

Original article

Molecular and immunological characterization of Asp f 34, a novel major cell wall allergen of *Aspergillus fumigatus*

Background: Although fungal spores have been recognized as triggers of respiratory allergy and asthma, only two allergenic fungal cell wall components have so far been described.

Methods: Eighty-one sequences derived from an *Aspergillus fumigatus* cDNA library encoding putative allergens were examined for the presence of cell wall components. A new allergen (Asp f 34) was evaluated by Western blots, enzyme-linked immunosorbent assay (ELISA), peripheral blood mononuclear cell (PBMC) proliferation assays, and skin prick test (SPT).

Results: The cDNA encoding Asp f 34 contained an open reading frame predicting a protein of 185 amino acids with a molecular weight of 19.38 kDa, showing sequence homology to phiA, an essential protein for the formation of conidia in the genus *Aspergillus*. The recombinant Asp f 34 was binding IgE from sensitized individuals in Western blots. An ELISA survey showed that 94% of the ABPA and 46% of the *A. fumigatus*-sensitized individuals tested had Asp f 34-specific serum IgE. Asp f 34 induced allergen-specific proliferation exclusively of PBMCs from patients sensitized to the allergen. Eight patients with anti-Asp f 34 serum IgE tested reacted positively in SPT, whereas four *A. fumigatus*-sensitized individuals without Asp f 34-specific IgE and eight healthy controls scored negatively.

Conclusions: A cell wall protein of the phialides of *A. fumigatus* was identified as a major allergen. Asp f 34 belongs to the *Aspergillus*-specific proteins of the phiA family and has relevant potential for a specific diagnosis of *Aspergillus* sensitization.

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Indoor and outdoor exposure to fungal spores is a recognized triggering factor for respiratory allergy and asthma (1). Among the over 100 000 fungal species reported (2), only a few hundred have been described as opportunistic pathogens (3) causing human illness through three specific mechanisms: elicitation of harmful immune responses, direct infection of the host, and toxic effects due to secondary metabolites (4). Although the exact prevalence of fungal sensitization is still unknown (5), a large skin-prick test (SPT) survey on a cohort of subjects with respiratory diseases with extracts from *Alternaria*, *Aspergillus*, *Candida*, *Cladosporium*, *Penicillium*, *Saccharomyces*, and *Trichophyton*, the most common fungi involved in allergy, indicates a prevalence of sensitization around 19% (6). However, the huge variability in skin test surveys performed on the general population (5) is mainly related to the large variation in the allergen content of standardized, even commercially available fungal extracts (7). To circumvent the limited reliability of fungal extracts, the use of recombinant allergens has been suggested (8, 9). Beside being producible as perfectly standardized molecules, recombinant

allergens allow a component resolved diagnosis of sensitization delivering patient-specific sensitization profiles (10), detailed studies on the mechanisms underlying allergic reactions (11), and extended studies on cross-reactivity (12). Without doubt, diagnosis of sensitization based on recombinant allergens is more specific than those based on fungal extracts (10, 13), as shown in serology and skin test studies (8). The unsatisfactory quality of the fungal extracts available has strongly stimulated cloning, production, and characterization of fungal allergens. The official list of allergens (<http://www.allergen.org>) reports 22 allergens isolated from five species of the Basidiomycota, and 76 allergens from 18 species of the Ascomycota kingdoms. However, much more fungal allergens have been described as summarized in a recent imperative review (9). At a first glance, the list of fungal allergens contains a wide variety of different molecular structures including enzymes, intracellular, and secreted proteins (14). Interestingly, the major allergens of *Alternaria alternata*, *Aspergillus fumigatus*, and *Malassezia sympodialis* Alt a 1 (15), Asp f 1 (16), and Mala s 1 (17), respectively, represent species-specific

proteins without orthologous allergens among the fungal kingdom (18). Other allergens like MnSOD (19), cyclophilin (20), thioredoxin (21), or enolase (22), are members of phylogenetically highly conserved pan-allergen families. It is notable, however, that among the long list of fungal allergens, only two represent cell wall components which are expected to be the first structures of the fungal spores encountering the immune system: a minor allergen of *Cladosporium herbarum* (23), and Mala s 1, a major allergen of *M. sympodialis* (17). Therefore, we re-examined all allergen sequences derived from a high throughput screening of an *A. fumigatus* cDNA library displayed on phage surface (24) for the presence of putative cell wall allergens.

