

Bioinformatics and Immunologic Investigation on B and T Cell Epitopes of Cur I 3, a Major Allergen of *Curvularia lunata*

Vidhu Sharma,[†] Bhanu P. Singh,[†] Shailendra N. Gaur,[‡] Santosh Pasha,[†] and Naveen Arora^{*,†}

Allergy and Immunology Section, Institute of Genomics and Integrative Biology (CSIR), and V. P. Chest Institute, Delhi University Campus, Delhi, India

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The knowledge on epitopes of proteins can help in devising new therapeutic modalities for allergic disorders. In the present study, five B (P1–P5) and five T cell (P6–P10) epitopes were predicted *in silico* based on sequence homology model of Cur I 3, a major allergen of *Curvularia lunata*. Peptides (epitopes) were synthesized and assessed for biological activity by ELISA, competitive ELISA, lymphoproliferation and cytokine profiling using *Curvularia* allergic patients' sera. B cell peptides showed higher IgE binding by ELISA than T cell epitopes except P6. Peptides P1–P6 achieved EC₅₀ at 100 ng, whereas P7–P10 required 10 μ g in inhibition assays. Peripheral blood mononuclear cells from *Curvularia* allergic patients ($n = 20$) showed higher lymphoproliferation for T cell epitopes than B cell epitopes except P6 confirming the properties of B and T cell prediction. The supernatant from these patients show highest interleukin-4 release on stimulation with P6 followed by B cell peptides. P4 and P6 together identified 35/37 of *Curvularia* positive patients by skin tests. In summary, experimental analysis confirmed *in silico* predicted epitopes containing important antigenic regions of Cur I 3. P6, a predicted T cell epitope, showed the presence of a cryptic B cell epitope. Peptides P4 and P6 have potential for clinical application. The approach used here is relevant and may be used to delineate epitopes of other proteins.

Keywords: Cur I 3 • Cytochrome c • Epitopes • Fungal allergens • *in silico*

1. Introduction

Fungal spores dominate the aerospora at various places in the world and are associated with respiratory allergy.¹ *Curvularia lunata* fungus induces sensitization in 7–16% of predisposed individuals in India and 18–28% in other countries.² It also shows cross-reactivity within species and with other fungal genera.^{3,4} Cur I 1 and Cur I 2 were identified as major allergens from *C. lunata*^{5,6} and showed enzymatic activity as serine protease and enolase, respectively. Cur I 3 (12 kDa) was identified as cytochrome c allergen and demonstrated cross-reactivity with grasses and fungi.²

Elucidation of B and T cell epitopes of allergen helps to understand the structure–function relationship and may predict the basis of cross-reactivity. The cross-reactive epitopes can be useful in reducing the number of allergens without compromising the efficacy of therapy. Immunodominant epitope of Asp f 1 could improve the diagnosis of aspergillosis,⁷ while Alt a 1 peptides with high IgE binding were proposed for allergy diagnosis/therapy.⁸ Immunotherapy with T cell epitopes of phospholipase A2 and Fel d1 showed improvement in lung and nasal symptoms by inducing Th-1 and T regulatory cells.⁹ T-cell

epitope of Bet v 1_{142–156} showed cross-reactivity with food allergens and was successfully employed for immunotherapy.¹⁰

Studies suggest that small synthetic peptides corresponding to T and B-cell epitopes are the next generation reagents for allergy treatment.^{9,11} Epitope (peptide) derivatives of Fel d 1 and PLA2 showed promise for effective immunotherapy without IgE mediated adverse reactions. The therapeutic potential of peptides can be increased using altered peptide ligands by amino acid substitutions to reduce IgE binding as demonstrated for a mammalian allergen lipocalin Bos d 2.¹² Hybrid peptides containing combination of important T cell repertoire can suit highly polymorphic HLA diversity as observed in a preclinical evaluation of Japanese cedar (*Cryptomeria japonica*) pollen major allergens, namely, Cry j 1 and Cry j 2.¹³ Intranasal administration of a dominant T cell epitope peptide (114–128 aa) of Der p 1 inhibited mucosal hyperresponsiveness and Th2 type immune responses in mice model.¹⁴

Epitopes have been deciphered using SPOTS membrane based immunoassay, gene-fragmentation, overlapping peptide synthesis, proteolytic digestion of proteins and random phage display or microarray immunoassay. These methods require high-throughput screening to identify relevant epitopes. Recently, various softwares have been suggested to predict B and T cell epitopes.¹⁵ The present study was undertaken to identify linear B and T cell epitopes of Cur I 3 using *in silico* approach and validated experimentally by standard *in vitro* and *in vivo* methods. These peptide epitopes have potential as therapeutics.

