

Immunodominant Peptide Epitopes of Allergen, Asp f 1 from the Fungus *Aspergillus fumigatus*

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KURUP, V.P., B. BANERJEE, P. S. MURALI, P. A. GREENBERGER, M. KRISHNAN, V. HARI AND J. N. FINK. *Immunodominant peptide epitopes of allergen Asp f 1 from the fungus Aspergillus fumigatus*. PEPTIDES **19**(9) 1469–1477, 1998—*Aspergillus fumigatus* ribotoxin Asp f 1 is a major allergen with IgE binding activity to serum of a majority of patients with allergic bronchopulmonary aspergillosis (ABPA). The IgE binding epitopes or the T-cell stimulatory peptides of this molecule have not been studied. In the present investigation, we have synthesized linear decapeptides spanning the whole molecule of Asp f 1 and analyzed their IgE binding properties. We have also synthesized peptides based on their possible T-cell stimulatory properties and studied the stimulation of peripheral blood mononuclear cells from ABPA patients and normal controls. Several peptides demonstrated distinct IgE antibody binding response against sera from ABPA patients and proliferative response against peripheral blood mononuclear cells from the patients. From the results, it can be concluded that the carboxy-terminal region of Asp f 1 representing amino acid residues 115–149 involved in both humoral and cell mediated immunoresponses in ABPA. © 1998 Elsevier Science Inc.

Aspergillus fumigatus Allergic bronchopulmonary aspergillosis B-cell epitope T-cell epitope

ALLERGIC bronchopulmonary aspergillosis (ABPA) is a complication of allergic asthma and is caused by the ubiquitous fungus *Aspergillus fumigatus* (Af). ABPA is a progressively disabling lung disease characterized by elevated total and anti-*Aspergillus* IgE, pulmonary and peripheral blood eosinophilia, central bronchiectasis, and an obstructive airway disease (7,22,23,37,45). Several crude and purified antigens from *A. fumigatus* have been used to demonstrate specific antibodies in the sera of patients with ABPA (2,5,25,26,36,38,39,40). Crude culture filtrate and mycelial extract antigens widely used in the past have not been satisfactory. However, recently concerted effort has been directed to obtain relevant purified Af antigens for immunodiagnosis. In the last few years there has been progress in isolating better characterized and potentially pure allergens/antigens from Af using molecular biologic

techniques (1,4,14,15,16,34,42,43). Of the several recombinant proteins from Af, Asp f 1, a ribotoxin, was found to be a major allergen (37). Understanding of B-cell epitopes may be of value both in the diagnosis and in the elucidation of the immunopathogenesis of the disease. In recent years, a number of more common allergens such as house dust mite and pollen allergens have been studied for B-cell epitopes (18,24,27,28,32). A careful evaluation of this information indicates that conserved linear and conformational epitopes exist among some of these allergens. These epitope mapping studies of various allergens indicate that the small linear epitopes bind specifically to IgE antibody of allergic patients.

In recent years, T-cell and B-cell epitopes have been mapped from bacteria, viruses, and parasites (3,6,19,44). Although the antibody binding to epitopes depends on many

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factors, the understanding of allergenic epitopes may be useful in improving immunodiagnosis and therapeutic intervention (10). In addition, the identification and study of epitopes binding to IgE antibody as well as inducing T-cell proliferative responses may be of value in understanding the immune mechanisms involved in ABPA (35). Until recently, T- or B-cell epitopes of the major recombinant allergens from *A. fumigatus* have not been mapped. In the present study, we mapped the linear B-cell epitopes of Asp f 1 and evaluated their IgE binding and cell mediated immune responses. The results indicate that there are several immunodominant regions distributed throughout the Asp f 1 molecule with a stronger IgE binding and peripheral blood mononuclear cell stimulatory responses mediated by carboxy-terminal region of the allergen.

METHOD

Subjects

We have studied eight ABPA patients and six normal control subjects for their serum IgE binding activity to various synthetic peptides of Asp f 1. All 14 subjects were also evaluated for their T- and B-cell binding ability to respond to stimulation with *Aspergillus* extracts and various peptides. These patients fulfilled the diagnostic characteristics of ABPA previously described (23,45). All the ABPA patients with characteristic central bronchiectasis, and the control subjects with no clinical evidence of respiratory diseases were included in this study. ABPA patients and four of six normal controls were from Northwestern University School of Medicine, Chicago, while the remaining two controls were from the Medical College of Wisconsin in Milwaukee, Wisconsin. The human research review committee of the respective institutions approved the study.

