

Antibody Binding of Deletion Mutants of Asp f 2, the Major *Aspergillus fumigatus* Allergen

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Asp f 2, a 268 amino acid major allergen from *Aspergillus fumigatus* (Af) demonstrated nine linear IgE binding regions. It is not known whether any of these linear epitopes are also conformational epitopes. Hence, we constructed deletion mutants of Asp f 2 devoid of one or more epitopes, and the IgE binding of these proteins with sera from patients with ABPA was compared with the full-length Asp f 2 expressed in *E. coli* and *Pichia*. The *Pichia* expressed protein reacted weakly with IgE, but strongly with IgG of ABPA sera compared to *E. coli* expressed Asp f 2. Weak IgE binding only was seen when the C-terminal or N-terminal was deleted, while depletion of both ends negated all reactivity. The monoclonal antibody IL-B8 and IgE and IgG of ABPA sera bound significantly to the Asp f 2 E-4 fragment indicating that the major B-cell epitope is located at the N-terminal end of Asp f 2. © 2000 Academic Press

Key Words: Asp f 2; deletion mutants; *Aspergillus fumigatus*; IgE; immune response.

Aspergillus fumigatus major allergen Asp f 2 has been cloned and the protein expressed (1). This protein reacted with IgE antibody in the sera of over 90% of patients with ABPA (2). When overlapping peptides covering the whole sequence of Asp f 2 were evaluated for IgE binding, nine linear regions were identified spanning the full-length of the allergen molecule (3). Although the antibody binding sites are sequestered based on the primary, secondary, and tertiary structures, the linear sequence may also be of significance particularly when such synthetic peptides can bind to

Abbreviations used: Asp f 2 E, *E. coli* expressed Asp f 2; Asp f 2 P, *Pichia* expressed Asp f 2; Asp f 2 E1-5, *E. coli* expressed Asp f 2 fragments; SDS–PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; IPTG, isopropyl-β-D-galactopyranoside; PAS, periodic acid Schiff; ELISA, enzyme-linked immunosorbent assay; ABPA, allergic bronchopulmonary aspergillosis.

the serum or membrane bound antibody. Since T-cell epitopes are predominantly linear, they are of value in understanding the T-cell response and activation cascades (4). The pathogenesis of ABPA could be better understood by the use of these relevant synthetic peptides representing T and B cell epitopes of the allergen (5, 6). The response of T-cell epitopes and their binding to specific receptors may lead to a better understanding of the regulatory events leading to the disease. As B-cell recognition and activation is based on the secondary and tertiary structures of the allergens and since such structures are lacking in the decamer synthetic peptides, we devised Asp f 2 proteins depleted of selected IgE binding regions. Asp f 2 gene was cloned as mutants by targeted depletion of N- and C-terminal sequences representing epitopes of IgE binding regions. Five truncated Asp f 2 proteins were compared with the full-length mature protein for their antibody binding reactivity. The results indicate decreased IgE binding when C-terminal or N-terminal ends were deleted, while depletion of both ends eliminated all reactivity. Both monoclonal antibody and ABPA sera reacted with the N-terminal IgE binding region of Asp f 2.

MATERIALS AND METHODS

Cloning of Aspergillus fumigatus gene encoding Asp f 2 and fragments. A cDNA library was constructed from *A. fumigatus* in a λZAP II vector and the protein expressed in a pET 23b(+) vector as described previously (1, 7). Primers used for amplification of Asp f 2 DNA and the different fragments of Asp f 2 are shown in Table 1. The sense and antisense primers had BamHI and XhoI at their 5' ends. The amplified fragments were subcloned into PCR II vector (Invitrogen, San Diego, CA). The isolated plasmid DNA was digested with BamHI and XhoI and ligated to 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. The cells carrying expressed proteins were sonicated and the recombinant expression protein was analyzed on 15% Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) (8). After electrophoresis, the gels were stained with Coomassie brilliant blue, and the proteins