* To whom correspondence should be addressed. Naveen Arora, Ph.D., Laboratory No.509, Allergy and Immunology Section, Institute of Genomics and Integrative Biology, Mall Road, Delhi 110007, India. Phone, 011-27666156, 27666157; fax, 011-27667471; e-mail, naveen@igib.res.in or navdelhi@hotmail.com.

[†] Institute of Genomics and Integrative Biology (CSIR).

[‡] V. P. Chest Institute, Delhi University Campus.

Table 1. Peptide Sequences and Position in Cur l 3^a

name of peptide	sequence	length of peptide	position (in Cur l 3)	prediction of the epitope (<i>in silico</i>)	IgE binding by ELISA (ODs)
P1	QGDAKKGANLFKTRC	15	5–19	B	0.314 ± 0.017
P2	LKAGEGNKIGPE	12	25–36	B	0.334 ± 0.090
P3	TDANKQKGIEWNHDT	15	54–68	B	0.214 ± 0.017
P4	ENPKKYIPGTK	11	74–84	B	0.431 ± 0.087
P5	LKKPKDRNDLI	11	90–100	B	0.215 ± 0.045
P6	TRCAQCHTLK	10	17–26	T	0.543 ± 0.109
P7	KIGPELHGL	9	32–40	T	0.135 ± 0.028
P8	GLFGRKTGSA	11	39–49	T	0.159 ± 0.012
P9	VAGYSYTDANK	11	48–58	T	0.172 ± 0.017
P10	RNDLITFLEQETK	13	96–108	T	0.152 ± 0.018

^a B and T cell epitopes were identified by *in silico* methods. For IgE binding, peptides were tested by ELISA using *Curvularia* positive patients' sera. The control serum samples were drawn from healthy individuals with no history of allergic symptoms. Control value IgE = 0.098 ± 0.023 (mean ± SE). The specific IgE values represented here are mean values of triplicates.

2. Experimental Section

2.1. Materials. General cell culture reagents, chemicals and antibodies were procured from Sigma-Aldrich (St. Louis, MO), Amersham (U.K.) or other companies stated in the text. Histamine assay kit was purchased from Immunotech (France). Cytokine estimation kits were obtained from BD Pharmingen, San Jose, CA.

2.2. Methods. **2.2.1. Epitope Mapping and Peptide Designing.** **2.2.1.1. Sequence Homology Based Molecular Modeling of Cur l 3.** The structure of Cur l 3 (cytochrome c) was derived from Swiss-Prot model building server (ProMod II)¹⁶ using *Saccharomyces cerevisiae* cytochrome c as template (1YEA Iso 2, reduced state cytochrome c). The query sequence had 69% identity and >80% sequence similarity. The model was analyzed for accuracy by WHAT_CHECK package.¹⁷ The model was visualized using Insight II, Rasmol and Cn3D softwares.¹⁸ The stereochemistry was tested using PROCHECK program and graphical processing as well as the calculation of the electrostatic potential mapped onto the molecular surface of the protein with Swiss-pdb Viewer. The values of total solvent-accessible surface area for the whole exposed surface and individual area contributions of the residues were computationally determined.

2.2.1.2. Epitope Mapping of Cur l 3. B and T cell epitopes of Cur l 3 were predicted using PROTEAN (DNASTAR)¹⁹ and Web based servers as ABCpred, BcePred, MHCpred2 and Propred.²⁰ These algorithms estimate hydrophilicity, surface probability/solvent accessibility, chain flexibility for B cell epitopes and presence of HLA II ligands and number of proteasomal cleavage sites for T cell epitopes. Finally, all these factors were combined to determine a surface contour value resulting in five B and five T cell epitopes (Table 1). The epitopes predicted were checked for their surface availability by superimposing them on the homology model of Cur l 3.