Aspergillus Antigen

The antigen used was a mixture of culture filtrate and mycelial extract from Af. The crude culture filtrate antigen was prepared as previously described (36,38,39). In brief, Af was grown as stationary culture in synthetic broth for 2–3 weeks at 37°C. The culture was separated and dialyzed against deionized water at 4°C and lyophilized. The mycelial extract was obtained by homogenizing the washed 72 h growth from aerated cultures with a French Press. Both mycelial and culture filtrate antigens were evaluated for their protein content, SDS-PAGE profile and reactivity with patient and normal sera. These antigens were used to evaluate the sera from patients for *Aspergillus* specific IgE.

Construction of cDNA Library and Expression of Asp f 1 Protein

A cDNA library was prepared from the mRNA of *Aspergillus fumigatus* as described before (34). The Asp f 1 gene was obtained by PCR amplification using 5'GCGACCTG-GACATGCATC3' sense and 5'ATGAGAACACAGTCT-

CAAGTC anti-sense primers. The amplified fragments were subcloned into PCR II (Invitrogen, San Diego, CA) and the plasmid DNA was isolated. The DNA was digested with BamHI and XhoI and ligated to a similarly digested expression vector pET 23b (pET Expression System, Novagen, Madison, WI). The induction and analysis of the fusion protein was performed according to the pET expression protocol of the manufacturer as previously described (4). In brief, *E. coli* strain HMS174 was transformed with the ligated product by electroporation with *E. coli* pulser apparatus (Bio-Rad, Hercules, CA). The transformed cells were plated on LB agar containing ampicillin (100 µg/ml). Plasmid isolated from overnight culture was further transformed into BL21DE3pLysS by electroporation followed by plating on LB agar containing ampicillin (100 µg/ml) and chloramphenicol (34 µg/ml). When the cells reached the log growth phase ($A_{600} = 0.4$), IPTG (isopropyl-β-D thiogalactopyranoside) was added to a final concentration of 1 mmol/l to induce the expression of fusion protein by the recombinant Af clone. Cells were harvested at intervals of 1 h to 4 h after IPTG induction and were sonicated. The cell lysates containing recombinant expression protein were analyzed on a 12% polyacrylamide gel containing 0.5% SDS under reducing and denaturing conditions. The gels were stained with Coomassie brilliant blue dye, and the expression at different periods of induction was compared (4). The expressed protein was purified as reported before (4). The purified Asp f 1 was used in ELISA to detect IgE in the sera of the patients and controls and for PBMC stimulation and peptide IgE binding inhibition studies. The purity of the protein preparation was ascertained by two-dimensional gel electrophoresis. Eluted protein was lyophilized and stored at –20°C.

Solid Phase Synthesis of Asp f 1 Decapeptides

Decapeptides were synthesized on derivatized cellulose membrane using F-moc amino acids, as reported (6), according to the procedure of the manufacturer of the membrane (SPOTs, Genosys Biotechnologies, Inc., The Woodlands, TX). SPOTscan software program was used to generate amino acid sequences of the decapeptides and schedule for each coupling cycles. Amino acids spanning the whole-length of Asp f 1 representing all the 149 amino acids (1,43) were used for synthesizing the peptides with one amino acid offset and nine amino acids overlap. All reagents used were of high purity as suggested by the protocol of the manufacturer.

IgE Reactivity of the Decapeptides of Asp f 1

The cellulose membranes were blocked overnight at 4°C, after the synthesis of peptides, using blocking buffer supplied by the manufacturer along with 5% sucrose (6). The membranes then were incubated with a 1:10 dilution of a pooled serum from ABPA patients for three hours at room

temperature. The serum pool was diluted in the blocking reagent. Next the membranes were washed three times, each for five minutes, in TBS containing 0.1% Tween-20 (TBS-T). The washed membranes then were treated with a 1:1000 dilution of a biotinylated goat anti-human IgE and incubated for 1 h at room temperature and the membranes were washed as before. Alkaline phosphatase coupled to streptavidin (1:27000 dilution in TBS) was added and the membrane was incubated for 45 min. The color then was developed using NBT/BCIP (Bio-Rad, Burlingame, CA) as previously described (6). The color developed on the cellulose membrane was analyzed by densitometric scanning of the membrane using Ambis Image Acquisition and Analysis software (Ambis, Inc., San Diego, CA).