TABLE 1
Primer Sequences Used for Expressing rAsp f 2 and Fragments

Asp f 2*	Molecular size (kDa)	Primers' sequences
rAsp f 2 P	72	1) 5'GAATTCAGACGCTGGCGCGGTGACCTC3' 2) 5'TCTAGAAAAGTGCAATGAAGCTGTCCACCTT3'
rAsp f 2 E	37	1) 5'GGATCCGGACGCTGGCGCGGTGACCTCGT3' 2) 5'CTCGAGAAAGTGCAATGAAGCTGTCCACCTTC3'
E1	35	1) 5'GGATCCGGAGCTCGCCCGGCACGCCA3' 2) 5'CTCGAGAAAGTGCAATGAAGCTGTCCACCTTC3'
E2	32	1) 5'GGATCCGGTCATCGTGAACGGGGACAAG3' 2) 5'CTCGAGAAAGTGCAATGAAGCTGTCCACCTTC3'
E3	24	1) 5'GGATCCGGGCTACACCGTCGCCGGCT3' 2) 5'CTCGAGAAAGTGCAATGAAGCTGTCCACCTTC3'
E4	24	1) 5'GGATCCGGACGCTGGCGCGGTGACCTCGT3' 2) 5'CTCGAGAATCCGACACCGGGAGCAGCAATAT3'
E5	20.5	1) 5'GGATCCGGTCATCGTGAACGGGGACAAG3' 2) 5'CTCGAGAATCCGACACCGGGAGCAGCAATAT3'

* rAsp f 2 P, *Pichia* expressed in full-length Asp f 2. rAsp f 2 E, *E. coli* expressed in full-length Asp f 2. E1-E5, Asp f 2 fragments expressed in *E. coli*.

expressed at different induction times were compared (3). The expressed proteins were affinity purified from the cell extracts using Ni-NTA agarose column (Qiagen Inc., Valencia, CA). The purity, molecular weights, and reactivity of the recombinant proteins with antibody were studied (1–3). The purified recombinant proteins were lyophilized and stored at -20°C .

The mature Asp f 2 E protein consists of 268 amino acids while the five deletion mutants expressed in *E. coli* using the pET vector varied from 135 to 238 amino acids (Figs. 1A and 1B). These deletion mutants were constructed based on the IgE binding regions determined in a previous study (3). Using the cDNA of Asp f 2, IgE binding "epitopes"-1, 2, 3, and 4 from the N-terminal end and "epitope"-9 from the C-terminal ends were deleted in different mutants. Another fragment was constructed by deleting two epitopes from the N-terminal end and one epitope from the C-terminal end (Fig. 1B). The primers used to construct these mutants and the truncated proteins expressed are shown in Table 1.

Sequencing the DNA. The DNA representing the full-length proteins and fragments were sequenced and the sequences were com-

pared (1, 3). The double stranded plasmid DNA of Asp f 2 and the deletion mutants were used as template for the sequencing. The sequences were compared and the integrity of the mutants designed was ascertained.

Expression of Asp f 2 in *Pichia pastoris*. The nucleotide sequence analysis of Asp f 2 gene demonstrated four possible glycosylation sites (3). Evidence at hand from the study of native *Aspergillus* allergens confirmed the presence of carbohydrates as constituent of this molecule. Hence, we used the yeast *Pichia pastoris* an eukaryotic organism to express the glycosylated Asp f 2 (9). Transformants carrying Asp f 2 gene were linearized with Pme I. The restriction enzyme site is located in the 5' AOX1 region of pPICZ- α . Transformation of GS115 with linearized constructs by electroporation resulted in single crossover recombination at the AOX1 locus. The Mut⁺ phenotype (Methanol utilization) of the transformed GS115 was confirmed by growing the organism on MDH (minimal dextrose histidine) and MMH (minimal methanol histidine) medium. Thereafter, 6–10 colonies of Mut⁺ phenotype were selected for small-scale expression. The small culture of each colony was induced with methanol and cell pellets at different time intervals were analyzed by SDS–PAGE. The time point exhibiting maximum expression of the fusion protein was selected for large-scale expression and purification of the recombinant Asp f 2 P.

Characterization of the various Asp f 2 proteins. All Asp f 2 proteins were characterized for their profile in 15% SDS–PAGE. The proteins were stained with Coomassie brilliant blue and the molecular weights compared with standards. The monoclonal antibody reacting to native Asp f 2 was prepared as reported previously (10). All the recombinant proteins were studied for their reactivity against the monoclonal antibody by Western blots and ELISA. The specificity of the reactivity was ascertained by absorbing the antibody with Asp f 2 E and testing it against Asp f 2 P by ELISA. The epitope directed against the monoclonal antibody was determined by the reactivity of different fragments with the monoclonal antibody. The glycosylation of the protein was determined by staining the SDS–PAGE gels with Periodic Acid Schiff (PAS) stain (11, 12).

Sera from patients and normal controls. ABPA patients diagnosed according to the criteria of Rosenberg *et al.* (13) were studied along with patients with asthma and immediate skin test reactivity to *Aspergillus fumigatus* antigen, but not having sufficient criteria for diagnosing ABPA. Normal healthy controls without skin test reactivity to *Aspergillus* species to other antigens also were studied.