2.2.1.3. Solid Phase Peptide Synthesis. B and T cell epitopes predicted *in silico* were synthesized by Wang resin batch method using an advanced Chemtech Act 90 peptide synthesizer following F-moc chemistry in inert environment. Cysteine containing peptides were synthesized in reducing atmosphere by applying N₂ through the system. Peptides were purified, sequenced (Procise 491, Applied Biosystems) and analyzed on MALDI-TOF (Kratos analytical). All the peptides obtained were >95% pure. One peptide from Cur l 3 protein sequence present in predicted nonpeptidic region was also synthesized and tested as control.

2.2.2. Assessment of Immunoreactivity of Peptides.

2.2.2.1. Patients' Sample. Patients of asthma, rhinitis or both were screened based on history, skin tests, clinical and laboratory investigations at Out Patients' Department, V. P. Chest Institute, Delhi, India. Asthma was diagnosed as per American Thoracic Society guidelines²¹ and rhinitis was assigned to patient(s) having two or more of the symptoms.²² Blood samples were collected from *Curvularia* hypersensitive patients showing positive skin test reaction to Cur l 3 for assessment of various immunological parameters.

2.2.2.2. ELISA. *C. lunata* extract and recombinant Cur l 3 were prepared as described earlier.^{2,3} IgE binding of peptides, *C. lunata* extract and Cur l 3 was measured quantitatively by ELISA. The optimum amount of antigen(s) for indirect ELISA was observed to be 1 µg/well for *C. lunata* extract, 40 ng/well for Cur l 3 and 100 ng/well for each peptide in a dose–response curve.

2.2.2.3. Competitive Inhibition. To determine the relative affinity of peptides toward patients' IgE, competitive ELISA was performed. Briefly, 40 ng of Cur l 3 was coated on ELISA plate. The plates were washed, blocked and incubated overnight at 4 °C with *C. lunata* hypersensitive patients' serum pool (1:10) preincubated with different peptides in the concentration range of 0.01–100 µg. The plates were washed and incubated with anti-human IgE peroxidase (1:1000) at 37 °C for 3 h and processed further as in ELISA.⁶ The control was the serum pool without any inhibitor.

2.2.2.4. Cytokine Profile and Lymphocyte Proliferation by Peptides. PBMCs isolated from heparinized blood of *C. lunata* hypersensitive patients by Ficoll-hypaque centrifugation (Sigma) were cultured in RPMI-1640 media (10⁵ cells/well) with 10% fetal calf serum (FCS) and *Curvularia* extract/Cur l 3/different peptides (10 µg) for 72 h in 5% CO₂ as described earlier.² The concentration of antigens for stimulating PBMC proliferation was optimized using dose response curve. Phytohemagglutinin (PHA, 5 µg) was used as positive control, whereas cells cultured without stimulant served as negative control.

IL-4, IL-5 and IFN-γ were measured in culture supernatants of above patients' samples using BD Pharmingen Opt EIA kits (BD Pharmingen, Germany) as described earlier.²

2.2.2.5. Intradermal Testing. Intradermal tests were performed with *C. lunata* and other allergen extract(s) on asthma and rhinitis patients as described earlier.² *C. lunata* extract was procured from Antigen Lab, Institute of Genomics and Integrative Biology, Delhi, India, approved by Drug controller. The

