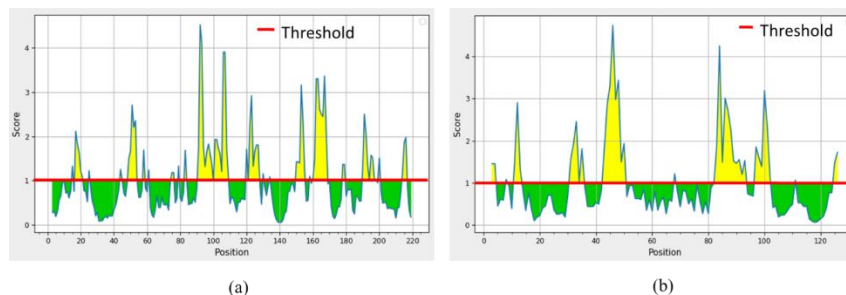


Fig. 6: Emini surface accessibility prediction, yellow peaks are predicted as B-cell epitope whereas green peaks are not. a) Der-p1 and b) Der-p2

Table 4: List of surface accessibility B-cell epitopes. a) Der-p1 and b) Der-p2

No.	Start	End	Peptide	Length
1	91	99	ESYYRYVAR	9
2	101	109	QSCRRPNAQ	9
3	122	127	PNANKI	6
4	150	155	FRHYDG	6
5	160	168	QRDNGYQPN	9

No.	Start	End	Peptide	Length
1	43	50	ANQNSKTA	8
2	83	93	GQQYDIKYTN	11
3	97	102	IAPKSE	6

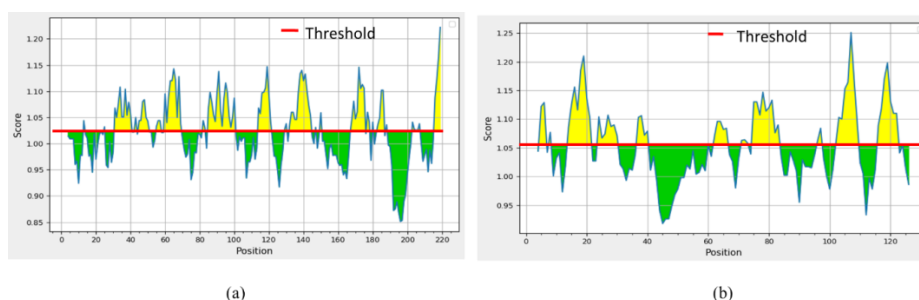


Fig. 7: According to the antigenicity prediction of Kolaskar and Tongaonkar, yellow peaks are recommended to be B-cell epitope. a) Der-p1 and b) Der-p2

Table 5: List of antigenicity B-cell epitopes. a) Der-p1 and b) Der-p2

No.	Start	End	Peptide	Length
1	31	40	CGSCWAFSGV	10
2	45	50	SAYLAY	6
3	60	69	QELVDCASQH	10
4	85	100	HNGVVQESYYRYVARE	16
5	114	121	SNYCIYP	8
6	132	144	AQTHSAIAVIIGI	13
7	168	175	NYHAVNIV	8
No.	Start	End	Peptide	Length
1	14	21	KKVLVPGC	8
2	24	30	SEPCIH	7
3	75	83	YMKCPLVKG	9
4	102	109	ENVVTVK	8
5	116	122	VLACAIA	7

DISCUSSION

Allergic-asthma is an IgE-mediated respiratory airway disease characterized by chronic airway inflammation, bronchoconstriction, airway hyperresponsiveness, and hypersecretion of mucus. Majorly, HDM allergens trigger allergic asthma in sensitized individuals with elevated specific-IgE titer. According to clinical

reports, the cysteine proteases and lipid-binding proteins are the primary allergens in HDM, which belong to groups 1 and 2 allergens, respectively (18).

Reports show that more than 80% of individuals are sensitized to Der-p1 and Der-p2 which are the most prevalent allergens and develop anti-human IgE against these allergens. The interaction between

allergens and immunoglobulins might be associated with three-dimensional structural principles interdependence among epitopes of antigen and paratopes of antibody (19). The report suggests that purified whole single allergen were used in clinics for developing immunotherapies, however, this approach has led to anaphylactic reactions due to the high chance of cross reactivity. Recently, epitope-based vaccines are shown to elicit an effective immune response without provoking undesired allergy symptoms, and hence, might be a favourable treatment option for allergen-specific immunotherapy (20). Moreover, peptide-based epitope therapies are shown to be more effective than standard vaccines because of their excessive levels of stability, safety, and accuracy (21).

According to the current study, we investigated both linear and discontinuous B-cell epitopes of both Der-p1 and Der-p2 allergens, respectively. Prediction of discontinuous B-cell epitopes is based on the allergenic structure of the allergens. In this regard, homology modelling was carried out for a desired structural confirmation. In addition, conformational epitope design might be useful for peptide-based immunotherapy. The implicated cysteines may also be a target for developing hypoallergens since disulfide linkages are crucial in the creation of protein structures (12, 22).

Further, antigenicity, surface accessibility, and threshold values were employed in the prediction of linear and discontinuous epitopes. In the *in silico* prediction, 6 linear epitopes above the threshold value of 0.516 were predicted in Der-p1 whereas 6 linear epitopes above the threshold score of 0.6 in the case of Der-p2 (19). In addition, the peptides emerge to be relatively part of the discontinuous conformational epitope region based on the allergen structure. These epitope-rich zones bind to B-cell receptors specifically and stimulate IgE reactions. The modelled structure of the Der-p1 sequence FM177224 showed 123 conformational epitopes above the threshold value of 0.559, which also contained linear epitope peptides whereas Der-p2 sequence FM177223 showed 72 conformational epitopes above the threshold value of 0.554 (23). In conclusion, predicted epitopes with the highest threshold score might be the strongest potential candidate for development as vaccine candidates.

CONCLUSION

The study signifies that the prediction of several major epitopes of Der-p1 and Der-p2 binding to surface B cells receptors might be employed in the design of epitope-based allergen-specific immunotherapy. However, *in vitro* and *in vivo* allergy-based studies are required to confirm the efficacy of these anticipated epitopes.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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