Synthesis of Peptides

Peptides were synthesized based on the results from the SPOTs assay or from the secondary and tertiary structure of the allergen using a computer software obtained through the courtesy of Dr. David C. Feller of MedImmune, Inc., Gaithersburg, MD (20). The synthetic peptides designed from T cell epitope mapping was based on the primary amino acid sequences of Asp f 1 in the region of a stable amphipathic structure in which the hydrophobic and hydrophilic residues tend to occur in opposite faces. The peptides were synthesized manually by a solid phase peptide synthesis method using F-moc (9-fluorenylmethoxycarbonyl) chemistry as reported before (21,35). F-moc amino acids were purchased from Bachem (Torrance, CA) or Peninsular Labs (Belmont, CA). The amino acid contained the following side-chains protective group: N9-Mtr for Arginine, trityl for cystine, and histidine, *ot*-butyl for aspartic acid, glutamic acid, serine, threonine, and tyrosine and boc for lysine (35).

The C-terminal amino acid of *p*-alcoxybenzyl resin cross-linked with divinyl benzene resin (Wang resin) in a 25 × 80 mm glass reaction vessel using dimethyl aminopyridine (DMAP) and diisopropyl carbodiimide (DIC). The dried amino acid conjugated resin was allowed to swell overnight in dimethyl formamide (DMF), washed three times with DMF and deprotected by reacting with 50% piperidine in DMF for 20 min and washed again in DMF prior to coupling with the next amino acid. Amino acid coupling involved the addition of a 4 M excess of the next F-moc amino acid activated ester freshly prepared by reacting the F-moc amino acid with HOBt/DIC. The coupling efficiency was monitored by the ninhydrin test (Pierce Chemical Co., Rockford, IL). After completion of the synthesis, the peptide was cleaved with 95% Trifluoroacetic acid (TFA) in the presence of the appropriate scavenger (21). The cleaved peptide was precipitated and washed with ether and dried.

The Specificity of the Antibody Binding

The specificity of the peptide IgE binding was evaluated using an ELISA inhibition assay. In this study, only pooled patient serum and selected peptides were used. The percentage of inhibition of IgE antibody binding to solid phase coated Asp f 1 was determined by using pooled serum from ABPA patients preincubated with various concentrations of Asp f 1 (10 ng to 1 mg) and compared with the IgE binding of unabsorbed serum. From the dose dependent inhibition in IgE binding of solid phase coated Asp f 1, the amount of Asp f 1 required for > 50% inhibition in IgE binding was calculated. Based on the results, 100 µg of Asp f 1 was used to absorb the pooled serum (1:10) for peptide inhibition study. The incubation was carried out overnight at 4°C under continuous shaking. After blocking the cellulose membrane strips containing the synthesized peptides, the strips were incubated with patient's serum. The reactivity was studied as described above using both absorbed and unabsorbed sera. The intensity of the color reaction was scanned using an optical scanner and the optical density of absorbed and unabsorbed sera was compared.

Peptide Specific Lymphocyte Proliferation

Proliferative assays were performed in 96-well flat bottom microtiter plates in medium alone and in presence of synthetic peptides of Asp f 1, purified Asp f 1, and crude *A. fumigatus* extract. Peripheral blood mononuclear cells (PBMCs) were obtained from eight ABPA patients and six normal controls. PBMCs were separated from heparinized blood according to the method of Boyum (8). PBMCs at a concentration of 2×10^5 per well were cultured in RPMI 1640 medium supplemented with 2 mmol/L glutamine, penicillin (100 units/ml), streptomycin (100 µg/ml), and 10% fetal bovine serum. The viability of the PBMCs were determined by Trypan blue dye exclusion method using various concentrations of the peptides (1–100 µg/ml) for stimulation. A concentration of 30 µg/ml synthetic peptide was used to stimulate the cells, while only 10 µg/ml of Asp f 1 and crude antigen were used. Asp f 1 was found to be toxic to the cells even at lower concentrations. The cell cultures were incubated at 37°C in a humidified CO₂ (5% CO₂ in air) incubator for 7 days. The cultures were then pulsed with 0.5 µCi/well of tritiated thymidine (specific activity of 6.7 Ci/mmol, New England Nuclear, Beverly, MA) for the final 18 h of culture. The cells were harvested on glass fiber filter and the radioactivity counted on a beta scintillation counter (Rack Beta, LKB, Sweden). Results are expressed as stimulation index as follows:

$$SI = \frac{\text{counts in peptide/antigen-stimulated culture}}{\text{counts in control culture}}$$