Here, we describe cloning, production, molecular and immunological characterization of Asp f 34, a major allergen of *A. fumigatus*, which belongs to the class of surface expressed cell wall proteins essential for phialide development and sporulation in filamentous Ascomycetes (25).

Materials and methods

Cloning, production, and purification of Asp f 34

Asp f 34 cDNA was amplified by standard PCR from clone U1c01 isolated by high throughput screening of a phage surface displayed *A. fumigatus* cDNA library (24) using the following primers: 5'-cgc gga tcc atg cag atc aag agc ttc gtc-3', and 3'-primer: 5'-ccc aag ctt act ggg tgt aga cac agc tg-3'. After *Bam*HI/*Hind*III restriction, the amplicon was ligated into likewise restricted pQE30 high-level expression vector (Qiagen, Hilden, Germany) and transformed into *Escherichia coli* M15/pREP4 electrocompetent cells (26). Inserts of single transformants were verified by DNA sequencing. The novel allergen was formally termed Asp f 34 according to the recommendations of the International Allergen Nomenclature Committee (<http://www.allergen.org>) and the sequence deposited at National Center of Biotechnology Information database (accession number AM496018).

For the production of [His]₆-tagged recombinant Asp f 34 protein, *E. coli* cells carrying the correct insert were grown at 37°C in 2xYT medium to an OD₅₅₀ of 0.6, induced with 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG), further incubated for 6 h in a shaking culture (37°C, 220 rpm), and harvested by centrifugation at 6000 g for 15 min at 4°C. Cell pellet was resuspended in 7 M guanidine hydrochloride, 100 mM NaH₂PO₄, 10 mM Tris pH 8.0, and lysed by four cycles of freezing and thawing. Insoluble material was removed by centrifugation (48 000 g, 4°C, 20 min).

His-tagged Asp f 34 was purified by nickel-affinity chromatography using an ÄKTAbasic system with a HisTrap FF column (GE Healthcare Life science, Uppsala, Sweden) under denaturing conditions (8 M urea, 100 mM NaH₂PO₄, 10 mM Tris pH 8.0, 10 mM imidazole). Bound protein was eluted according to the supplier's recommendation. After dialysis against distilled water, protein concentrations were determined spectrophotometrically using an ε₂₈₀ absorption coefficients of 27 180 /M/cm calculated from the predicted amino acid sequence (27). Correct size and purity of the recombinant protein preparations were tested by SDS-PAGE (NuPage, 12% Bis-Tris, Invitrogen Ltd, Paisley, UK) under denaturing, reducing conditions as described elsewhere (20, 21). The LPS

content was determined by QCL-1000 endotoxin test (Cambrex Bio Science, Walkersville, MD, USA).

Immunoblots, IgE immunoassays, and inhibition ELISA

For Western blot analysis, 0.5 µg of recombinant protein was subjected to SDS-PAGE (NuPage, 12% Bis-Tris, Invitrogen) and electrotransferred onto a Hybond PVDF membrane (GE Healthcare) at 30 V for 1 h and processed as described (23). Bands were visualized with ECL Western Blotting Detection Reagents by exposing the membrane to Amersham Hyperfilm ECL (GE Healthcare).

Asp f 34-specific serum IgE was determined by a standard direct solid-phase enzyme-linked immunosorbent assay (ELISA) using Maxisorp™ polystyrene microtiter plates (Nunc, Roskilde, Denmark) coated and processed as described (16). Results were expressed as ELISA units per milliliter (EU/ml) calibrated against absorbency (OD_{405 nm}) of an in-house reference standard arbitrarily defined as 100 EU/ml (16). The cut-off level for the background value was set at 15 EU/ml which corresponds to three times the mean EU/ml values of the healthy control group.

For inhibition ELISA, coating and blocking were performed as for standard ELISA. In a separate blocked plate, antigens were serially diluted 1 : 3 in blocking buffer starting with a concentration of 15 µg/ml and incubated with 1 : 20 diluted serum pool for 1 h at 37°C. The preincubated sera were transferred to Asp f 34 or *A. fumigatus* extract-coated plates and incubated for 1 h at 37°C. Residual IgE binding capacity was determined as described above. Relative inhibition was calculated from the absorbency values of samples where serum was preincubated in blocking buffer without antigen in the liquid phase.

