

Intracutaneous Tests with Recombinant Allergens in Cystic Fibrosis Patients with Allergic Bronchopulmonary Aspergillosis and *Aspergillus* Allergy

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Allergic bronchopulmonary aspergillosis (ABPA), an intensive inflammatory reaction to *Aspergillus fumigatus*, can cause irreversible lung damage in patients with cystic fibrosis (CF). The aim of this study was to assess if intracutaneous testing with recombinant *A. fumigatus* allergens (rAsp f) allowed a reliable diagnosis of ABPA. Fifty patients with CF were tested, 12 suffering from ABPA, 21 with allergy to *A. fumigatus*, and 17 CF control patients not sensitized to *A. fumigatus*. All patients with ABPA reacted to at least one of the two intracellular *A. fumigatus* allergens rAsp f 4, a 30-kD protein of unknown biologic function, and rAsp f 6, a 23-kD manganese superoxide dismutase, at a concentration of 10^{-2} μ g/ml. The intracutaneous tests were negative or only marginally positive in the patients with allergy to *A. fumigatus* and completely negative in the CF control patients. The differential responses to the recombinant *A. fumigatus* allergens were in perfect agreement with our previous serologic results, so that rAsp f 4 and rAsp f 6 can be considered specific markers for ABPA. Early diagnosis of the disease might help to prevent irreversible lung damage and minimize possible steroid-mediated side effects as a consequence of an optimized control of the disease.

Keywords: cystic fibrosis; recombinant allergens; *Aspergillus fumigatus*; allergic bronchopulmonary aspergillosis

Allergic bronchopulmonary aspergillosis (ABPA) was first described in 1952 in adults with asthma (1) and in 1965 in two children with cystic fibrosis (CF) (2). ABPA has been recognized as a severe pulmonary complication caused by *Aspergillus fumigatus* (3). The importance of detecting specific IgE and IgG antibodies to *A. fumigatus* in establishing the diagnosis was emphasized (4). The clinical manifestation of ABPA is wheezing and chest radiographs revealing pulmonary infiltrates (5). In addition to these clinical and radiographic features, the diagnosis of ABPA is based on a positive skin test to *A. fumigatus*, the presence of specific IgE and IgG antibodies to *A. fumigatus*, elevated concentrations of total IgE, and a positive culture of *A. fumigatus* from sputum (6). Early diagnosis of ABPA is of pivotal clinical relevance, because untreated ABPA may lead to substantial lung damage such as proximal bronchiectasis or end-stage pulmonary fibrosis (7, 8). Currently, the only effective treatment is high-dose corticosteroids with or without the addition of itraconazole, bearing the

well-known risks of side effects, especially in patients with CF. In a multi-center study of patients with CF in the United States, the high dose arm (prednisone 2 mg/kg body weight on alternate days) had to be terminated early due to an increased occurrence of diabetes mellitus, cataracts, and growth retardation (9). Even at a middle dose of prednisone 1 mg/kg body weight on alternate days, growth retardation was observed beginning after 24 months of treatment (10). These data clearly confirm the necessity to reliably diagnose ABPA. However, the differentiation of full-blown ABPA from simple allergy to *A. fumigatus* is complicated by a number of characteristics shared between the two diseases. In a cross-sectional study including 105 patients with CF aged from 9 months to 18 years, the incidence of full-blown ABPA was 8.6%, whereas 30% of non-ABPA patients had positive skin prick tests to *A. fumigatus*, 23% had serum IgE antibodies to *A. fumigatus*, and 19% had precipitins to *A. fumigatus* (11). The quality of serologic tests depends mostly on the composition of the antigen preparations used. In commercial extracts, the content of *A. fumigatus* allergens and the quantity of IgE-binding components can vary considerably from batch to batch (12) and is dependent on the different raw materials used for preparation such as mycelia, spores, or culture filtrates (13). Even during culture, the expression of IgE-binding components can change rapidly (14). Attempts at standardization (14, 15) or biochemical purification (16, 17) are not satisfactory, and internationally standardized reference extracts are not yet available (18).

