

Identification of Allergic Bronchopulmonary Aspergillosis in Cystic Fibrosis Patients by Recombinant *Aspergillus fumigatus* I/a-Specific Serology

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Before specific therapy, such as oral corticosteroids, can be commenced it is essential to distinguish full-blown allergic bronchopulmonary aspergillosis (ABPA) from allergy to *A. fumigatus* in patients with cystic fibrosis (CF). For this purpose we have evaluated the diagnostic value of recombinant *A. fumigatus* allergen I/a (rAsp f I/a)-specific serology in 55 patients with CF. Based on clinical presentation and laboratory data, 10 CF patients had ABPA, 27 had *Aspergillus* allergy, and 18 were not allergic to *A. fumigatus* (CF control group). The serologic assays revealed a 10-fold increase in rAsp f I/a-specific IgE, a 5-fold increase in rAsp f I/a-specific IgG₁, and a 4-fold increase in rAsp f I/a-specific IgG₄ antibodies in ABPA patients compared with the *Aspergillus* allergy and CF control groups. Sera from 11 CF patients were analyzed without knowledge of their clinical state or diagnosis of ABPA. All ABPA patients (4 of 11) were accurately identified. We conclude that rAsp f I/a-specific serology is a highly sensitive and specific test that can be used to identify ABPA reliably in CF patients. Nikolaizik WH, Moser M, Cramer R, Little S, Warner JO, Blaser K, Schöni MH. Identification of allergic bronchopulmonary aspergillosis in cystic fibrosis patients by recombinant *Aspergillus fumigatus* I/a-specific serology.

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Allergy to *Aspergillus fumigatus* is common in patients with cystic fibrosis (CF) (1), but only a few patients develop allergic bronchopulmonary aspergillosis (ABPA) (2). Differentiation between full-blown ABPA and *A. fumigatus* allergy is difficult but of immense clinical relevance, because untreated ABPA may lead to substantial lung damage, including proximal bronchiectasis or total lung collapse (3, 4). Significant correlations of decreased lung function with both IgE and IgG antibody titers to *Aspergillus* have been reported (5, 6). Currently, the only effective treatment of ABPA is high-dose corticosteroids (7), which bear the well-known risk of severe side effects, such as hyperglycemia, cataracts, and growth retardation (8).

ABPA in CF was first described in 1965 (9). Since then, the importance of detecting both IgE and IgG antibodies to *Aspergillus* have been emphasized in establishing the diagnosis (10). However, the quality of serologic tests depends mostly on the antigen preparations used. Variability of *A. fumigatus* preparations can be a result of different isolates (11), batch-to-batch variations (12), rapid changes in IgE binding components during culture (13), or different sources used to prepare allergen extracts, such as mycelia, spores, or culture filtrates (14). Most allergen preparations are heterogeneous mixtures of proteins and glycoproteins and also contain such components as lectins, endotox-

ins, hemolytic toxins, and histamine (15, 16). To overcome the problems of impurity of allergen preparations, attempts have been made to standardize the production of *A. fumigatus* extracts (13, 17) or to purify various *Aspergillus* antigens biochemically (16, 18). Even these apparently homogeneous allergens may be contaminated by degradation products or other allergenic components in trace amounts but of similar immune reactivity.

The production of purified recombinant allergens of defined biologic activity has been a major advance (19), and recently recombinant *A. fumigatus* antigen I/a (rAsp f I/a), a major allergenic protein of *A. fumigatus*, has been cloned and expressed (20). rAsp f I/a, expressed in *Escherichia coli* and purified by absorption chromatography, has the same functional characteristics as the native 18 kD ribotoxin and can therefore be used as a defined allergen or antigen for clinical and serologic diagnosis of *A. fumigatus*-associated diseases (20). We have evaluated the diagnostic value of rAsp f I/a in CF patients with ABPA and compared the results with *A. fumigatus*-allergic patients and CF control subjects not allergic to *A. fumigatus*.