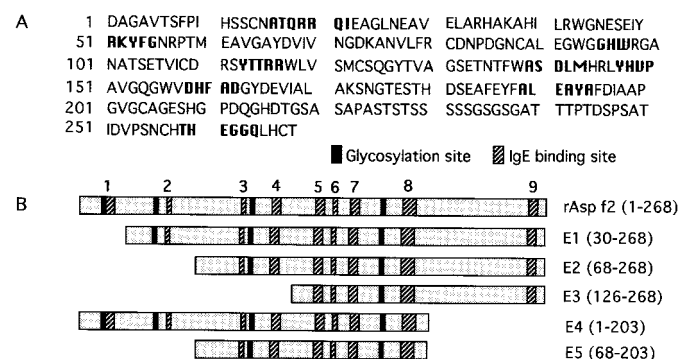


FIG. 1. (A) The deduced amino acid sequence of Asp f 2 E. Nine IgE binding sites identified by epitope mapping are shown in bold letters. (B) Schematic representation of six different cDNA constructs of various fragments of Asp f 2 having IgE binding regions and glycosylation sites selectively depleted. These clones encoding recombinant polypeptides were over expressed in *E. coli* (pET-23⁺ vector). The full-length protein was also expressed in *Pichia pastoris* (for details see Materials and Methods).

Ten ABPA, 10 skin test positive asthmatics, and 10 normal healthy controls were studied. All sera were tested for specific IgE and IgG antibodies by ELISA using the different Asp f 2 antigens. The institutional review committees approved the human study envisaged in this study.

Antigen-specific ELISA for IgE and IgG in the sera of patients and controls. The binding of IgE antibody in the sera of ABPA patients, skin test positive asthmatics, and normal controls was studied against Asp f 2 and the various fragments by a solid-phase ELISA (14–17). Immulon II polystyrene plates (Fisher Scientific, Itasca, IL) were coated overnight at 4°C with 2.5 µg/ml of the various recombinant proteins in PBS (pH 7.4). The subsequent steps of incubations with serum, biotinylated anti-human IgE and IgG, and streptavidin peroxidase were carried out as described previously (15). The color developed with the chromogen OPD (orthophenylene diamine) was read at 490_{nm} in an automatic ELISA plate reader (Dynatech, Chantilly, VA). All dilutions of the reagents were selected empirically after checkerboard titrations with positive and negative control sera. Appropriate controls were included in all plates.

Competitive inhibition ELISA. In order to determine the cross-reactivity between the glycosylated recombinant Asp f 2 P and *E. coli* expressed Asp f 2 E, a competitive ELISA was used. Microplates were coated overnight at 4°C with 100 µl of 5 µg/ml of the rAsp f 2 P. Mouse monoclonal antibody (IL-B-8) specific to native Asp f 2 and pooled sera from patients with ABPA were incubated for 2 hours at room temperature with rAsp f 2 E at concentrations ranging from 0.40 to 200 µg/ml. After incubation, 100 µl of the absorbed serum was transferred to the ELISA plates coated with recombinant Asp f 2 P. ELISA was performed as described above, and the OD values of pre-incubated serum samples without rAsp f 2 E was taken as 100% binding. Dose dependent inhibition was determined from the serum absorbed with various concentrations of rAsp f 2 E.

Western blot analysis of Asp f 2 and fragments. Five µg of rAsp f 2 E, rAsp f 2 P, and the five fragments were subjected to SDS-PAGE. The proteins separated in the gel were transferred to nitrocellulose membrane (Schleicher & Schuell) using a TransBlot apparatus (Bio-Rad) as described previously (7, 16). The free binding sites on the membranes were blocked by PBS-T with 3% BSA. The membranes then were incubated overnight at 4°C in a 1:500 dilution of the monoclonal antibody (IL-B8) or in a 1:10 dilution of the ABPA serum pool. The membrane strips were treated with biotinylated rabbit anti-mouse IgG₁ antibody or biotinylated rabbit anti-human IgE antibody for 1 hour. This was followed by incubation with streptavidin alkaline phosphatase and the color development with 5-bromo-4-chloro-3-indolylphosphate toluidinium-nitroblue tetrazolium substrate (Boehringer Mannheim, Indianapolis, IN) as described before (2, 3). The developed reactivity of the bands were compared.

Statistical analysis. The optical density (O.D.) values of the different groups of subjects were compared by using Student's *t* test (two-tailed independent samples). A *P* value of <0.05 was taken as significant.