Recombinant allergens can be produced from cloned cDNA as very pure protein reagents with defined biologic activity and concentration suitable for diagnostic applications (19, 20). The recombinant *A. fumigatus* allergen 1 (rAsp f 1), a 16.9-kD ribotoxin, was cloned and expressed in *Escherichia coli*, purified to homogeneity, and demonstrated to be equivalent to biochemically purified Asp f 1 (21). The diagnostic value of rAsp f 1 for serologic and skin prick testing was evaluated in CF patients with ABPA or allergy to *A. fumigatus* and in control CF patients (22, 23). The serologic assays revealed a 10-fold increase in rAsp f 1-specific IgE, a 5-fold increase in rAsp f 1-specific IgG₁, and a 4-fold increase in rAsp f 1-specific IgG₄ antibodies in ABPA patients compared with *Aspergillus*-allergic patients and control patients (22), but the results show some overlap between the different patient groups. Skin prick test reactions to rAsp f 1 were 8.5-fold stronger in *A. fumigatus*-allergic patients and 3.3-fold stronger in ABPA patients compared with the CF control patients (23). However, only three out of six well-characterized ABPA patients had positive skin prick test reactions to rAsp f 1, although all patients reacted to crude *A. fumigatus* preparations. Further recombinant *A. fumigatus* allergens have been cloned, expressed, and purified (24, 25). The most important among these are rAsp f 3, a 18.5-kD peroxisomal protein, rAsp f 4, a 30-kD protein of unknown biologic function, and rAsp f 6, a 23-kD manganese superoxide dismutase. Differential IgE responses to the allergens in *A. fumigatus*-sensitized CF patients with or without ABPA and CF control patients without sensitization to *A. fumigatus*

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were demonstrated (26). rAsp f 1 and rAsp f 3 were recognized by sera from *A. fumigatus*-sensitized CF patients with or without ABPA, whereas rAsp f 4 and rAsp f 6 were recognized exclusively by IgE from sera of CF patients with ABPA. Therefore, the latter two allergens can be considered as specific markers for ABPA. It was the aim of this study to evaluate if intracutaneous testing with the recombinant allergens *A. fumigatus* allergens Asp f 1, 3, 4, and 6 allows a specific and sensitive diagnosis of ABPA in patients with CF.

METHODS

Patients

A total of 50 patients with CF, 30 males and 20 females, was entered into the study. The age range of the patients was 21.5 ± 8.4 years. The diagnosis of CF had been substantiated by the sweat iontophoresis test, with sweat sodium concentrations greater than 70 mmol/L on at least two specimens of 100 mg or more of sweat (27) and/or by the presence of two gene mutations specific for CF. All patients studied were informed verbally and in writing about the purpose and the procedure of the study including intracutaneous testing with recombinant allergens. Approval from the official local ethics committee was obtained before starting the study.

Classification of Patients

Classification into the following groups occurred according to clinical and biochemical criteria.

ABPA patients. The diagnosis of ABPA was based on a minimum of six of the seven criteria as described by Nelson and colleagues (6): (1) Wheezing, (2) new pulmonary infiltrate, (3) positive sputum culture for *A. fumigatus*, (4) positive immediate reaction to *A. fumigatus* extracts in skin prick testing (wheal ≥ 3 mm in diameter) (28), (5) increased total IgE concentrations (IgE greater than 2 standard deviations [SD] of the normal values for age) (29), (6) specific IgE to *A. fumigatus* by radioallergosorbent test (RAST) (RAST score ≥ 2), and (7) increased concentration of IgG antibodies specific to *A. fumigatus* (enzyme-linked immunosorbent assay above 40 EU/ml) (30).

Aspergillus-allergic CF patients. CF patients were assigned to the group with *Aspergillus* allergy if routine skin prick tests with commercial *A. fumigatus* extract (SmithKline Beecham, Neuss, Germany) were positive (wheal ≥ 3 mm in diameter) (28) or specific IgE to *A. fumigatus* by RAST was ≥ 2 , but the patients did not otherwise fulfill the criteria for ABPA (6).