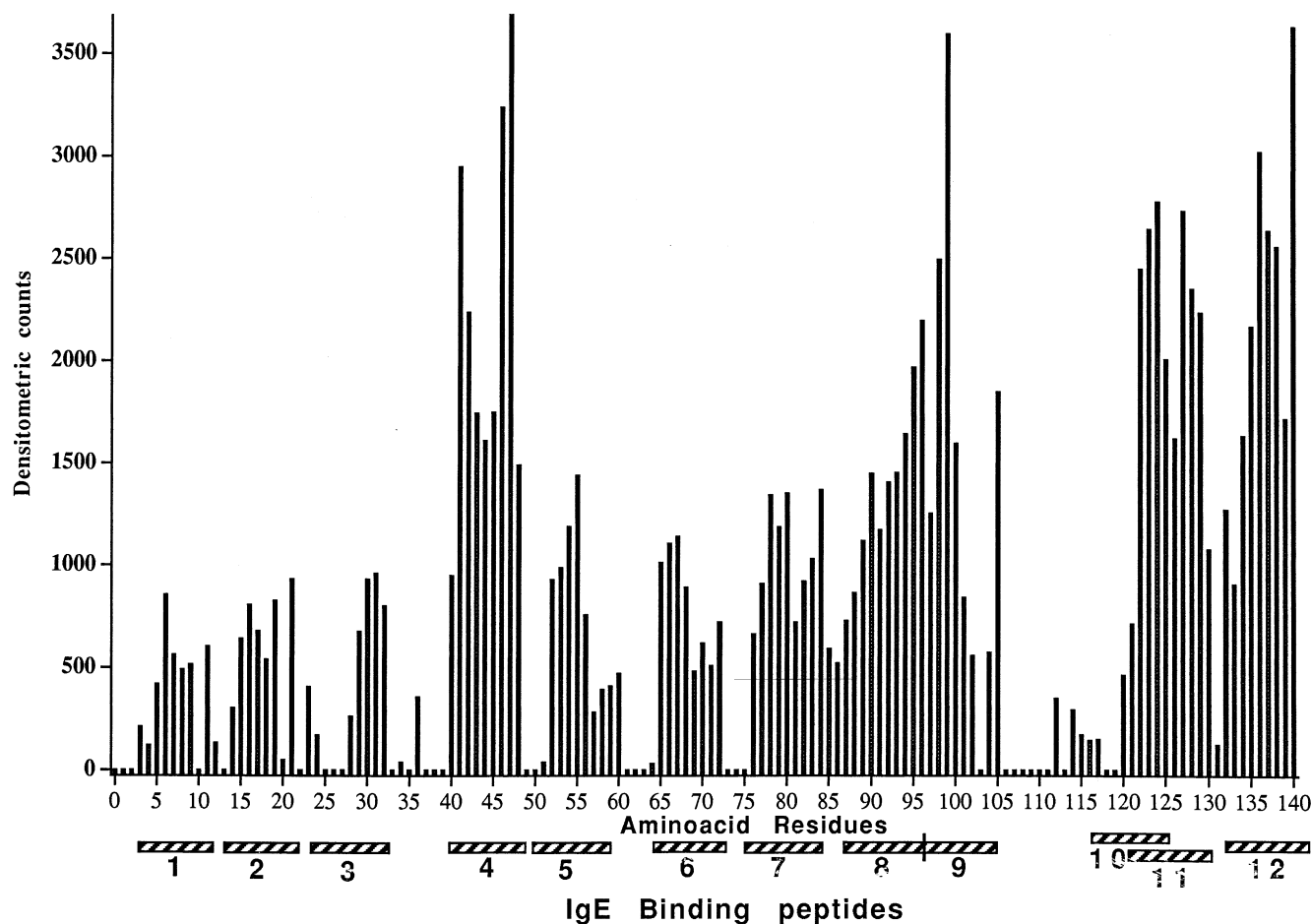


FIG. 1. The intensity of the decapeptide reaction with IgE antibody in the pooled sera from ABPA patients. The decapeptide spots are shown in the abscissa and each number represent the position of the first amino acid residue of the decapeptides. The complete linear amino acid residues of Asp f 1 are represented from 1–140 spots. Out of 13 IgE binding epitopes 1–12 are shown in the abscissa and epitope 13 (Table 1) represents the spot number 140 (encompassing amino acid residues 140 to 149).

Statistical Analysis

The data were analyzed using student's *t*-test (two-tailed, independent samples). A *p* value of ≤ 0.05 was considered statistically significant.