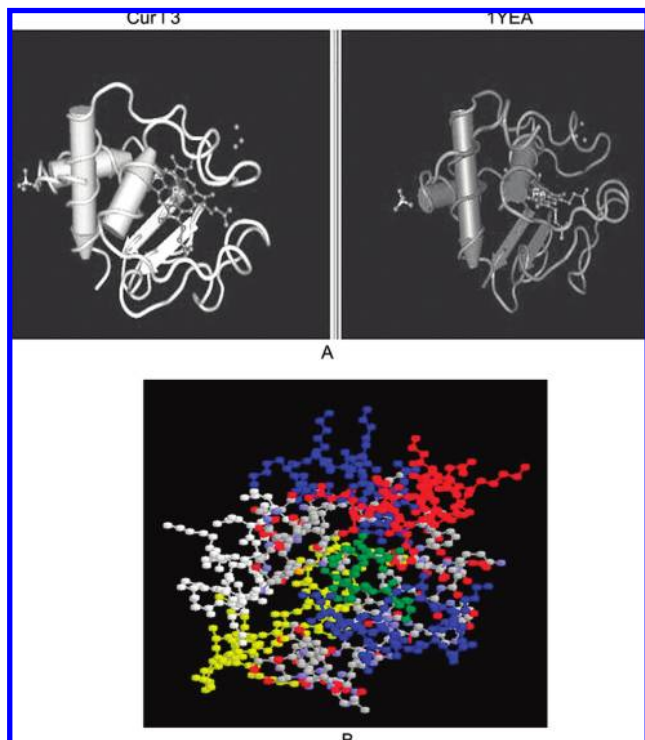


Figure 1. (A) Comparison between structures of template and query sequence: Sequence homology based model of Cur l 3 of *C. lunata* and three-dimensional structure of template sequence (i.e., 1 YEA, yeast cytochrome c Iso 2, Chain 32). (Swiss-Prot homology modeling server). The structural folds and motifs were comparable in both. (B) Molecular surface of Cur l 3 and antigenic regions: Sequence based homology model of Cur l 3 was viewed using Rasmol. Different B cell epitopes as predicted by DNASTAR software were superimposed on this model and shown here as colored domains. The small stretch corresponding to green color is the conserved motif of Cur l 3 predicted *in silico* to be buried in the core and a predicted T cell epitope.

purified protein (Cur l 3)/peptides were tested intradermally on 37 patients hypersensitive to *Curvularia*. Nonatopic healthy individuals ($n = 10$) were also tested as controls. Skin test reactions were graded as described earlier.^{2,6} Human Ethics committee approved the protocol and informed consent of patients was obtained for skin test and blood collection.

2.2.2.6. Statistical Analysis. GraphPad Prism 5.0 and MS Excel 2003 were used for all the statistical analysis. Nonparametric statistical analysis was performed for comparison between healthy controls and allergic individuals using unpaired *t* test (Welch's correction). For multiple comparisons, one-way ANOVA was used and *P*-value was assigned based on Kruskal–Walis test with Dunn's post test. $P < 0.05$ was considered significant.

3. Results

Cur l 3 structure predicted *in silico* showed comparative tertiary structure with Y1EA of *S. cerevisiae* (Figure 1A). The epitopes identified by various bioinformatics tools were superimposed on Cur l 3 modeled structures. Three-D model viewer program Rasmol and Cn3D were used to identify the surface accessible regions. Presuming that an antibody binding region exhibits an exposed solvent accessible surface with electrostatically active molecular domain, five such regions were defined in Cur l 3. The predicted B cell epitopes were

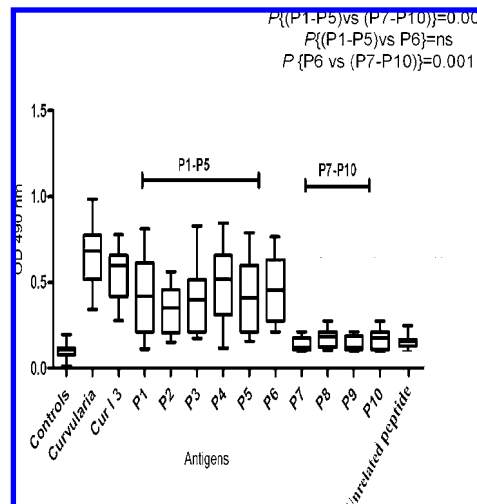


Figure 2. IgE binding of peptides: *Curvularia*, Cur l 3 or peptides (100 ng each) were coated on ELISA plate and incubated with serum samples of 20 individual *Curvularia* hypersensitive patients and IgE affinity detected using anti-human IgE peroxidase labeled. The data is presented as box and whiskers plots showing variation of IgE values from median. The significance of difference is represented by *P*-value.

present on the surface of Cur l 3 depicted as colored domains (except green region) in homology model (Figure 1B). The green region is the conserved active domain of cytochrome family proteins. T cell epitopes were clustered in the hydrophobic core and were potential HLA-II ligands. The conserved motif of cytochrome c was partially buried inside showing T cell activity *in silico*. The sequences of predicted B and T cell epitopes are presented in Table 1.