CF control group. Patients with CF were assigned to the CF control group if routine skin prick tests with commercial *A. fumigatus* extract (SmithKline Beecham) were completely negative (no wheal and no flare) and specific IgE to *A. fumigatus* by RAST was below the detection limit (< 0.35 kU_A/L).

Routine Assessment

Each patient had the routine assessment when entering the study. Skin test reactions to *A. fumigatus* (Bencard skin test antigens, SmithKline Beecham) were recorded after 15 minutes by measuring the diameter of the wheal, and the results were considered positive with a wheal ≥ 3 mm and no reaction to the control solution (28). The radioallergosorbent test for IgE antibodies to *A. fumigatus* (Pharmacia, Uppsala, Sweden) used the Pharmacia allergen-specific CAP system. Results were converted into RAST classes from 0 to 6 as described by the manufacturer (RAST class 0, < 0.35 kU/L; class 1, 0.35–0.69 kU/L; class 2, 0.70–3.49 kU/L; class 3, 3.50–17.49 kU/L; class 4, 17.50–49.99 kU/L; class 5, 50.00–99.99 kU/L; class 6, ≥ 100 kU/L). Specific IgG antibodies to *A. fumigatus* were analyzed by enzyme-linked immunosorbent assay using standardized DPC antigens (Diagnostic Products Corporation, Los Angeles, CA) bound to solid phase (30).

Production of rAsp f

The following allergens were produced as previously described (31): rAsp f 1, a secreted 16.9-kD ribotoxin; rAsp f 3, an 18.5-kD peroxisomal protein; rAsp f 4, a 30-kD protein of unknown biologic function; and rAsp f 6, a 23-kD manganese superoxide dismutase. Briefly, a cDNA library from *A. fumigatus* was displayed on the surface of a fil-

amentous phage and screened for gene products binding to human serum IgE. The cDNA gene products were covalently linked to the surface of the phage by the strong interaction of the Jun and Fos leucine zippers, allowing a rapid and simplified screening of cDNA libraries by gene product–ligand interaction. Phages expressing IgE-binding proteins from *A. fumigatus* were selectively enriched from unspecific phages using serum IgE from *A. fumigatus*-allergic individuals, immobilized to solid phase supports (24, 25). Subsequently, the proteins were produced in *E. coli* and purified for usage in allergy testing (32). Purity of the protein preparations was assessed by sodium dodecyl sulfate gel electrophoresis and silver staining and the absence of IgE-binding contaminants shown by Western blot analysis. The four cDNA encoding *A. fumigatus* allergens yielded between 36 and 220 mg pure recombinant allergens per liter culture.

Intracutaneous Testing

The patients included in the study had intracutaneous tests to the following four rAsp f: rAsp f 1, rAsp f 3, rAsp f 4, and rAsp f 6. The recombinant allergens were used at protein concentrations of 10^{-4} , 10^{-3} , 10^{-2} , and 10^{-1} μ g/ml according to the recommendations of the European Academy of Allergology and Clinical Immunology (28). The results were recorded after 15 minutes by measuring the maximal longitudinal (D1) and transversal (D2) diameters of the wheal. The surface (S) of the wheal was calculated according to the following formula: $S = ([D1 + D2]/2)^2$. The results were considered positive if the surface of the wheal was at least half the size of the wheal of the histamine reaction. A negative control was included with saline 0.9%.

Statistics

Data were analyzed with the Systat computing system for statistics (Systat Inc., Evanston, IL). The paired Wilcoxon test was used for comparison of repeated values within the same subject and the Mann-Whitney U test for comparison of data between different subjects. Categorical data were compared by the Pearson χ^2 test with Yates' correction for small numbers. Correlation coefficients were determined using Pearson's linear regression analysis. Sensitivity and specificity were assessed by determination of the confidence intervals for binomial parameters. Differences associated with probabilities p less than 0.05 were considered significant.