Proliferation assay of peripheral blood mononuclear cells

Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized peripheral venous blood samples by Ficoll density gradient centrifugation, washed three times in phosphate-buffered saline and resuspended in RPMI 1640 medium supplemented with 1 mM sodium pyruvate, 2 mM L-glutamine, 1% MEM non-essential amino acids and vitamins, 100 µg/ml streptomycin, 100 U/ml penicillin, 100 µg/ml kanamycin (all from Invitrogen, Switzerland), and 10% heat-inactivated fetal calf serum (BioConcept, Switzerland). Aliquots thereof (10⁶ cells/ml) were stimulated in triplicate with serial dilutions of Asp f 34, starting with 1 µM for 6 days in humidified atmosphere with 5% CO₂. Proliferation was measured as tritiated thymidine incorporation (Hartmann Analytics, Braunschweig, Germany) during the final 8 h of culture (28).

Subjects, routine assessment, and skin prick tests

Sera from 64 asthmatic patients with ABPA and 24 *A. fumigatus*-sensitized asthmatic patients without ABPA were analyzed together with sera from 32 allergic patients without *A. fumigatus*-sensitization, and 32 healthy controls. The *A. fumigatus*-sensitized asthmatics had a history of sensitization to the fungus and ABPA was excluded according to the clinical history. Patients suffering from ABPA fulfilled at least six of the seven criteria required to diagnose the disease (29). Allergic patients not sensitized to *A. fumigatus* had positive immediate SPT-reactivity to at least one of the following allergen panel: house dust mite, cat, dog, grass, or birch pollen but a negative SPT to *A. fumigatus* extract. The healthy controls had no history of allergy or asthma and normal IgE levels.

Total serum IgE and allergen-specific IgE to *A. fumigatus* extract (m3) were quantitatively determined using the ImmunoCAP system (Phadia, Uppsala, Sweden).

SPT with recombinant Asp f 34 was performed using a solution of 100 µg/ml in PBS as described (8, 16). The study was carried out according to a clinical protocol approved by the local ethical committee and all participants gave written informed consent after a full explanation of the procedure given individually before testing.

Results

Molecular cloning and production of Asp f 34

Screening of *A. fumigatus* cDNA libraries displayed on phage surface with IgE from *A. fumigatus*-sensitized patients yielded 81 different clones carrying distinct inserts encoding putative IgE-binding proteins (24). Among these, one clone coded for a cDNA sharing high sequence identity with a PhiA protein of *Aspergillus nidulans* (25). This cDNA contained an open reading frame of 555 bp, predicting a protein of 185 aa (Fig. 1) with a molecular mass of 19.38 kDa and a calculated pI value of 5.20. The allergen formally termed Asp f 34 was

cloned into the high-level expression vector pQE30, produced as [His]₆-tagged recombinant protein, and purified by Ni-NTA affinity chromatography. The protein yield varied from 10 to 25 mg/l depending on the production batch. The purified recombinant protein was free of contaminants as shown by SDS-PAGE and Coomassie blue staining. The apparent molecular mass for [His]₆-tagged Asp f 34 was in good agreement with the calculated value of 19.38 kDa (Fig. 2). All recombinant protein preparations were tested for LPS contaminations and the values were <0.1 ng/µg protein.

Allergenicity of Asp f 34 *in vitro*

The IgE-binding capacity of recombinant Asp f 34 was first assessed by Western blot analysis (Fig. 2). Binding of IgE was confirmed and surveyed by standard ELISA (Fig. 3). Signals were assigned positive when the value of Asp f 34-specific IgE was >15 EU/ml. Sixty out of 64 of the APBA patients (94%) reacted positively to Asp f 34, whereas 11 out of 24 (46%) *A. fumigatus*-sensitized patients without ABPA recognized the allergen. The 32 allergic individuals without *A. fumigatus*-specific IgE and