RESULTS

The characteristics of *Pichia* expressed and *E. coli* expressed Asp f 2. The *E. coli* expressed Asp f 2 appeared as a 35–37 kDa protein, while the protein expressed through *Pichia pastoris* (Asp f 2 P) appeared as a protein with a molecular mass of 70–75 kDa (Fig. 2A). The *Pichia* expressed protein showed twice the molecular mass of the *E. coli* expressed Asp f 2. Since there were four possible glycosylation sites identified in the sequence, Asp f 2 P was analyzed for the presence of carbohydrates. SDS-PAGE stained with PAS demonstrated the presence of carbohydrates in the Asp

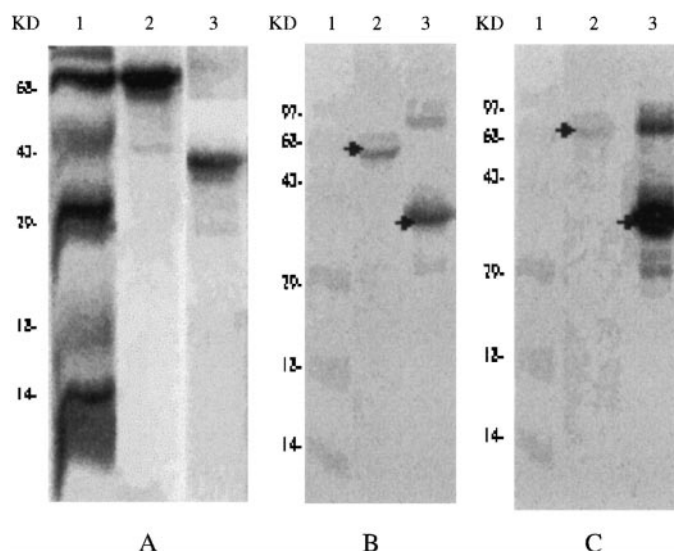


FIG. 2. SDS-PAGE and Western blots of rAsp f 2. (A) SDS-PAGE analysis of purified rAsp f 2 P and rAsp f 2 E. Five micrograms of purified antigens were loaded and subjected to electrophoresis before staining with Coomassie brilliant blue R250. (B) Western blot analysis of rAsp f 2 P and rAsp f 2 E using pooled sera from ABPA patients. (C) Western blot analysis of rAsp f 2 P and rAsp f 2 E using mouse mAb (IL-B8). Lane 1: molecular weight standard; Lane 2: rAsp f 2 P expressed in *Pichia pastoris*; Lane 3: rAsp f 2 E expressed in *E. coli*.

f 2 P, while the staining was negative for the *E. coli* expressed proteins (data not shown).

The Reactivity of Asp f 2 P and Asp f 2 E with Specific Antibody in the Sera

Enzyme linked immunosorbent assay (ELISA). The *E. coli* expressed and the *Pichia* expressed Asp f 2 proteins reacted with the sera from patients with ABPA by ELISA (Fig. 3). The reactivity of IgE with Asp f 2 E was stronger than with rAsp f 2 P (Fig. 3A). Both allergens demonstrated very weak binding to the specific IgE in the sera of asthmatics and normal control subjects. The IgG antibody in the sera of patients with ABPA showed stronger reactivity with Asp f 2 P compared to Asp f 2 E (Fig. 3B). Both skin test positive asthmatics and normal control sera also reacted with Asp f 2 P, although the levels were considerably lower compared to ABPA (Fig. 3B). Significantly high titers were detected with both Asp f 2 P and E when monoclonal antibody was studied (Fig. 4).

The structural conformation of Asp f 2 P and Asp f 2 E was compared using ELISA inhibition with mouse mAb to Asp f 2 and sera from ABPA patients. A competitive inhibition assay demonstrated a dose-dependent inhibition when the ABPA serum pool or monoclonal antibody was absorbed with different concentrations of Asp f 2 E and evaluated against solid phase coated with Asp f 2 P (Fig. 5). A dose-dependent