RESULTS

Fifty patients with CF, 30 males and 20 females, completed the study. The mean age ± 1 SD was 21.5 ± 8.4 years. Twelve patients with CF were diagnosed as having ABPA due to exacerbation of the condition at the time of the study, eight of them with six of Nelson's criteria, and four with seven of them (6) (Table 1). Twenty-one patients with CF belonged to the group with documented allergy to *A. fumigatus* (Table 2), and 17 were assigned to the control group (Table 3). The mean Shwachman score, assessing the severity degree of the disease (33), from all patients was 71.1 ± 19.2 , indicating that the patients were moderately affected. There was no significant difference between the study groups regarding this clinical score ($p > 0.45$, Mann-Whitney U test). There was no significant difference either regarding the nutritional status or the Crispin-Norman score (34) as radiologic score. The mean vital capacity of all patients was $68.4 \pm 21.9\%$ of predicted values, the forced expiratory volume in 1 second was $52.1 \pm 23.4\%$ of predicted values. There was no significant difference between the study groups: vital capacity was $66.9 \pm 22.3\%$ in the ABPA group, $68.5 \pm 20.3\%$ in the patients with allergy to *A. fumigatus*, and 69.3% in the CF control patients. Forced expiratory volume was $49.3 \pm 19.4\%$ in the ABPA group, $50.7 \pm 20.6\%$ in the patients with allergy to *A. fumigatus*, and $55.8 \pm 29.4\%$ in the CF control patients. Altogether, 10 patients were on low-dose steroid treatment because of bronchial obstruction. Six of them belonged to the ABPA group and had prednisone at a median dose of 7.5 mg (range 2.5–10 mg); four belonged

TABLE 1. NELSON'S CRITERIA IN THE 12 CYSTIC FIBROSIS PATIENTS WITH ALLERGIC BRONCHOPULMONARY ASPERGILLOSIS

Patient	Age (yr)	Sex	Wheezing	New Pulmonary Infiltrate	Aspergillus in sputum	Aspergillus Prick (mm)	Total IgE (IU/ml)	Aspergillus RAST (0-4)	Aspergillus IgG (EU/ml)
1	20	W	+	+	-	4	861	4	81
2	13	M	+	+	+	3	1,390	3	236
3	11	M	+	+	+	4	936	4	191
4	19	M	+	+	+	3	218	3	186
5	12	W	+	+	-	4	2,390	4	321
6	29	M	+	+	+	5	354	3	258
7	19	W	+	+	-	9	671	4	100
8	12	W	+	+	-	3	4,000	4	232
9	14	W	+	+	-	4	1,860	4	172
10	11	W	+	+	-	3	1,960	4	278
11	19	M	+	+	-	3	649	4	169
12	10	W	+	+	-	4	1,313	4	151

to the group of patients with allergy to *A. fumigatus* and had prednisone at a median dose of 5.0 mg (range 5.0–15 mg).

As suggested by Nelson's criteria (6), routine skin prick test reactions to *A. fumigatus* with Bencard allergens were positive in all ABPA patients, as well as in all patients allergic to *A. fumigatus*, but were negative in all control patients (Tables 1–3). Total IgE was significantly elevated in ABPA ($1,384 \pm 1,060$ IU/ml) compared with CF control patients (38.7 ± 60.5 IU/ml, $p < 0.0001$) and *Aspergillus*-allergic patients (325.8 ± 235.6 IU/ml, $p < 0.0002$). *Aspergillus*-allergic patients had significantly higher IgE concentrations than the CF control group ($p < 0.0001$).

IgG antibodies to *A. fumigatus* were significantly elevated in ABPA (213.3 ± 74.4 EU/ml) compared with *Aspergillus*-allergic patients (132.5 ± 112.5 EU/ml, $p < 0.05$), but not with CF control patients (175.1 ± 135.4 EU/ml, $p > 0.45$). The difference between the last two groups was not statistically significant ($p > 0.33$).

The intracutaneous test reactions to rAsp f 1 were positive in the ABPA group at a concentration of 10^{-4} μ g/ml in one patient, at 10^{-3} μ g/ml in two, at 10^{-2} μ g/ml in four, and at 10^{-1} μ g/ml in one. Four ABPA patients had negative intracutaneous test results to rAsp f 1 (Figure 1).

Twelve patients with allergy to *A. fumigatus* had positive reactions at a concentration of 10^{-1} μ g/ml; eight patients showed negative test results. The test results in patients with allergy to *A. fumigatus* were significantly smaller than in patients with ABPA ($p < 0.05$, Mann-Whitney U test).