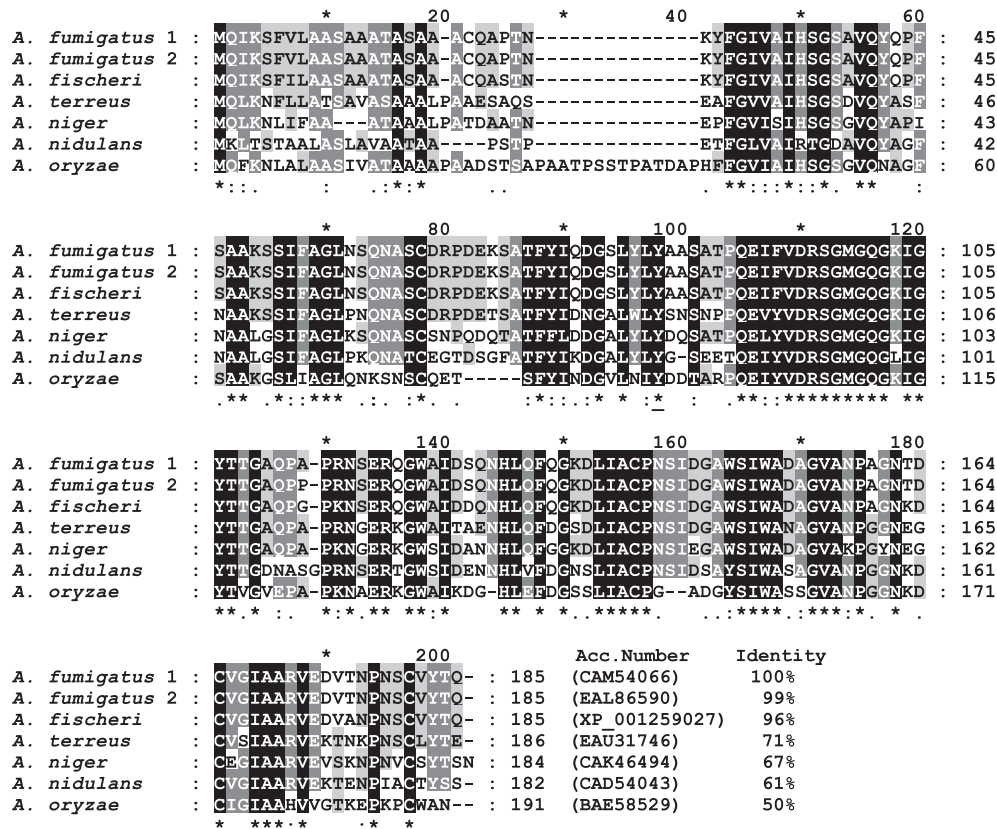


Figure 1. Amino acid sequence alignment of Asp f 34 (first line) with the sequences of homologous phiA proteins which belongs to a large family of cell wall proteins of the genus *Aspergillus*. Identical amino acids in all sequences are shown on black background. Eighty percent and 50% conserved amino acids are highlighted in dark and light grey, respectively. Gene Bank accession numbers (in parentheses) and % identity in relation to Asp f 34 are given at the end of the sequences.

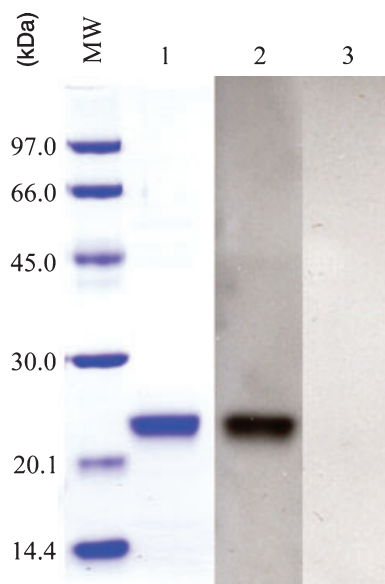


Figure 2. SDS-PAGE and Western blot analysis of Asp f 34. Molecular weights of the standards (kDa) are indicated on the left site. Line 1 shows the purity of Asp f 34 (2 µg) after separation by SDS/PAGE and Coomassie Blue staining. Lane 2 shows the specific IgE-binding of the recombinant *A. fumigatus* allergens Asp f 34 (0.5 µg) analyzed by Western blotting using a serum pool from individuals sensitized to *A. fumigatus*. In lane 3, 0.5 µg of Asp f 34 were separated by SDS-PAGE, blotted, and probed for IgE-binding with serum of healthy individuals as a negative control.

the 32 healthy individuals all showed values below the cut-off value of the ELISA (15 EU/ml), demonstrating that Asp f 34 is a specific allergen for *A. fumigatus*-sensitized individuals.