RESULTS

Major IgE Binding Epitopes of Asp f 1 are at the C-terminal End of the Protein

The linear decapeptides of Asp f 1, synthesized on derivatized cellulose membrane exhibited distinct IgE binding with the pooled sera from patients with ABPA. The densitometric scanning of the spots representing individual peptides are shown in Figure 1. The spots representing peptides in the region of amino acid residues 40 to 48, 76 to 102, and in the C-terminal end of the protein from 120 to 149 exhibited strong peptide antibody interaction. The amino acid sequences of the 13 decapeptides showing IgE binding by SPOT analysis are listed in Table 1.

TABLE 1
THE AMINO ACID RESIDUES OF VARIOUS EPITOPES OF ASP f 1 BINDING TO IgE

Number	Amino Acid Residues
1	3-WTCINQQLNP-12
2	13-KTNKWEDKRL-22
3	24-YNQAKAESNS-33
4	40-DGKTGSSTPH-49
5	50-WFTNGYDNG-59
6	64-GRTPKFKSQA-73
7	75-CDRPPKHSQN-84
8	87-GKDDHYLLEF-96
9	96-FPTFPDGHDI-105
10	116-PGPARVIYTY-125
11	121-VIYTPNPKVF-130
12	132-GIVAHQRGNQ-141
13	140-NQGDRLRLCSH-149

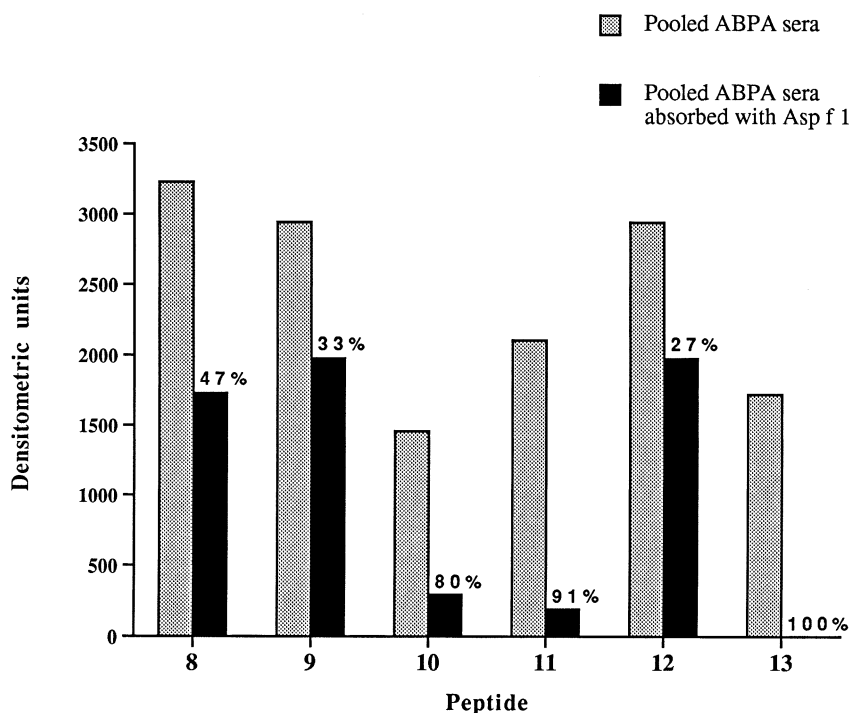


FIG. 2. The selected peptides (8 to 13, Table 1) showed reactivity with pooled ABPA serum were tested for their IgE binding specificity. The specificity was determined by inhibiting the IgE antibody reactivity of the pooled serum with Asp f 1 and using the Asp f 1-specific, IgE-depleted serum to demonstrate the reactivity with the six selected synthetic peptides. Out of the six peptides studied peptides #8 to #12 showed reduced titers, while peptide #13 failed to react with the absorbed serum.