The intracutaneous test results were completely negative in the 17 CF control patients. This difference was significant compared with the ABPA group ($p < 0.0001$) and the patients with allergy to *A. fumigatus* ($p < 0.0005$).

The intracutaneous test reactions to rAsp f 3 were positive in the ABPA group at a concentration of 10^{-4} μ g/ml in one patient, at 10^{-3} μ g/ml in one, at 10^{-2} μ g/ml in four, and at 10^{-1} μ g/ml in five. One ABPA patient had negative intracutaneous test results to rAsp f 3 (Figure 2).

One patient with allergy to *A. fumigatus* had a positive reaction at a concentration of 10^{-3} μ g/ml, two at a concentration of 10^{-2} μ g/ml, and six at a concentration of 10^{-1} μ g/ml, and eight patients showed negative test results. The test results in patients with allergy to *A. fumigatus* were significantly smaller than in patients with ABPA ($p < 0.005$, Mann-Whitney U test).

The intracutaneous test results were completely negative in the 17 CF control patients. This difference was significant

TABLE 2. NELSON'S CRITERIA IN THE 21 CYSTIC FIBROSIS PATIENTS WITH ALLERGY TO *A. FUMIGATUS*

Patient	Age (yr)	Sex	Wheezing	New Pulmonary Infiltrate	Aspergillus in sputum	Aspergillus Prick (mm)	Total IgE (IU/ml)	Aspergillus RAST (0-4)	Aspergillus IgG (EU/ml)
13	20	W	-	+	-	4	779	4	95
14	24	M	+	-	-	5	599	4	18
15	12	M	-	-	+	3	130	2	161
16	20	W	-	-	-	11	108	3	19
17	27	M	+	-	-	9	201	3	29
18	32	M	+	-	-	12	248	4	48
19	17	W	-	-	-	12	277	4	48
20	25	M	-	-	-	3	580	4	184
21	16	W	+	+	-	3	211	4	163
22	17	M	-	-	+	3	657	4	189
23	40	M	+	-	-	7	466	3	167
24	18	W	-	-	+	3	20	0	45
25	34	M	+	-	-	6	534	4	82
26	15	W	-	-	-	6	98	3	31
27	23	M	-	+	-	3	300	1	33
28	33	M	+	-	-	5	120	2	20
29	44	W	-	-	-	5	748	2	48
30	23	M	+	-	+	4	80	2	17
31	26	M	-	+	-	3	316	2	51
32	18	M	-	-	-	3	223	2	9
33	22	M	-	-	+	5	147	2	30

TABLE 3. NELSON'S CRITERIA IN THE 17 CYSTIC FIBROSIS CONTROL PATIENTS WITHOUT ALLERGY TO *A. FUMIGATUS*

Patient	Age (yr)	Sex	Wheezing	New Pulmonary Infiltrate	<i>Aspergillus</i> in sputum	<i>Aspergillus</i> Prick (mm)	Total IgE (IU/ml)	<i>Aspergillus</i> RAST (0–4)	<i>Aspergillus</i> IgG (EU/ml)
34	35	M	+	–	+	0	9	0	21
35	12	M	+	–	–	0	37	0	25
36	16	W	+	–	+	0	3	0	122
37	15	M	–	+	+	0	68	0	222
38	15	W	–	–	+	0	56	0	224
39	28	M	–	+	+	0	55	0	170
40	17	W	+	+	–	0	28	0	117
41	16	W	+	–	–	0	50	0	79
42	16	W	–	–	+	0	3	0	130
43	37	W	–	+	+	0	258	0	83
44	33	W	–	–	+	0	13	0	220
45	15	M	–	+	+	0	7	0	78
46	12	M	–	–	–	0	35	0	20
47	32	M	–	–	+	0	4	0	20
48	30	W	–	–	+	0	3	0	19
49	27	W	–	–	+	0	5	0	15
50	22	M	–	–	–	0	26	0	52

compared with the ABPA group ($p < 0.0001$) and the patients with allergy to *A. fumigatus* ($p < 0.005$).