Synthetic peptides of predicted B cell epitopes (P1–P5) and T cell epitopes (P6–P10) were tested for IgE binding by ELISA using *Curvularia* positive patients' sera. The cutoff value for ELISA positive was taken as 3 times of the control value (control $\text{OD } 0.098 \pm 0.023$). Peptide P6, a T cell epitope showed highest IgE binding ($\text{OD} = 0.543$) compared to other T and B cell epitopes and was recognized by all *Curvularia* hypersensitive patients' sera tested. Among the predicted B cell epitopes, peptides P1, P2 and P4 showed significant IgE binding than controls (Table 1, Figure 2). In general, IgE binding of B cell epitopes was 2- to 3-fold higher (mean $\text{OD } 0.38 \pm 0.087$) than the predicted T cell epitopes (mean $\text{OD } 0.154 \pm 0.015$). This data indicates that B cell epitopes and P6 have higher IgE binding than other T cell epitopes.

Competitive ELISA was performed to evaluate the potency of peptides. It was observed that B cell epitopes required ~ 100 ng of peptides to achieve 50% IgE binding inhibition, whereas T cell epitopes required $10 \mu\text{g}$ peptide(s). P6 having higher affinity for IgE required 100 ng to achieve 50% inhibition. The maximum inhibition achieved by B cell epitopes was 89% ($P < 0.05$) and that for T cell epitopes was 62% ($P < 0.05$). Among B cell epitopes, EC_{50} of P4 was lowest (80 ng), while P10 achieved inhibition at $10 \mu\text{g}$, indicating its low IgE binding (Figure 3). The unrelated peptide from nonpeptide region tested as control could not inhibit IgE binding.

The predicted T cell epitopes, P6–P10, showed significantly higher PBMCs proliferation compared to B cell peptides ($P < 0.05$) as observed by ODs at 570 nm. B cell epitopes (P1–P5) showed mean stimulation of 0.213 ± 0.041 , more than 2-fold of the control ($P < 0.05$), whereas T cell epitope peptides

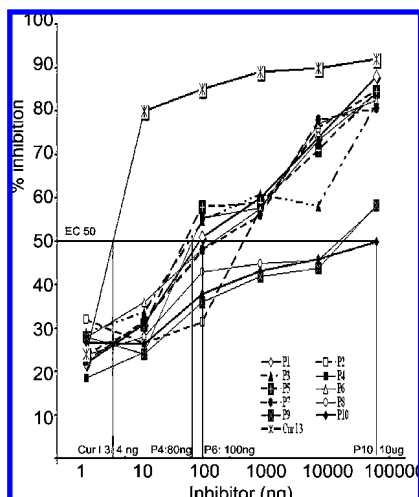


Figure 3. Allergenic potency of peptides: Competitive ELISA was done using different peptides as inhibitor. The peptides were preincubated with *Curvularia* hypersensitive pooled patients' sera in the concentration range 0.01–100 000 ng, added to Cur 1 3 coated plate (40 ng) and detected using anti-human IgE peroxidase labeled (1:1000). The values were plotted on semi-log graph to calculate percent inhibition. EC_{50} for P4, P6 and P10 marked by vertical line.

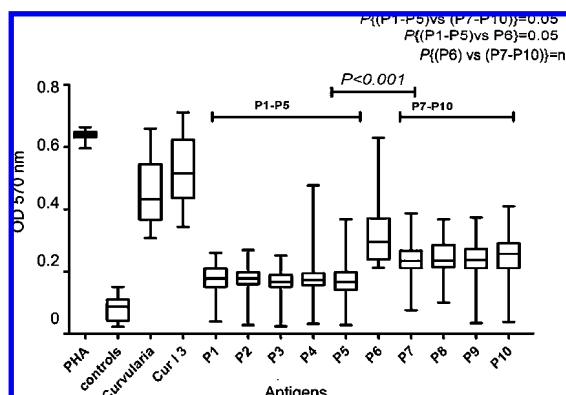


Figure 4. Lymphocyte proliferation assay with peptides: PBMCs from 20 *Curvularia* hypersensitive patients were cultured with 10 μ g of *Curvularia* extract, Cur 1 3 or peptides, in RPMI with supplements. Phytohemagglutinin (PHA, 5 μ g) was used as positive control for stimulation, while cells without stimulant served as negative control. The proliferation was measured by MTT assay. The data is represented as box and whiskers plots. $P < 0.05$ was considered significant.