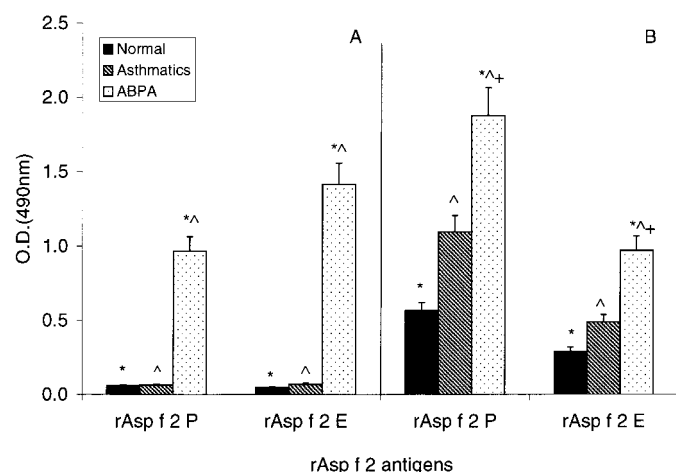


FIG. 3. IgE (A) and IgG (B) binding of rAsp f 2 P and rAsp f 2 E using 10 each of serum samples from ABPA, skin test positive asthmatics, and normal control groups by ELISA. The statistical significance between different groups are shown as “*” for normal vs ABPA; “^” for asthmatics vs ABPA and significant reactivity among antigens are shown by “+”.

inhibition of the binding reactivity of 30% or more suggested that the monoclonal antibody and human ABPA sera reacted with the same epitopes of Asp f 2.

Western blot. Recombinant Asp f 2 E and P reacted with IgE from ABPA sera (Fig. 2B). However, the *Pichia* expressed allergen demonstrated a weaker reaction when compared to the *E. coli* expressed protein (Fig. 2B). Asp f 2 E demonstrated two IgE reacting bands in the Western blots (Fig. 2B). One was a strong band at the 35–37 kDa region and the other was at the 72–74 kDa region (Fig. 2B). Both Asp f 2 P and Asp f 2 E also reacted with anti-Asp f 2 monoclonal antibody IL-B8 in Western blots; Asp f 2 E showed strong reaction compared to Asp f 2 P (Fig. 2C). Similar to the reactivity with ABPA sera in Western blot, Asp f 2 E showed two bands reacting with the monoclonal antibody (Fig. 3C). This suggests the possibility of the respective protein existing as a dimer or as a result of aggregation.

The immunochemical characteristics of Asp f 2 fragments. The SDS profile of the five fragments of Asp f 2 E cloned and expressed are shown in Fig. 6. The truncated Asp f 2 proteins demonstrated molecular sizes of 20 to 37 kDa. The nucleotide sequence analyses of the five cDNA constructs agreed with the expected sequences. There were differences in the expected molecular weight calculated from the amino acid residues and the size determined from SDS–PAGE. All bands stained with Coomassie blue, but not with PAS stain.

The reactivity of rAsp f 2 E fragments against sera from humans and monoclonal antibodies. The deletion fragments with varying number of IgE binding regions expressed in *E. coli* (E-1 to E-5) demonstrated

slightly decreased reactivity with ABPA sera compared to Asp f 2 E when studied by ELISA (Fig. 7A). The fragment (E-5) with two glycosylation sites and six of nine IgE binding regions but no N- and C-terminal ends failed to react with sera from ABPA patients. Similarly, E-3 lacking N-terminal end and with three glycosylation sites and four IgE binding regions also failed to react with ABPA sera (Fig. 7A). Among the five fragments studied, three fragments, namely E-1, E-2, and E-4, reacted significantly ($P < 0.05$) with ABPA sera. The reactivity of the fragment E-2 was comparable to Asp f 2 E IgE binding to ABPA sera (Fig. 7A). However, sera from *Aspergillus* skin test positive asthmatics failed to react with any of these allergens. The lack of IgE or IgG response against Asp f 2 and the deletion mutants may be due to the fact that the patients were tested with crude *Aspergillus* antigen, which may lack Asp f 2 or no apparent Asp f 2 response in asthmatics. The other possibility is that the skin test is considered a more sensitive indicator than the *in vitro* tests, which detects only high levels of antibody. Among the Asp f 2 E fragments, only E-4 showed significantly enhanced IgG in the sera from patients with ABPA compared to asthmatics and normal controls (Fig. 7B). However, no significant IgG reactivity was detected with remaining Asp f 2 fragments tested against any of the sera studied.

Asp f 2 E and fragments E-2 and E-4 reacted with IgE in the sera of ABPA by Western blot (Fig. 6B). E-1 and E-3 showed very weak reaction in Western blot, while E-5 was negative. The result indicates that for the binding of IgE antibody to the allergen either the “C” terminal or “N” terminal containing relevant IgE binding region is essential. Mutants lacking both the “C” and “N” terminal failed to react with IgE antibody in the sera of ABPA patients by both ELISA and Western blot.