The intracutaneous test reactions to rAsp f 4 were positive in the ABPA group at a concentration of 10^{-3} $\mu\text{g/ml}$ in three patients, at 10^{-2} $\mu\text{g/ml}$ in four, and at 10^{-1} $\mu\text{g/ml}$ in five. None of the ABPA patients had intracutaneous test results negative to rAsp f 4 (Figure 3).

Three patients with allergy to *A. fumigatus* had positive reactions at a concentration of 10^{-1} $\mu\text{g/ml}$; 18 patients showed negative test results. The test results in patients with allergy to *A. fumigatus* were significantly smaller than in patients with ABPA ($p < 0.0001$, Mann–Whitney U test).

The intracutaneous test results were completely negative in the 17 CF control patients. This difference was significant compared with the ABPA group ($p < 0.0001$), but not in comparison with the patients with allergy to *A. fumigatus* ($p > 0.10$).

The intracutaneous test reactions to rAsp f 6 were positive in the ABPA group at a concentration of 10^{-4} $\mu\text{g/ml}$ in one patient, at 10^{-3} $\mu\text{g/ml}$ in five, at 10^{-2} $\mu\text{g/ml}$ in four, and at 10^{-1} $\mu\text{g/ml}$ in one. One ABPA patient had intracutaneous test results negative to rAsp f 6 (Figure 4).

Twelve patients with allergy to *A. fumigatus* had positive reactions at a concentration of 10^{-1} $\mu\text{g/ml}$; nine patients

showed negative test results. The test results in patients with allergy to *A. fumigatus* were significantly smaller than in patients with ABPA ($p < 0.0001$, Mann–Whitney U test).

The intracutaneous test results were completely negative in the 17 CF control patients. This difference was significant compared with the ABPA group ($p < 0.0001$) and the patients with allergy to *A. fumigatus* ($p < 0.0005$).

The surface of the histamine wheal was recorded as 2.04 ± 1.10 cm^2 in the ABPA group, as 1.81 ± 0.78 cm^2 in patients with allergy to *A. fumigatus*, and as 1.87 ± 0.66 cm^2 in the CF control patients. The difference between the groups was not statistically significant ($p > 0.68$).

Altogether, 28 of the 33 patients with previously positive skin prick tests to commercial *A. fumigatus* extract also showed positive results to at least one of the recombinant allergens 1, 3, 4, and 6 by intracutaneous testing. The sensitivity of the diagnosis of *A. fumigatus* sensitization reached 84.9% (95% confidence interval 68.1–94.9%), whereas the specificity was 100% (95% confidence interval 80.5–100%) because intracutaneous testing with recombinant allergens to *A. fumigatus* was completely negative in the CF control patients.

Intracutaneous testing with the two intracellular recombinant allergens rAsp f 4 and rAsp f 6 revealed negative or bor-

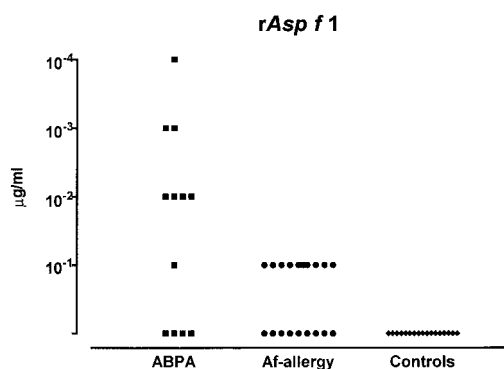


Figure 1. Intracutaneous test results with rAsp f 1. Positive results are shown at the equivalent protein concentration, negative results at the bottom line without protein concentration. Significance for ABPA versus *A. fumigatus* allergy, $p < 0.05$; ABPA versus control patients, $p < 0.0001$; *A. fumigatus* allergy versus control patients, ($p < 0.0005$).