P7–P10 showed mean stimulation (0.410 ± 0.031) 4-fold of the control, $P < 0.05$ (Figure 4). The mean proliferation induced by P6 was also higher (0.391 ± 0.021) than that by other peptides. P1–P5 released IL-4 $\sim 141 \pm 17.1$ pg/mL and P7–P10 released 82 ± 11.23 pg/mL, while P1–P5 released IL-5 $\sim 142.68 \pm 36.74$ pg/mL and P7–P10 released 66.8 ± 24.32 pg/mL in culture supernatant. Stimulation with P6 induced significantly higher Th-2 cytokines release than other T cell epitopes with mean OD $\sim 163 \pm 34.2$ pg/mL ($P < 0.05$). IFN- γ levels were, however, low in all the samples ($\sim 80 \pm 12.5$ pg/mL) comparable to controls indicating predominant Th-2 response (Figure 5).

Cur 1 3, P4, P6 and P10 (10 μ g/mL) peptides were skin tested on 37 *C. lunata* positive patients (2+ or above). All 37 patients showed marked positive skin reaction to Cur 1 3. P4 and P6 peptides elicited marked positive skin reaction in 26/37 and

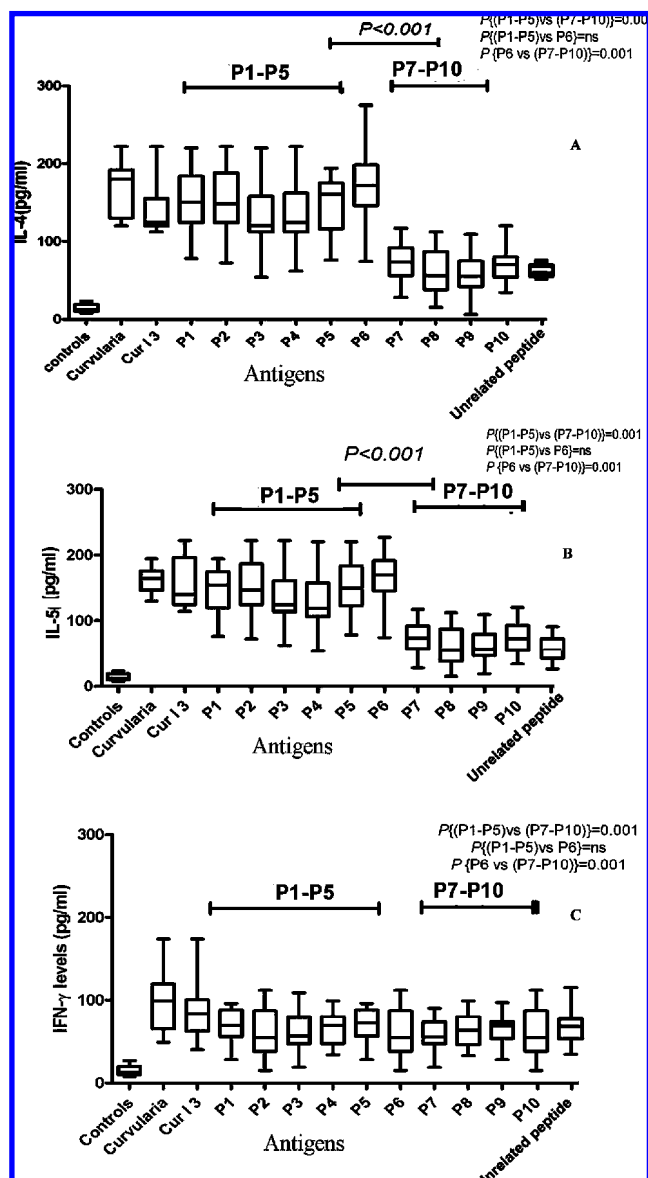


Figure 5. Cytokine profile: (A) IL-4, (B) IL-5, or (C) IFN- γ cytokines secreted in culture supernatants were measured by ELISA ($n = 20$). Briefly, capture antibodies (1:250) for each cytokine were coated on plates, incubated overnight and blocked with PBS containing 10% FCS for 1 h. Standards (5–50 ng), 100 μ L of supernatant of each sample (1:2 v/v) or controls were added to the wells and incubated at 37 $^{\circ}$ C for 3 h. The plates were washed, incubated with biotinylated secondary antibody, color developed using *o*-phenylene diamine and read at 490 nm in an ELISA reader. The data is presented as box and whiskers plots showing variation of cytokine levels from median. Values are plotted from minimum to maximum. The significance of difference is represented by P value.

28/37 of patients, respectively, while only 4/37 of patients were positive to P10. The results of skin testing were analyzed by unpaired t test with Welch correction (Table 2).

4. Discussion

X-ray crystallography of protein–antibody complex is the most accurate method of identifying B-cell epitopes that requires several experimental efforts. Previously, Cla h 6 structure was modeled based on yeast enolase, its surface