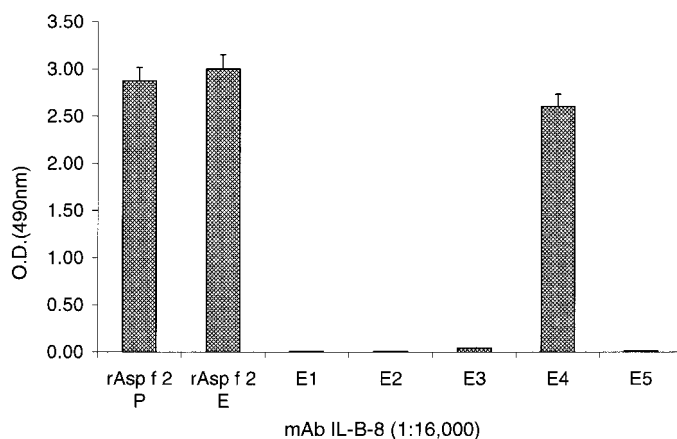


FIG. 4. IgG₁ binding activity of rAsp f 2 P, rAsp f 2 E, and fragments against mouse mAb (IL-B8) by ELISA.

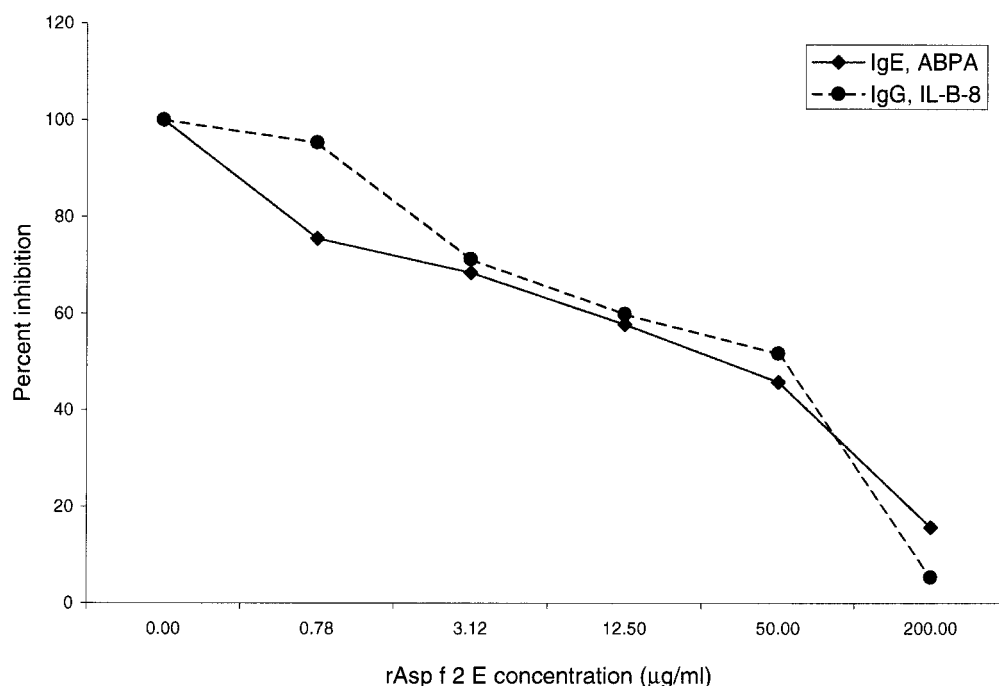


FIG. 5. Inhibition of Asp f 2 P specific IgE and IgG₁ by Asp f 2 E. Solid-phase was coated with rAsp f 2 P. ABPA patient's serum and mouse monoclonal antibody were preincubated overnight with different concentrations of rAsp f 2 E. The preincubated serum samples were transferred to rAsp f 2 P coated wells and antibody binding was studied by ELISA. The inhibition of titer was calculated. The titer of serum pre-incubated without antigen was taken as 100% binding.

Reactivity of Asp f 2 fragments with monoclonal antibody. Although Asp f 2 E reacted strongly with the monoclonal antibody IL-B8 by ELISA, only one frag-

ment, Asp f 2 E-4, showed strong reactivity with the monoclonal antibody (Fig. 4). The binding was comparable to that obtained with Asp f 2 E, while none of the