IgE binding of positive to *A. fumigatus* Asp f 34 could be inhibited in a dose-dependent manner by preincubation with increasing amounts of Asp f 34 (Fig. 4A). Due to the great number of allergens produced by *A. fumigatus*, IgE binding to solid-phase coated extract with Asp f 34 in fluid phase was in the range of 20% (Fig. 4B), as observed also for other fungal allergens (30). BSA used as negative control does not show any inhibition.

Primary proliferative response of PBMCs

As allergen-specific IgE production is dependent on T cell help, we measured the proliferative responses of PBMCs from individuals sensitized to Asp f 34. Five ABPA patients and two *A. fumigatus* allergic patients with detectable Asp f 34-specific IgE had positive proliferative responses to the allergen (Fig. 5) as well as to *A. fumigatus* extract. In contrast, two *A. fumigatus*-sensitized asthmatics lacking detectable IgE antibodies against Asp f 34 responded to the fungal extract only, indicating that the recombinant allergen did not induce nonspecific effects. In additional experiments, the mean proliferative responses to Asp f 34 of four allergic individuals not

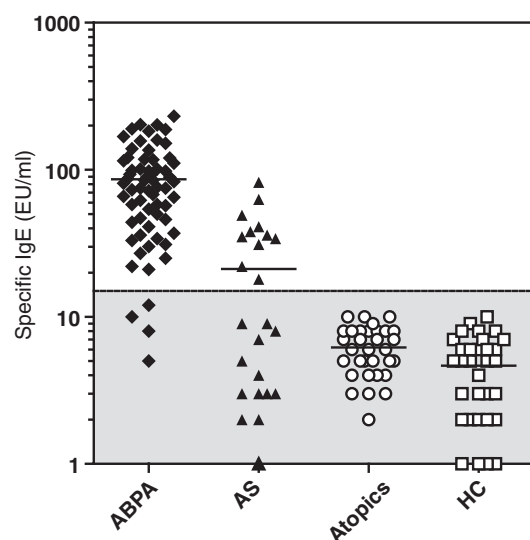


Figure 3. IgE-binding to Asp f 34 in an antigen-specific ELISA calibrated against the binding to a reference serum pool arbitrarily assigned to 100 EU/ml (16). Sixty-four patients with ABPA (◆), 24 patients sensitized to *A. fumigatus* without ABPA (▲), 32 atopic individuals without *A. fumigatus* sensitization, (○) and 32 healthy controls (□) were analyzed. Mean values are indicated by lines. The grey area represents the cut-off value (15 EU/ml) defined as the threefold value of the mean EU/ml value of the healthy control group. The reliability of the cut-off value was confirmed by skin prick tests (Table 1).

sensitized to *A. fumigatus* and eight healthy controls were determined (Fig. 5). All showed a stimulation index below three which is considered relevant for an allergen-specific proliferation.

Skin-test reactivity to Asp f 34

Although IgE-binding, inhibition ELISA, and allergen-specific proliferation of PBMCs are suitable *in vitro* methods to show the allergenicity of a protein, the biological relevance of an allergen can only be shown *in vivo*. We have performed SPT to demonstrate that Asp f 34 is able to cross-link allergen-specific IgE on the surface of effector cells. Six *A. fumigatus*-sensitized patients with and six without ABPA were tested together with eight healthy controls. All individuals with ABPA had specific IgE to *A. fumigatus* extract and Asp f 34-specific serum IgE levels > 15 EU/ml and reacted to both, fungal extract and Asp f 34 skin challenge (Table 1). Only two *A. fumigatus*-sensitized individuals without ABPA had Asp f 34-specific serum IgE levels > 15 EU/ml and reacted positively in SPT. The remaining four *A. fumigatus*-sensitized patients reacted only to *A. fumigatus* extract. They had, like the healthy controls, Asp f 34-specific serum IgE levels < 15 EU/ml and scored negatively in SPT with the recombinant allergen (Table 1).