METHODS

Subjects

A total of 55 patients with CF, 30 males and 25 females, were entered into the study: 44 were patients admitted to the Alpine Children's Hospital in Davos, Switzerland, and 11 patients were inpatients of the Royal Brompton National Heart and Lung Hospital in London. The latter served as a blind control group for the test to detect ABPA through rAsp f I/a-specific serology. Both groups had the same routine assessment for CF and allergy, including routine skin prick testing. The mean age of the patients was 19.2 ± 7.9 yr. The diagnosis of CF had been substantiated by the sweat iontophoresis test with sweat sodium concentrations > 70 mM on at least two specimens of > 100 mg sweat (21). All

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patients studied were informed verbally and in writing about the purpose and the procedure of the study. Approval from the official ethics committee of Davos was obtained before starting the study.

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

ABPA patients. The diagnosis of ABPA was based on a minimum of six of seven criteria as described by Nelson and colleagues (22): (1) wheezing, (2) pulmonary infiltrate, (3) positive sputum culture for *A. fumigatus*, (4) positive immediate reaction to *A. fumigatus* extracts in skin prick testing (wheal $\geq$ 3 mm in diameter) (23), (5) increased total IgE levels (IgE more than 2 standard deviations [SD] greater than normal values for age) (24), (6) specific IgE to *A. fumigatus* by RAST (RAST score $\geq$ 1), and (7) increased level of IgG antibodies specific to *A. fumigatus* (ELISA $>$ 40 EU/ml) (25, 26) or the presence of *Aspergillus* precipitins.

Aspergillus-allergic CF patients. CF patients were assigned to the group with *Aspergillus* allergy if routine skin prick tests with Bencard antigens (SmithKline Beecham, Neuss, Germany) were positive to *A. fumigatus* (wheal $\geq$ 3 mm in diameter) (23) but did otherwise not fulfill the criteria for ABPA (22).

CF control group. CF patients were assigned to the CF control group if routine skin prick tests with Bencard antigens (SmithKline Beecham, Neuss, Germany) were completely negative to *A. fumigatus* (no wheal and no flare).

Routine Assessment

Each patient had his or her routine assessment when admitted to the hospital, including sputum cultures for bacteria and fungi, chest radiograph, lung function test, and skin prick testing to *A. fumigatus* and a range of other allergens (Bencard skin test antigens, SmithKline Beecham, Neuss, Germany). The skin test reactions were recorded after 15 min by measuring the diameter of the wheal, and the results were considered positive with a wheal $\geq$ 3 mm and no reaction to the saline control solution (23). A positive control was included with 0.1% histamine.

A blood sample was taken for routine blood cell count, routine IgE and IgG antibody determination, and radioallergosorbent test for IgE antibodies to *A. fumigatus* (Pharmacia, Uppsala, Sweden) using the Pharmacia allergen-specific CAP system and converting the results into RAST classes from 0 to 4 as described by the manufacturer (RAST Class 0, $<$ 0.35 IU/ml; Class 1, 0.35 to 0.69 IU/ml; Class 2, 0.70 to 3.49 IU/ml; Class 3, 3.50 to 17.49 IU/ml; Class 4, $\geq$ 17.50 IU/ml). In patients admitted to the Alpine Children's Hospital in Davos, total IgG antibodies to *A. fumigatus* were analyzed by ELISA using standardized DPC antigens (Diagnostic Products Corporation, Los Angeles, CA) bound to solid phase (25) because ELISA has been shown to be more sensitive for detecting IgG antibodies to *A. fumigatus* than the measurement of *Aspergillus* precipitins (26). In patients admitted to the Royal Brompton National Heart and Lung Hospital, total IgG antibodies to *A. fumigatus* were analyzed as serum precipitins to *Aspergillus* using double immunodiffusion with Bencard standard Antigens 1 and 2 (SmithKline Beecham, Neuss, Germany).

Production of rAsp f I/a

rAsp f I/a was produced as previously described (20). Briefly, the gene encoding the Asp f I/a protein was amplified by polymerase chain reac-

tion from a cDNA library constructed into the λ zap II vector (Stratagene, La Jolla, CA). The amplification product was subcloned into the high-level expression vector pDS 56 to produce a hexahistidine-tagged fusion protein (27). *E. coli* M15 transformants produced about 50 mg/L of recombinant protein, which was readily purified to homogeneity by metal chelate affinity chromatography in a single step. The rAsp f I/a protein was refolded *in vitro* to reach biologic activity and assayed by analyzing specific cleavage of the α fragment from 28S ribosomal RNA.