Table 2. Skin Test Reactivity of *Curvularia lunata* Hypersensitive Patients with Cur l 3 and Peptides^a

patients no.	clinical history	<i>C. lunata</i>	Cur l 3	P4	P6	P10
1	BA	2+	2+	-	2+	-
2	BA	2+	2+	-	2+	-
3	AR	2+	2+	2+	2+	-
4	BA	2+	2+	2+	2+	-
5	BA	2+	2+	-	2+	2+
6	BA	2+	2+	2+	3+	2+
7	Both	2+	2+	2+	-	-
8	AR	2+	2+	2+	3+	-
9	BA	2+	2+	2+	2+	-
10	Both	2+	2+	2+	2+	-
11	AR	2+	2+	-	2+	-
12	BA	3+	2+	2+	2+	-
13	BA	2+	2+	2+	2+	-
14	BA	2+	2+	2+	2+	-
15	AR	2+	2+	2+	2+	-
16	Both	2+	3+	2+	2+	-
17	AR	2+	3+	2+	2+	-
18	BA	2+	2+	-	2+	-
19	AR	2+	2+	-	2+	-
20	Both	2+	3+	2+	2+	2+
21	BA	3+	2+	2+	2+	2+
22	BA	2+	2+	2+	2+	-
23	AR	2+	2+	2+	+	-
24	Both	2+	2+	-	+	-
25	BA	2+	2+	-	2+	-
26	BA	2+	2+	-	2+	-
27	AR	2+	2+	-	2+	-
28	Both	2+	2+	2+	2+	-
29	BA	2+	2+	2+	2+	-
30	BA	2+	2+	2+	+	-
31	AR	2+	2+	2+	2+	-
32	BA	2+	2+	2+	-	-
33	Both	2+	2+	-	-	-
34	AR	2+	2+	2+	-	-
35	BA	2+	2+	2+	-	-
36	BA	2+	2+	2+	2+	-
37	AR	2+	2+	2+	-	-

^a Patients were suffering with allergic rhinitis (AR), bronchial asthma (BA) or both. The patients were tested intradermally with *C. lunata*, Cur l 3 and three peptides. 2+, skin reaction (wheal) equal to positive control, i.e., histamine diphosphate; 3+, more than size of positive control with 1–2 pseudopodia.

exposed IgE epitopes were deciphered, and these were validated by mutational analysis.²³ The availability of limited 3D structures for epitope elucidation is overcome by sequence based homology models providing good approximation of the structure. Recently, microarray-based immunoassay was employed successfully to map peanut epitopes using small quantities of serum.²⁴ In the present study, B and T cell epitopes of Cur l 3, a major allergen of *C. lunata* were predicted by *in silico* methods. Cur l 3 structure was modeled on yeast cytochrome c to identify five B and five T cell epitopes. B cell epitopes superimposed on the surface of this model exhibited solvent accessible regions predicted by other computational tools, while the hydrophobic interior core was composed of most of the T cell epitopes.