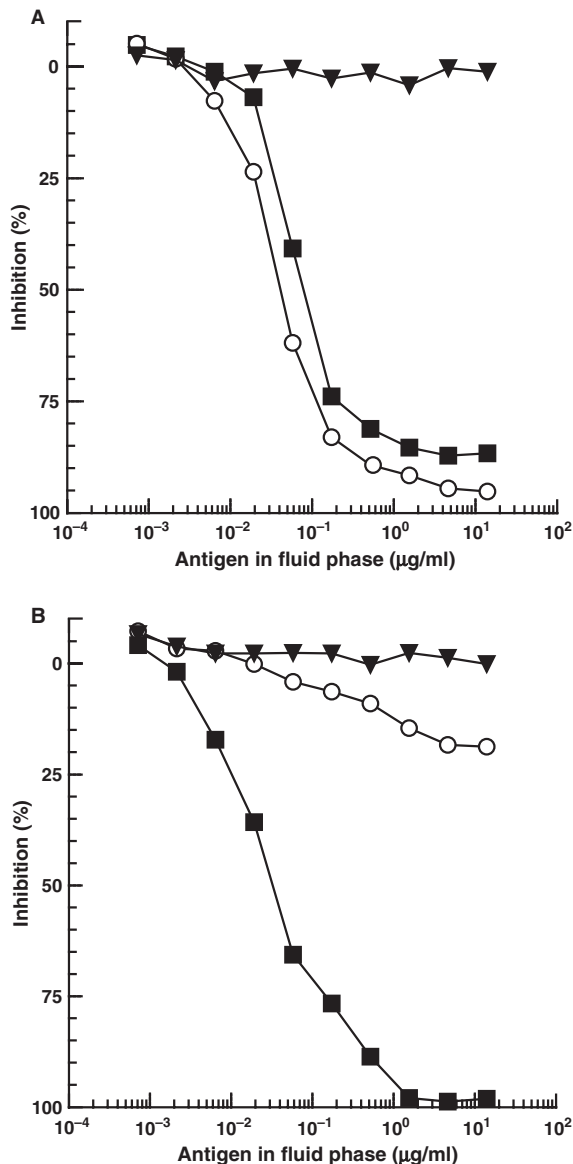


Figure 4. Competitive inhibition of IgE-binding to recombinant Asp f 34 and *A. fumigatus* extract coated on the solid phase. Serum from Asp f 34-sensitized patients was preincubated with increasing amounts of recombinant Asp f 34 (○), *A. fumigatus* extract (■), or BSA as a negative control (▼). Preincubated serum samples were transferred to wells coated with Asp f 34 (A) or *A. fumigatus* extract (B), and residual IgE-binding (% of the control measurement without inhibitor) was analyzed by ELISA.

Discussion

Fungal allergy, although recognized as a relevant problem, is still poorly defined (5). The disease is caused by allergens present in spores, mycelial fragments, and whole fungal extracts (9). They cover a wide variety of molecular structures including secreted and intracellular proteins. Some of the allergens are highly species-specific like Asp f 1 or Alt a 1, the major allergens of *A. fumigatus* and

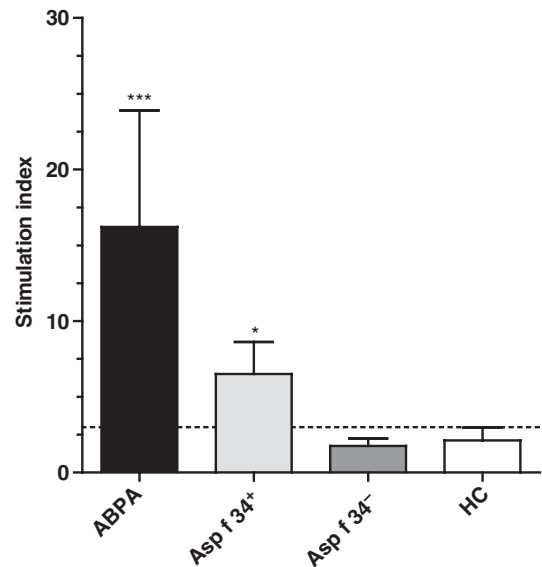


Figure 5. Proliferative responses of PBMCs from five patients with ABPA and two *A. fumigatus* allergics sensitized to Asp f 34 (Asp f 34⁺), four *A. fumigatus* sensitized individuals without Asp f 34-specific IgE (Asp f 34⁻) and eight healthy controls induced by recombinant rAsp f 34. Proliferation was measured by incorporation of tritiated thymidine (cpm) during the last 8 h of culture and converted to stimulation indices as described (28). Each bar represents the mean of the respective group $\pm$ SD. Significantly higher proliferation was observed in Asp f 34-sensitized patients with (***) $P > 0.001$ and without (*) $P > 0.05$ ABPA compared to healthy controls and *A. fumigatus*-sensitized individuals without Asp f 34-specific serum IgE. The dotted line represents the cut-off value of > 3 SI which is considered to be below the level of relevance.