rAsp f I/a-specific Antibodies in Serum

Anti-rAsp f I/a antibodies were measured by ELISA (20). In brief, microtiter plates, Maxisorp microtiter plates (Nunc, Roskilde, Denmark), were coated for 2 h at 37°C with 10 μ g/ml of rAsp f I/a in phosphate-buffered saline (PBS), pH 8. The remaining free sites were blocked with PBS, pH 7.4, containing 5% (wt/vol) nonfat dry milk powder at 37°C for 1 h (blocking buffer). The wells were then washed three times with PBS, pH 7.4, containing 0.05% Tween® 20 (Fluka, Buchs, Switzerland). The plates were incubated with serially twofold diluted sera (100 μ l) in blocking buffer including 5% Tween 20 (dilution buffer) at 37°C for 2 h. Secondary monoclonal antibodies (MAB RJ4 antihuman IgG₄ and NL16 antihuman IgG₁ [Oxoid, Basingstoke, UK], alkaline phosphatase-labeled goat antihuman IgA α chain [Tago, Burlingame, CA], and MAB TN-142 antihuman IgE [kindly provided by Dr. C. H. Heusser, Ciba, Ltd., Basel, Switzerland]) were appropriately diluted in buffer and incubated at 37°C for 2 h. After washing, the plates were incubated at room temperature in 1 M diethanolamine buffer, pH 9.8, containing 0.5 mM MgCl₂ and 4-nitrophenylphosphate (1.5 mg/ml) as substrate. The resulting absorbance at 405 nm was measured in an ELISA reader (Molecular Devices, Menlo Park, CA). Pooled sera from two adult ABPA patients with asthma were used as reference standard, with absorbency and dilution values converted into ELISA units (EU) and arbitrarily set as 100 EU/ml for each isotype analyzed.

Statistics

Data were analyzed with the SYSTAT® computing system for statistics (SYSTAT, Inc., Evanston, IL). Statistics employed the Mann-Whitney U test for comparison of rank data between the different groups and the Pearson's chi-square test with Yates' correction for comparison of categorical data. Correlation coefficients were determined using Pearson's linear regression analysis. Differences associated with probabilities $p < 0.05$ were considered significant.

RESULTS

Of 55 CF patients, 10 were diagnosed as having ABPA: 6 showed six and another 4 expressed seven of Nelson's criteria. Of these 10 patients, 6 were from Davos and 4 from London. The diagnostic Nelson's criteria for the 10 ABPA patients are shown in Table 1. As shown in Table 2, 27 patients belonged to the group with documented allergy to *A. fumigatus* and 18 patients to the control group. Mean Shwachman score, assessing the severity degree of the disease (28), from all patients was 60.9 ± 15.4 , indicating that the patients were moderate to severely affected.

TABLE 1
PATIENT DATA ACCORDING TO NELSON'S CRITERIA IN THE 10 PATIENTS WITH ABPA

Patient	Age (yr)	Sex	Wheezing	Infiltrates	<i>Aspergillus</i> in Sputum	<i>Aspergillus</i> Prick (mm)	IgE (IU/ml)	<i>Aspergillus</i> RAST (0-4)	<i>Aspergillus</i> IgG (EU/ml)
1	11	F	+	+	+	3	2,398	4	162
2	7	M	+	+	+	3	3,804	4	50.0
3	22	F	+	+	-	4	1,532	4	50.2
4	17	M	+	+	-	4	5,423	4	40.6
5	15	F	+	+	+	3	1,017	4	82.2
6	12	F	+	+	-	5	5,226	4	147
46	20	F	+	+	-	4	396	2	1*
49	8	F	+	+	+	4	560	2	2*
51	9	M	+	+	-	4	5,736	2	1*
53	11	F	+	+	+	3	145	1	4*

* Number of *Aspergillus* precipitin lines.