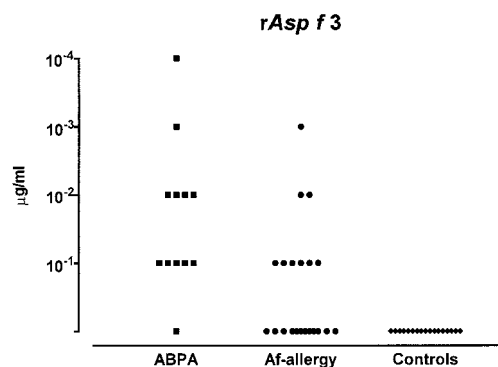


Figure 2. Intracutaneous test results with rAsp f 3. Positive results are shown at the equivalent protein concentration, negative results at the bottom line without protein concentration. Significance for ABPA versus *A. fumigatus* allergy, $p < 0.005$; ABPA versus control patients, $p < 0.0001$; *A. fumigatus* allergy versus control patients, $p < 0.005$.

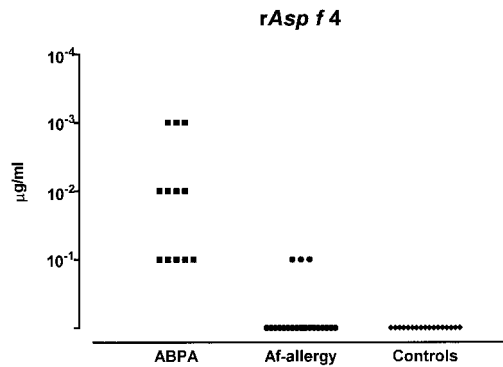


Figure 3. Intracutaneous test results with rAsp f 4. Positive results are shown at the equivalent protein concentration, negative results at the bottom line without protein concentration. Significance for ABPA versus *A. fumigatus* allergy, $p < 0.0001$; ABPA versus control patients, $p < 0.0001$.

derline results in the patients with allergy to *A. fumigatus*, e.g., the patients showed positive reactions only at a concentration of 10^{-1} µg/ml. Using cutoff values of 10^{-2} µg/ml, corresponding to the next test concentration, five patients with ABPA showed positive reactions to both allergens, rAsp f 4 and rAsp f 6, two reacted only to rAsp f 4, and five only to rAsp f 6. The highly specific detection of ABPA on the basis of a positive reaction to rAsp f 4 and/or rAsp f 6 reached a sensitivity of 100% (95% confidence interval 73.5–100%) and a specificity of 100% (95% confidence interval 83.9–100%).

DISCUSSION

CF is a complex systemic disease involving respiratory, pancreatic, hepatic, gastrointestinal, and reproductive tract abnormalities. *A. fumigatus* has the ability to colonize the respiratory tract of patients with CF, which is probably favored by local factors in the lungs of the patients such as hypersecretion of viscous phlegm, chronic infection and inflammation of the bronchial walls, and bronchiectatic lung destruction. In addition, fungal antigens, including rAsp f 1, are potent inhibitors of protein synthesis (35), and low molecular mass metabolites are thought to facilitate fungal growth through immunosuppression and inhibition of macrophage phagocytosis (36). Colonization of the respiratory tract by *A. fumigatus* and sensitization to *A. fumigatus* have been shown to occur in up to 60% of patients with CF (37–39). However, allergy to *A. fumigatus* must clearly be distinguished from ABPA, which requires systemic steroid therapy to control the ongoing inflammation and lung destruction. Therefore, it is surprising that in our study only 33% of CF patients with ABPA had a positive sputum culture for *A. fumigatus*, compared with 24% of patients with allergy to *A. fumigatus* and 71% of the CF control patients not sensitized to *A. fumigatus*. This might be explained by the bronchial obstruction in ABPA patients and those sensitized to *A. fumigatus*; *A. fumigatus*, although present in the lungs, will not appear in the sputum. It is also an argument not to use a positive sputum culture of *A. fumigatus* as a major criterion for the diagnosis of ABPA. In a previous study, differential IgE responses to four recombinant allergens of *A. fumigatus* were demonstrated in *A. fumigatus*-sensitized CF patients with and without ABPA (26). Although rAsp f 1-specific serology has been shown to be helpful in the diagnosis of ABPA (22, 40), rAsp f 1 and rAsp f 3 were also recognized by IgE from sera of *A. fumigatus*-sensitized CF patients, but rAsp f 4 and rAsp f 6 were recognized exclusively by IgE from sera of CF patients with ABPA (26). Therefore, the last two intracellular proteins may be re-