A. alternaria, but most of the fungal allergens belong to the class of phylogenetically highly conserved proteins (12, 19–22). At present, only two genuine cell wall proteins, a minor allergen of *C. herbarum* (23), and Mala s 1, a major allergen of *M. sympodialis* (17), have been described. This is surprising because spores and fungal fragments represent the major source of exposure to fungi (1) and, therefore, it would be expected that cell wall structures are the first immunogenic fungal components encountered by the immune system. To identify cell wall-associated fungal allergens, we have re-examined all sequences derived from a high throughput screening of an *A. fumigatus* cDNA library expressed on phage surface. Among the 81 identified sequences encoding potential IgE-binding proteins identified, all described 22 *A. fumigatus* allergens were present, together with 59 additional sequences encoding proteins not yet characterized for their allergenicity (24). Out of this pool, one sequence is reported in the allergen database (<http://www.allergen.org>) as Asp f 17 and shows homology to a cell wall antigen of *Penicillium marneffei*. One clone shows homology to a spore coat precursor protein of *Neurospora crassa*, whereas two other clones are

Table 1. Induction of immediate skin reactions by Asp f 34 in ABPA patients, *Aspergillus fumigatus*-sensitized allergics without ABPA (AS), and healthy control individuals (HC)

Patient	Diagnosis	Sex*	Age (y)	Total IgE (kU/ml)	m3† IgE (kU/ml)	SPT‡			IgE§		IgE¶		
						Asp f 34	Asp f extract	Histamin	Asp f 34	Asp f 1	Asp f 3	Asp f 4	Asp f 6
1	ABPA	M	57	780	43.5	36	59	60	44	58	184	25	196
2	ABPA	M	59	3315	23.1	36	39	81	73	15	398	11	29
3	ABPA	M	55	1887	58.4	25	30	30	27	4	351	74	165
4	ABPA	F	48	1150	15.6	64	33	49	47	10	248	87	2
5	ABPA	M	49	1641	14.7	64	72	49	41	76	277	23	49
6	ABPA	F	37	1132	53.0	182	156	53	76	229	237	89	0
Median			52	1395.5	33.5	50	49	51	45.5	36.5	262.5	44.5	36
7	AS	M	55	471	1.06	0	9	56	9	7	9	6	4
8	AS	F	47	846	96	49	56	64	38	9	168	8	4
9	AS	F	37	366	1.94	0	12	20	8	8	7	4	7
10	AS	F	41	140	0.71	0	10	25	9	8	6	5	5
11	AS	F	55	47	14.7	0	49	64	3	12	56	5	6
12	AS	M	34	315	28	81	53	100	63	8	232	5	1
Median			44	340.5	8.32	0	32.5	60	9	9	32.5	5	4.5
13	HC	F	25	32	<0.35	0	0	64	8	8	9	7	0
14	HC	F	43	104	<0.35	0	0	16	3	5	2	3	3
15	HC	F	45	223	<0.35	0	0	56	5	9	6	7	4
16	HC	M	54	163	<0.35	0	0	25	1	5	1	2	6
17	HC	M	67	64	<0.35	0	0	90	2	9	3	6	3
18	HC	M	68	305	<0.35	0	0	90	3	1	2	0	0
19	HC	F	32	133	<0.35	0	0	64	2	4	7	4	0
20	HC	F	32	92	<0.35	0	0	90	1	0	3	1	1
Median			44	98	<0.35	0	0	64	2.5	5	3	3.5	2

*M, male; F, female.

†*A. fumigatus* ImmunoCAPm3 (Phadia AB, Uppsala, Sweden).‡SPT values are given in mm² calculated according to the formula [(D1 + D2)/2]² [14].