TABLE 2
PATIENT DATA ACCORDING TO NELSON'S CRITERIA IN THE 17 PATIENTS WITH *ASPERGILLUS* ALLERGY

Patient	Age (yr)	Sex	Wheezing	Infiltrates	<i>Aspergillus</i> in Sputum	<i>Aspergillus</i> Prick (mm)	IgE (IU/ml)	<i>Aspergillus</i> RAST (0-4)	<i>Aspergillus</i> IgG (EU/ml)
7	9	M	-	-	-	6	410	2.5	47.0
8	19	M	+	-	-	3	27	0	89.3
9	23	F	-	-	-	10	491	4	26.2
10	20	M	-	-	-	4	166	2.8	57.3
11	10	F	-	+	-	5	146	2.8	23.4
12	15	M	-	-	-	3	23	1.2	22.9
13	20	M	-	-	-	6	323	3.8	13.8
14	26	M	-	-	-	4	17	0.9	5.0
15	23	M	-	-	-	4	554	3.5	19.4
16	26	F	+	-	-	7	423	3.8	73.1
17	17	M	-	-	-	7	304	4	14.7
18	4	F	+	-	-	5	61	3.0	17.0
19	28	M	-	-	-	6	90	2.9	248
20	25	M	-	-	+	3	866	4	40.6
21	9	M	-	-	+	4	435	3.0	95.4
22	32	F	-	-	+	5	< 16	1.5	29.5
23	24	M	+	-	+	4	47	2.0	38.7
24	19	M	-	-	-	8	55	3.2	74.1
25	26	F	-	-	-	5	270	3.3	29.1
26	30	M	-	-	-	7	372	3.3	20.0
27	28	M	-	-	-	8	409	3.5	25.1
28	21	M	-	+	-	3	103	3.1	9.3
29	32	M	-	+	-	3	1,079	0	27.8
50	7	M	-	-	-	3	534	1.0	0*
52	16	F	-	-	-	7	71	2.0	0*
54	15	M	-	-	+	5	3,875	2.0	1*
55	10	F	-	-	-	3	90	0	1*

* Number of *Aspergillus* precipitin lines.

There was no significant difference between the study groups regarding this clinical score ($p > 0.05$, Mann-Whitney U test). Of 55 patients, 43 (78%) were colonized with *Pseudomonas aeruginosa* and 15 (27%) had *A. fumigatus* in their bronchial secretion; 7 patients (13%), including 3 ABPA patients (Patients 1, 6, and 49, Table 1), used oral corticosteroids in low doses (prednisolone 5 to 10 mg/d), and 15 patients (27%) inhaled corticosteroids. Again there was no significant difference between the study groups concerning the distribution of patients with steroid therapy ($p > 0.05$, Pearson's chi-square test).

The data from the routine clinical assessment are compiled in Table 3, the results between study groups are compared. As suggested by Nelson's criteria, skin prick test reactions to *A. fumigatus* with Bencard antigens were positive in all ABPA patients, as well as in all patients allergic to *A. fumigatus*. The size of the wheal (mm) was significantly larger in ABPA patients compared

with the CF control group ($p < 0.0001$, Mann-Whitney U test) and in *Aspergillus*-allergic patients compared with the CF control group ($p < 0.0001$). The size of the wheal (mm) was also larger in *Aspergillus*-allergic patients than in ABPA patients ($p < 0.05$).

Erythrocyte sedimentation rates, leukocyte counts, and neutrophil bands showed no significant difference between the study groups (data not shown), but there was a significantly higher eosinophil count in *Aspergillus*-allergic patients compared with the CF control subjects ($p < 0.05$) (Table 3). Total IgE was significantly raised in ABPA compared with CF control subjects ($p < 0.0001$) and *Aspergillus*-allergic patients ($p < 0.005$). *Aspergillus*-allergic patients had significantly higher IgE levels than the CF control group ($p < 0.005$). *Aspergillus* RAST was highest in ABPA patients and negative in the control group. However, there was no statistically significant difference between ABPA and *Aspergillus*-allergic patients. IgG antibodies to *A. fumigatus* as

TABLE 3
RESULTS OF COMMON SKIN AND SEROLOGY TESTING IN PATIENTS WITH CF AS ROUTINELY DONE DURING HOSPITALIZATION TO ASSESS NELSON'S CRITERIA

	CF Control (n = 18)	<i>Aspergillus</i> Allergy (n = 27)	ABPA (n = 10)
<i>Aspergillus</i> prick, mm	0.0 ± 0.0 (0 of 18 positive)	5.3 ± 1.9* (27 of 27 positive)	3.7 ± 0.7*§ (10 of 10 positive)
Eosinophil count, ×10 ⁹ cells/L	0.23 ± 0.23	0.33 ± 0.20†	0.70 ± 0.72
IgE, IU/ml	64 ± 112	416 ± 741‡	2,624 ± 2,233*§
<i>Aspergillus</i> RAST, score 0-4	0.53 ± 1.07	2.49 ± 1.27*	3.10 ± 1.20*
<i>Aspergillus</i> IgG antibodies, EU/ml	37.3 ± 37.5	45.5 ± 50.8	88.7 ± 53.1§

* $p < 0.0001$ versus CF control group.