To further evaluate the specificity of the IgE antibody binding of these linear peptides, a competitive inhibition assay using membrane bound peptides and sera from ABPA patients preincubated with Asp f 1 was carried out (Fig. 2). Of the 13 epitopes identified, six peptides (8 to 13) representing C-terminal end of the protein demonstrated strong IgE antibody binding reactivity were selected for the inhibition study. The specificity of the binding with sera was determined by inhibition of the reaction by preincubation with Asp f 1. Peptides #10 and #11 representing amino acid residues from 116 to 130 showed inhibition of IgE binding by 80% and 91%, respectively, whereas peptide #12 of amino acid sequence from 132 to 141 exhibited only 27% inhibition. However, the maximum inhibition (100%) could be achieved by the decapeptide representing the extreme C-terminal end of Asp f 1, from amino acid residues 140 to 149 (peptide #13). The results indicate that the major IgE binding epitopes of Asp f 1 are at the C-terminal end of the protein. Although, the decapeptides representing the N-terminal amino acid sequences of Asp f 1, synthesized on derivatized cellulose membrane demonstrated antibody binding, they failed to show significant inhibition in IgE antibody binding in ELISA inhibition study (data not shown).

T-cell Stimulation of ABPA Patient with Various Synthetic Peptides of Asp f 1

In T-cell proliferation studies, 15 synthetic peptides encompassing the entire sequence of Asp f 1 were used (Table 2). These peptides were designed on the basis of T-cell epitopes

TABLE 2
PEPTIDES SYNTHESIZED BASED ON THEIR SECONDARY
STRUCTURE ANALYSIS FOR T-CELL RESPONSES

A1	1-10:	ATWTCINQQL
A2	1-15:	ATWTCINQQLNPKTN
A3	6-21:	INQQLNPKTINKWEDKR
A4	21-36:	RLLYNQAKAESNSHHA
A5	36-51:	APLSDGKTGSSYPHWF
A6	53-68:	NGYDGNGLIKGRTP
A7	69-84:	KFGKADCDRPPKHSQN
A8	85-100:	GMGKDDHYLLEFPTFP
A9	101-114:	DGHDKYKFDSSKPKPE
A10	115-130:	DPGPARVIYTYPNKVF (Asp f 1)
A11	122-137:	IYTPNPKVFCGIVAHQ
A12	122-137:	IYTPNPKVFCGIVALQ*
A13	128-140:	KVFCGIVAHQGRN
A14	135-146:	AHQGRNQGDRL
A15	134-149:	VAHQGRNQGDRLRLCSH

A11-A15 are arbitrarily synthesized based on their IgE binding characteristics.
*A12 is same as A11 except that aa at 15th position has been mutated from H to L.