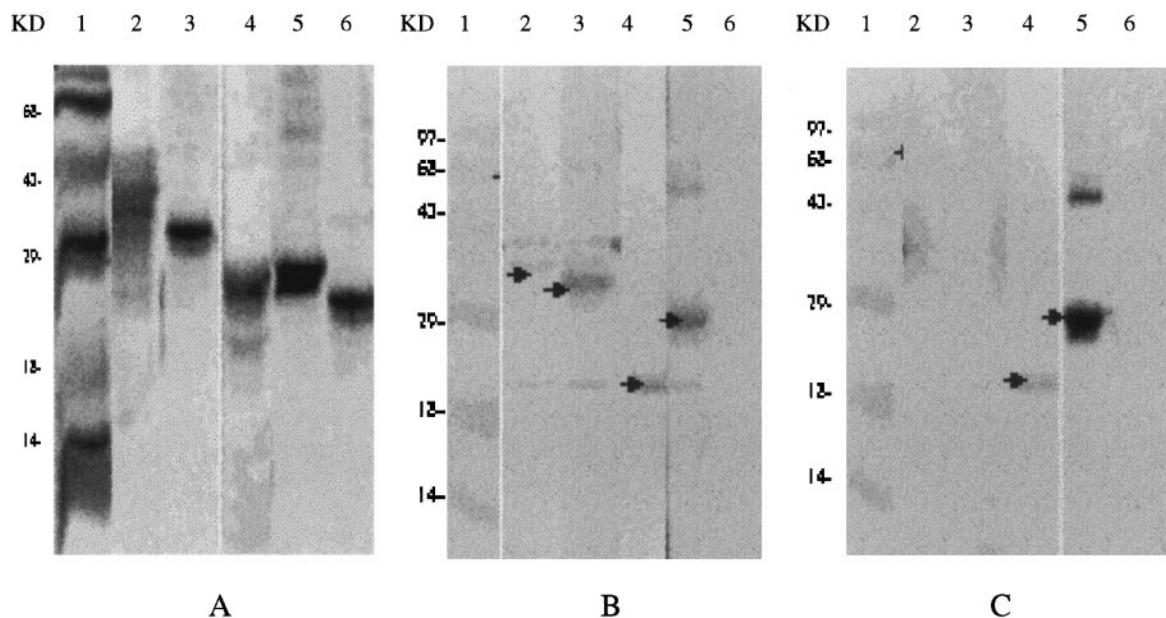


FIG. 6. SDS-PAGE and Western blots of rAsp f 2 E fragments. (A) SDS-PAGE analysis of purified rAsp f 2 E fragments. Five micrograms of purified proteins were subjected to electrophoresis, and the gels were stained with Coomassie brilliant blue R250. (B) Western blot analysis of rAsp f 2 E fragments using pooled sera from ABPA patients. (C) Western blot analysis of rAsp f 2 E fragments using mouse mAb. Lane 1: molecular weight standard; Lanes 2-6: rAsp f 2 E fragments, E-1, E-2, E-3, E-4, and E-5.

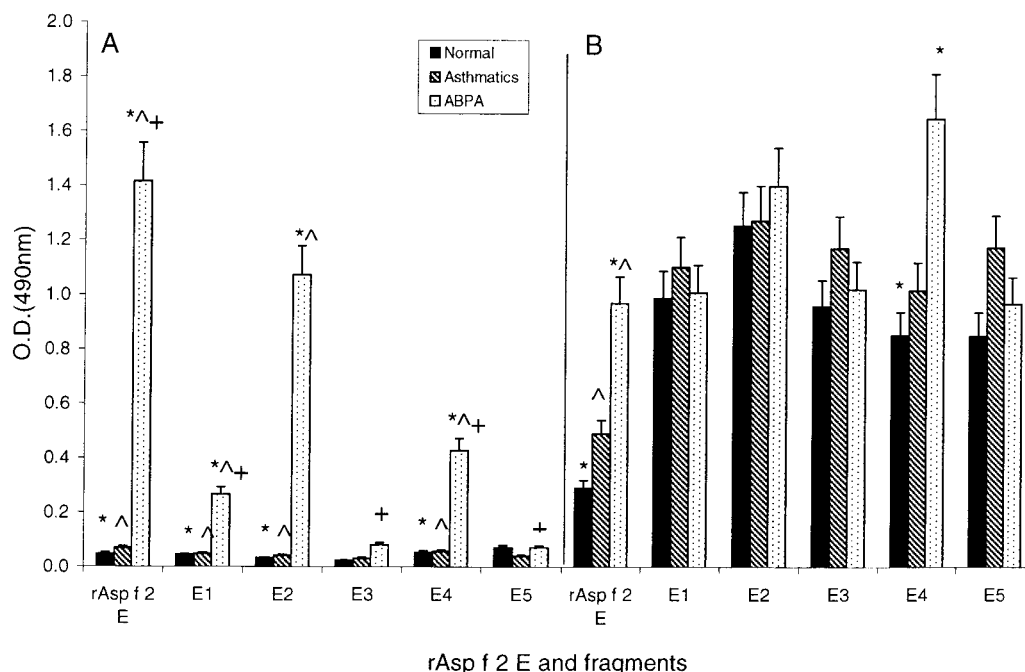


FIG. 7. IgE (A) and IgG (B) binding of rAsp f 2 E and five fragments using 10 serum samples each from ABPA, *A. fumigatus* skin test positive asthmatics, and normal control groups by ELISA. The significance between different groups of patient is indicated by the symbols “*” for normal vs ABPA, “^” for asthmatic vs ABPA, and “+” for significant reactivity among different antigen fragments.

other fragments showed any binding to IL-B8. In Western blot both E-3 and E-4 showed reactivity, although the former reacted weakly (Fig. 6C).