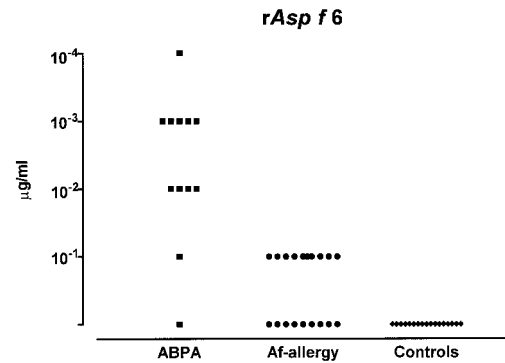


Figure 4. Intracutaneous test results with rAsp f 6. Positive results are shown at the equivalent protein concentration, negative results at the bottom line without protein concentration. Significance for ABPA versus *A. fumigatus* allergy, $p < 0.0001$; ABPA versus control patients, $p < 0.0001$; *A. fumigatus* allergy versus control patients, $p < 0.0005$.

garded as specific markers for ABPA. In this study, we were able to confirm that intracutaneous testing with rAsp f 1 and rAsp f 3 was sufficient to diagnose sensitization to a complex allergenic system like *A. fumigatus* in patients with CF. Moreover, the two intracellular *A. fumigatus* allergens, rAsp f 4 and rAsp f 6, allowed a reliable discrimination between the two IgE-mediated disorders, ABPA and allergy to *A. fumigatus*. Every single CF patient with ABPA reacted to rAsp f 4 and/or rAsp f 6 at a concentration of 10^{-2} µg/ml or less. None of the patients with allergy to *A. fumigatus* reacted to these two allergens in this concentration range.

Our results raise the question about the nature of the specific immune responses to distinct fungal allergens in patients with CF. The high incidence of IgE antibodies to secreted *A. fumigatus* allergens can be attributed to the prolonged exposure to the fungus growing in the respiratory tracts of patients predisposed to allergic reactions (41). The specific IgE responses to the cytoplasmic allergens rAsp f 4 and rAsp f 6 in patients with ABPA require further explanation. Specific sensitization to proteins in patients with ABPA suggests substantial differences in the pathogenesis of immune responses during exposure to *A. fumigatus*. It has been shown that infection by parasites can induce differential immune responses in infected individuals (42). However, a single pathogen has not yet been described that can elicit differential IgE responses in clinically related diseases. *In vitro* hyphal growth of *A. fumigatus* is inhibited by *Pseudomonas aeruginosa*, leading to ultrastructural abnormalities such as plasmolysis, vacuolation, and degeneration of organelles (43, 44). These ultrastructural abnormalities may increase the release of cytoplasmic antigens and thus trigger a more intense antibody response. This theory is supported by the observation that the development of ABPA is significantly associated with colonization of the respiratory tract by *P. aeruginosa* (11).

In our study, the mean age of patients was 15.6 years in the ABPA group, compared with 24.4 years in the patients with allergy to *A. fumigatus* and 22.1 years in the CF control patients. Despite the difference, these results are in accordance with data of a major European epidemiologic study in patients with CF who had a peak prevalence of 11.2% ABPA in the age group 13–18 years (45). In this study, ABPA was associated with a poor nutritional status, which was not the case in our patients. However, our patients have recently been diagnosed as having ABPA, so that the disease status might not have affected the nutritional status yet.

Our results clearly demonstrate that the immune responses in CF patients with ABPA can reliably be detected by intracu-

taneous testing with recombinant *A. fumigatus* allergens. The differential skin test reactivity to different *A. fumigatus* allergens allows a reliable diagnosis of ABPA, discriminating these patients from nonreactive *A. fumigatus* allergics. A trial on a large scale as well as longitudinal studies will be required in the CF population to confirm our data and to assess if quantitative test results can be used as a measure of activity and severity of ABPA, enabling the adjustment of steroid treatment. Then it might be possible to prevent irreversible lung damage by early intervention and to minimize possible side effects, optimizing steroid treatment.

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