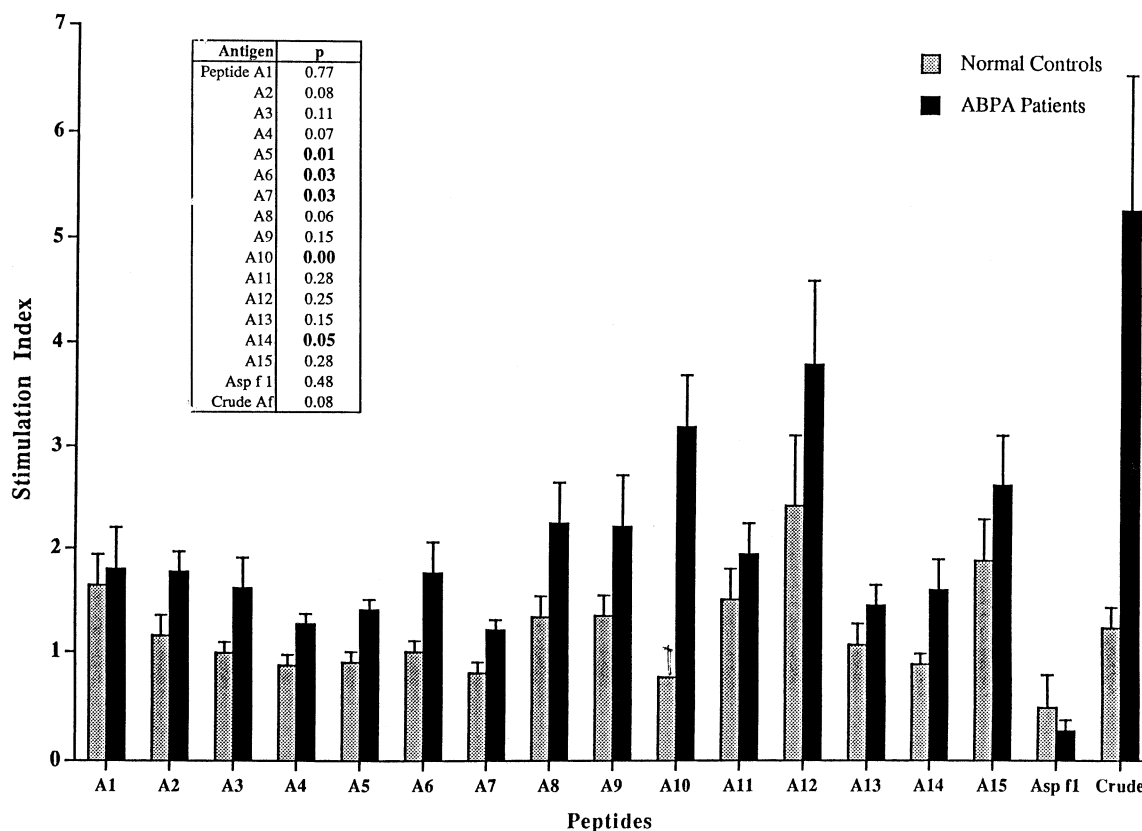


FIG. 3. The mean PBMC stimulation indices for the ABPA patients compared to normal controls is shown. The statistical analysis of the results is shown as insert in figure. The counts in unstimulated PBMC cultures of patients and controls varied from 167 to 405 cpm (mean = 237 ± 63).

predicted by computer algorithm and different from the membrane bound linear peptides identified by SPOT analysis. The stimulation index of PBMC from ABPA patients and normal control against various synthetic peptides of Asp f 1 is shown in Figure 3. All these peptides showed slight enhancement in their stimulation indices for ABPA patients compared to normal controls although statistically significant stimulations could be seen only with five peptides. Out of 15 peptides, A5 to A7, A10, and A14 encompassing amino acid residues from 36 to 84, 115 to 130, and 135 to 146, representing regions of Asp f 1 demonstrated strong proliferative responses. The histidine at position 136 of peptide A12 (as in peptide A11) believed to be involved in cytotoxicity of Asp f 1 is replaced with leucine. However, no significant differences in the proliferative responses could be detected between peptides A11 and A12 indicating H136 is not involved in PBMC proliferation. On the other hand, the complete Asp f 1, a ribotoxin, showed toxic effects on the PBMC at most concentrations used in the study compared to the crude Af extract and peptide A10 (115–130).

DISCUSSION

In the present study, we have identified eight major IgE binding regions representing 13 decapeptides spanning the whole molecule of Asp f 1. The majority of the strong IgE binding epitopes are concentrated at the C-terminal end. However, none of these epitopes showed any homology with the B-cell epitopes mapped from different allergens (3,12,13,18,28,41,47). The previous reports by other investigators indicate that there is yet no agreed upon pattern with regard to the antibody binding areas of the molecules (18,19,32). Increased antibody binding has been attributed to glycosylation and presence of proline rich sequences (48). However, in Asp f 1 no glycosylated or proline rich areas have been identified. The IgE binding by Cyn d 1 and related allergens has also been reported to be at the C-terminal end (27). Similarly with the house dust mite antigen Der p 2, the C-terminal end showed binding to IgE antibody, while the N-terminal end failed to show any reactivity to IgE (32). In this study the linear Asp f 1 peptides were synthesized on derivatized cellulose membrane and evaluated for IgE binding with ABPA sera. In

fact, distinct IgE binding peptides could be detected near the C-terminal region of the protein. The available data suggests that antigenic sites are generally located on the protein surface, in regions of relatively high segmental flexibility and hydrophilicity. The majority of the exposed surface of a protein may be antigenic for antibodies. In order to determine whether these IgE binding linear peptide sequences are present in native Asp f 1 molecule as such or are under conformational constraint, we carried out an ELISA inhibition study using Asp f 1 incubated ABPA sera. The peptides A10, A11, and A13 (present at the C-terminal end of Asp f 1) exhibited more than 80% inhibition in IgE binding with Asp f 1 pre-absorbed ABPA sera indicating that these peptides represent IgE binding epitopes comparable to the native Asp f 1, most likely these linear peptides are exposed to the surface of Asp f 1 and hence are available for antibody binding. On the other hand, peptides A8, A9, and A12 with less than 50% inhibition in IgE binding indicates either they are not properly exposed to the surface of Asp f 1 or under some conformational constraint to interact with Asp f 1 specific antibodies.

Asp f 1, a ribotoxin, has been identified as a major allergen in ABPA (38). It has been reported that IgE from over 80% of the patients with ABPA bind to this allergen, and there is good correlation between skin test and ELISA reactivity using this allergen (1,17,42,43). Previous reports also indicate that Asp f 1 stimulates T-cells from ABPA patients (11). A few T-cell epitopes from Asp f 1 have been identified and characterized (29,30,31). The present study demonstrated that some of the selected epitopes with 10–15 amino acid residues are not toxic to the PBMCs, while the whole molecule was found to be toxic to PBMC of ABPA and normal controls.