Analysis of overlapping peptides gives the best indication of IgE binding epitopes.²⁵ But *in silico* methods can reduce the effort of synthesizing and screening large number of peptides. Earlier, membrane bound peptides have been used for antibody binding studies. However, this limits the sites available for antibody binding. The synthetic peptides can possibly provide

an equal opportunity to all possible antibody binding sites. The specificity of IgE binding was ascertained from the lack of reaction to peptides with random sequences and the synthetic peptides representing nonepitopic regions. In the present study, B cell epitopes (P1–P5) elicited higher IgE binding than the T cell epitopes (P7–P10) because of their solvent accessibility. One of the T cell epitopes (P6), however, showed higher IgE binding than all B and T cell epitopes by ELISA. IgE binding of P6 was further assessed by surface plasmon resonance studies showing its high IgE affinity ($K_D = 5.67 \times 10^{-9}$ M) comparable to Cur l 3 ($K_D = 1.27 \times 10^{-9}$ M) (data not shown). Small peptides sometimes display lower binding with polystyrene plates in ELISA because of their size. To overcome this, calcium chloride has been used to increase the binding efficiency due to the formation of hydrophobic pockets in the protein/peptides.²⁶ P4 and P6 together demonstrated marked positive skin reaction in 35/37 of *C. lunata* hypersensitive patients tested and may have potential for peptide based diagnosis. Recently, two epitopes of Cuc m 2, a profilin predicted by solvent accessibility data and electrostatic potential, showed strong IgE binding.²⁴

Allergen specific CD4⁺ T cells produce Th2 cytokines and play an important role in progression of allergic diseases. Bet v 1_{142–156} was deciphered as a dominant T cell epitope by assessing proliferation of T cell lines in 61% of *Betula verrucosa* pollen allergic individuals.¹⁰ T cell epitopes of Lol p 5a predicted by 'TEPITOPE' demonstrated T cell reactivity in diverse HLA-DR alleles.²⁷ In the present study, P6–P10 epitopes showed significant PBMCs proliferation compared to controls or B cell epitopes ($P < 0.05$). P6 and P8 were identified *in silico* as potential HLA II ligands for DRB1_0402, DRB1_0801–6, DRB1_0813 and DRB1_0817 alleles. This information can potentially help in assessing the clinical relevance of T cell peptides.

Experimental validation of *in silico* predictions is required to know clinical relevance of epitopes. In the present study, P6 colocalizing with the conserved motif of cytochrome c (Thr-Arg-Cys-Ala-Gln-Cys-His-Thr) showed higher affinity to IgE both by competitive ELISA and BIA core (data not shown), and higher stimulation of T cells and effector cell function. This may be due to the limitation of *in silico* methods or it could be the simultaneous occurrence of B and T cell epitope as observed in a T cell epitope peptide of Der p 2.²⁸ In the hypothetical model of Cur l 3, the motif CAQCHT was seen partially buried and as ligand for several HLA-II alleles predicted *in silico*. The residues Arg, Gln and His in this sequence could provide net charge, probably giving it net antigenicity, whereas Thr and Cys being solvent inaccessible could contribute to the T cell epitope activity as demonstrated in an earlier study with Der p 2.²⁸ However, these presumptions require validation by specific mutational analysis experimentally. Here, T cell epitopes showed potential for peptide based allergen immunotherapy, whereas B cell epitopes can help in elucidating residues involved in IgE binding.

5. Conclusions

B and T cell epitopes of Cur l 3 were identified using bioinformatics tools and validated experimentally. *In silico* observations obtained were in agreement with the experimental data except for the conserved motif of Cur l 3. The sequence of peptide P6 is highly conserved and may provide cross-protection against other cross reactive allergens. Further, P6 (T cell epitope) containing a cryptic B cell epitope can be

modified to abrogate IgE binding while retaining T cell activity to serve as therapeutic agent. The approach used here is relevant and may be used to delineate epitopes of other allergenic proteins.

Abbreviations: Cur l 3, 12 kDa recombinant allergen from *Curvularia lunata*; IDT, intradermal testing; MALDI-ToF, matrix assisted laser desorption ionization-time-of-flight; PBMCs, peripheral blood mononuclear cells; PBST, phosphate buffered saline with Tween-20; PHA, phytohemagglutinin; CSIR, Council of Scientific and Industrial Research.

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