§, ¶ IgE to Asp f 34, Asp f 1, Asp f 3, Asp f 4, and Asp f 6 (EU/ml). The cut-off value for Asp f 34 was set to three times the mean of 32 healthy controls (15 EU/ml).

homologous to the PIR1 cell wall protein of *S. cerevisiae* and to a cell wall protein of *Schizosaccharomyces pombe*, respectively. One clone encodes a complete sequence for a protein with homology to a cell wall protein of *Aspergillus nidulans*. This sequence was subcloned, expressed as recombinant protein and evaluated for allergenicity *in vitro* and *in vivo*. As shown in Fig. 1 the protein termed Asp f 34 displays a high degree of sequence identity (50–96%) to PhiA cell wall proteins of the genus *Aspergillus* (25). PhiA is essential for the correct formation of phyalides and conidia and is exposed to the surface of the cell wall. Therefore, the protein is likely to be among the first antigens getting in contact with the immune system after inhalation of fungal spores. In view of the high degree of sequence identity between the homologous Phi A proteins reported in Table 1, cross-reactivity between this protein family has to be expected. However, because Asp f 34 homologous proteins are found only among the genus *Aspergillus* (25), it is not expected that sensitization to this particular allergen will affect allergic individuals sensitized to different mold species. The IgE-binding capability of Asp f 34 was first demonstrated by Western blot analysis (Fig. 2). A serological evaluation of *A. fumigatus*-sensitized individuals by ELISA showed that 94% of the ABPA patients and

46% of the *A. fumigatus*-sensitized allergics without ABPA had detectable serum IgE against Asp f 34 (Fig. 3). Therefore, Asp f 34 can be considered as a major allergen of *A. fumigatus*. IgE-binding to solid phase coated Asp f 34 showed a concentration-dependent inhibition by addition of Asp f 34 in the fluid phase and partially by the addition of *A. fumigatus* extract (Fig. 4). Because *A. fumigatus* produces many allergens (24) and variable patient-specific sensitization patterns (31), it cannot be expected that a single allergen can completely inhibit IgE-binding to the extract. However, the complete inhibition of IgE binding to Asp f 34 by itself demonstrates a highly specific interaction, and the partial inhibition of IgE-binding to extract by Asp f 34 indicates the presence of the allergen in the extract used. As expected, Asp f 34 was able to induce a strong proliferation exclusively in PBMCs of patients with detectable amounts of Asp f 34-specific serum IgE (Fig. 5) showing that the allergen did not induce nonspecific effects. Although these experiments clearly demonstrate that Asp f 34 is able to bind IgE *in vitro*, they do not provide any information about the *in vivo* biological relevance. Therefore, we investigated whether Asp f 34 elicits allergic reactions *in vivo* by skin tests (16). Six ABPA patients, six *A. fumigatus* allergic individuals

without ABPA, and eight healthy controls were tested for their ability to respond to SPT challenges with Asp f 34 and *A. fumigatus* extract (Table 1). Positive skin reactions to Asp f 34 were observed only in individuals who had detectable Asp f 34-specific serum IgE. These individuals also reacted to *A. fumigatus* extract. In contrast, all *A. fumigatus*-sensitized individuals without Asp f 34-specific IgE reacted to the extract only, whereas all healthy controls scored negative by both challenges (Table 1). Immediate skin reactions depend on mast cells degranulation and released mediators which are triggered through antigen-dependent cross-linking of FcεRI receptor-bound IgE on their surface (32). The results presented in Table 1 show that Asp f 34 is able to cross-link IgE on mast cells *in vivo* providing the demonstration that Asp f 34 acts as a biologically relevant allergen. Because *A. fumigatus* sensitization is crucial in the development of fungal-dependent asthma (33) and ABPA, a life-

threatening disease (29), the availability of Asp f 34 as a further *Aspergillus*-specific allergen will, together with the already described disease-specific *A. fumigatus* allergens (31), further contribute to improve the diagnosis of *A. fumigatus* sensitization. An early and reliable diagnosis of *A. fumigatus* sensitization in asthmatic patients, which is hardly achievable using currently available *A. fumigatus* extracts (7), is essential to detect patients at risk for the development of ABPA which, if untreated, can lead to the development of lethal pulmonary fibrosis (29).

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