Aspergillus fumigatus and related species secrete ribotoxins, which include Asp f 1, mitogillin, restrictocin, and alpha sarcin. These toxins act on the peripheral blood lymphocytes as evidenced by cell death in PBMC cultures. However, Knutsen et al. showed increased stimulation with all the five T-cell lines generated from ABPA patients (31). They used 5 µg/ml of Asp f 1 for T-cell generation, although this concentration was lethal for the majority of PBMCs in our study. The specific T-cells are frequently capable of stimulation using high concentrations of the specific allergens. However, several of our synthetic peptides were not lethal to PBMCs compared to Asp f 1 and some of them showed significant stimulation (A5, A6, A7, A10, and A14). This may be due to the toxic part of the molecule not being represented in those peptides stimulating PBMCs. The involvement of H136 in toxicity of Asp f 1 was evident from less toxicity of mutant H136L of Asp f 1 (9). In one of the T-cell stimulatory peptide A12, we replaced H136 by L136. However, both A11 (with H136) and A12 (with L136) exhibited similar proliferative re-

sponses indicating that native conformation of Asp f 1 is essential for its toxic effect.

The T-cell epitopes of Asp f 1 previously identified stimulated T-cell clones obtained from ABPA patients. The amino acid residues 1–20 failed to show stimulation of PBMC in the present study, while Chauhan et al. showed that 2/21 T-cell clones were stimulated by this peptide (11). Major epitopes identified by them namely aa 46–65 and aa 106–125 showed some overlap in amino acids with peptides A5 (aa 36–51), A6 (aa 53–68), and A10 (aa 115–130) used for stimulation in our study. Although our results and Chauhan et al. results cannot be compared as we have studied PBMC from ABPA patients while they used T-cell clones from patients. Thirteen of 21 clones from all three patients reacted with the three epitopes (11). In our previous study we have shown that a synthetic peptide from Asp f 1 with amino acid residues from 53 to 68 stimulated spleen cells of immunized mice and produced IL-4 (35). This peptide also was reported to be a major epitope reported by Chauhan and associates (11).

In the present study, we have identified linear IgE binding epitopes and used the same epitopes and those identified as T-cell epitopes based on computer algorithms for T-cell stimulation. Asp f 1 is a major allergen with strong binding to IgE antibody of sera from ABPA patients and may also cause T-cell stimulation. The production of IgE is dependent on elaboration of IL-4 in response to stimulation by the antigen (31,46). Because of toxicity this antigen cannot be used in T-cell studies. From the T-cell epitope analysis it was found that at least some of the regions of Asp f 1 molecule are non toxic. The peptide A10 (aa 115–130) did not have toxic effects on cells and moreover exhibited a distinct proliferative response to PBMCs from patients. At the same time the ELISA inhibition study also indicates a major IgE binding epitope in this region of Asp f 1. The synthetic peptides involved in both antibody binding and cell-mediated immune responses may be explored further for developing therapeutic strategies. It thus may be possible to delete the toxic regions of the molecule or mutate the putative amino acids responsible for the toxicity and devise fragments for T-cell stimulation studies. Such non-toxic antigens can also be successfully used for skin testing patients.

Knowledge of peptide interactions with T- and B-cells might provide tools for immunotherapy (10,33,47). Peptides that contain T-cell epitopes of allergens have been proposed as useful for immunotherapy, only if they do not bind to IgE. In the present study, peptide A5 (aa 36–51) showed less binding to IgE, but demonstrated stimulation of PBMC from ABPA patients. As we have not used T-cell clones, it is not possible to conclude that the stimulation studies are completely representative due to the heterogeneity of the cell population used in the present study. The results, however, indicate the possibility of developing immunotherapy

protocols using specific synthetic peptide epitopes. Nevertheless, it is essential to have a better understanding of T- and B-cell epitopes from Asp f 1 as well as from other major allergens associated with ABPA. Peptides that bind to T cell receptors without causing activation may result in a change in the production of cytokine from a predominantly Th2 to a Th1 might be useful in devising immunotherapeutic peptides (10).

In conclusion, we have identified immunodominant

epitopes of Asp f 1 based on the binding to serum IgE and stimulation of PBMC from ABPA patients. The carboxy-terminal regions were found to bind to IgE more strongly than N-terminal regions.

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