DISCUSSION

The two expression systems used in the present study yielded recombinant Asp f 2 allergens with several significant differences. The molecular size of the *E. coli* expressed Asp f 2 was almost half the size that of the *Pichia* expressed glycoprotein. The reactivity of ABPA serum IgE was much stronger with Asp f 2 E when compared to Asp f 2 P, both by ELISA and Western blot, while the reverse was true with the IgG antibody in the ABPA sera. The results indicate that the IgE binding to glycosylated Asp f 2 was weaker when compared to unglycosylated antigen expressed in *E. coli*. Similar results have been reported with several other allergens (18, 19). Only one Asp f 2 fragment, namely E-4, reacted significantly with ABPA serum IgG, while 3/5 fragments, E-1, E-2, and E-4, reacted with IgE in the sera of patients with ABPA evaluated by ELISA. Since glycosylation leads to a lesser binding of IgE than with Asp f 2 E, it can be concluded that no IgE binding epitopes are present in the carbohydrate portion of the glycosylated Asp f 2. Similar results have also been reported with other allergens including *Aspergillus fumigatus* allergen gp55 (20–23). The cross-reactive carbohydrates may at times result in discrep-

ant *in vitro* IgE and skin test results and such findings may lead to false interpretation (24).

The sizes of rAsp f 2 expressed in the two vector systems differed and this difference could be attributed to the glycosylation of the protein. The molecular sizes of the glycosylated and non-glycosylated Asp f 2 were 72 kDa and 37 kDa respectively. The glycosylation was confirmed by staining with PAS. Currently, there is no information available regarding the site and type of glycosylation of this antigen. Further studies are needed to understand the nature of the carbohydrate content and the role of glycosylation in the overall immune responses in ABPA patients, as significant pathophysiological role for glycosylated protein antigen have been reported (24, 25).

In a previous study, we have demonstrated nine IgE binding sites on the Asp f 2 molecule (3). The reactivity of various deletion mutants indicates that either the “C” or “N” terminal is essential for significant binding of IgE from patients with ABPA. Asp f 2 E-1 (aa 30–268) showed weaker reactions compared to Asp f 2 E-2 (aa 68–268) with two N-terminal epitopes lacking. This difference in their binding may be due to the conformational changes resulting from the N-terminal rearrangements of secondary structures of the fragments. Mutant Asp f 2 E-4 (aa 1–203) also showed strong binding to serum IgE from ABPA patients suggesting that intact N-terminal is essential for retaining the IgE binding conformational structure.

It is conceivable from the results of the ELISA using various Asp f 2 mutants that epitopes 1 and 2 from the N-terminal end and epitope 9 from the C-terminal are not essential for IgE binding. However, when epitopes 3–8 are expressed without the C- or N-terminal end, no reactivity was detected, indicating the need of intact N- or C-terminal end for strong binding to antibody. Similarly, epitopes 5 to 9 with the C-terminal end also failed to bind to IgE or IgG in the sera of ABPA. The observation is consistent with epitopes 3 and 4 representing major antibody binding region of Asp f 2. Based on the reactivity of the monoclonal antibody to the various fragments, it is likely that the N-terminal region encompasses the epitope binding site for the monoclonal antibody. From the reactivity of the various Asp f 2 fragments with ABPA serum IgE and monoclonal antibody and from the cross-inhibition studies, it can be concluded that epitopes 3 and 4 are involved in the binding of human IgE and mouse monoclonal antibody.

In conclusion, the glycosylated recombinant Asp f 2 reacted weakly with IgE from ABPA patients, but showed strong IgG reactivity, while rAsp f 2 expressed in *E. coli* showed strong IgE binding and comparatively less IgG binding. Epitope mapping studies indicate that the monoclonal antibody IL-B8 binds specifically to N-terminal epitopes of Asp f 2 similar to IgE in the sera from patients with ABPA. Understanding the specific reactivity of the epitopes may be useful in devising fragments, which may be more specific in immunodiagnosis and may be of value in specific immunotherapy and in devising vaccines.

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