† $p < 0.05$ versus CF control group.

‡ $p < 0.005$ versus CF control group.

§ $p < 0.05$ versus *Aspergillus* allergy.

|| $p < 0.005$ versus *Aspergillus* allergy.

TABLE 4
RESULTS OF *rAsp f* I/a-SPECIFIC SEROLOGY FROM THE
29 SWISS CF PATIENTS WITH ABPA (n = 6) AND
ASPERGILLUS ALLERGY (n = 23)

Patient	<i>rAsp f</i> I/a-specific				Final Diagnosis
	IgA (EU/ml)	IgE (EU/ml)	IgG ₁ (EU/ml)	IgG ₄ (EU/ml)	
1	286	429	448	1,024	ABPA
2	603	688	680	906	ABPA
3	93.7	137	80.1	883	ABPA
4	63.0	4.0	24.0	41.0	ABPA
5	54.0	87.0	199	303	ABPA
6	325	513	584	191	ABPA
7	172	55.9	21.3	17.1	<i>Aspergillus</i> allergy
8	52.5	5.0	5.0	5.0	<i>Aspergillus</i> allergy
9	95.2	38.3	75.6	17.6	<i>Aspergillus</i> allergy
10	184	13.5	46.7	118	<i>Aspergillus</i> allergy
11	331	18.0	119	28.1	<i>Aspergillus</i> allergy
12	143	5.0	32.2	6.0	<i>Aspergillus</i> allergy
13	694	5.0	15.7	5.0	<i>Aspergillus</i> allergy
14	114	5.0	5.0	5.0	<i>Aspergillus</i> allergy
15	411	47.4	63.5	176	<i>Aspergillus</i> allergy
16	309	5.0	9.1	5.0	<i>Aspergillus</i> allergy
17	349	34.5	66.3	310	<i>Aspergillus</i> allergy
18	20.0	< 1.0	16.4	< 1.0	<i>Aspergillus</i> allergy
19	38.0	117	78.0	170	<i>Aspergillus</i> allergy
20	268	89.0	419	1,003	<i>Aspergillus</i> allergy
21	31.0	5.0	126	65.0	<i>Aspergillus</i> allergy
22	131	1.0	6.0	1.0	<i>Aspergillus</i> allergy
23	221	42.0	230	684	<i>Aspergillus</i> allergy
24	27.0	7.0	24.0	10.0	<i>Aspergillus</i> allergy
25	34.0	26.0	9.0	5.0	<i>Aspergillus</i> allergy
26	69.0	25.0	65.0	23.0	<i>Aspergillus</i> allergy
27	197	10.0	236	6.0	<i>Aspergillus</i> allergy
28	828	10.0	40.0	4.0	<i>Aspergillus</i> allergy
29	72.0	12.0	60.0	12.0	<i>Aspergillus</i> allergy

measured by ELISA using DPC antigens were substantially increased in ABPA patients compared with *Aspergillus*-allergic patients ($p < 0.05$).

Total IgG and IgG subclasses did not differ significantly between groups (data not shown).

The most significant findings were highly elevated levels of *rAsp f* I/a-specific IgE, IgG₁, and IgG₄ in the ABPA patients (Table 4). As indicated in Figure 1, *rAsp f* I/a-specific IgE as measured by ELISA was 213 ± 245 EU/ml (mean $\pm$ SD) in ABPA patients, compared with 22.4 ± 28.1 EU/ml in *A. fumigatus*-allergic patients ($p < 0.01$, Mann-Whitney U test) and 23.0 ± 40.3 EU/ml in CF control patients ($p < 0.005$). *rAsp f* I/a-specific IgG₁ was $764 \pm 1,504$ EU/ml in ABPA patients compared with 76.5 ± 92.3 EU/ml in *A. fumigatus*-allergic patients ($p < 0.001$) and 169 ± 266 EU/ml in CF control subjects ($p < 0.05$), and finally, *rAsp f* I/a-specific IgG₄ was 745 ± 720 EU/ml in ABPA patients, 130 ± 264 EU/ml in *A. fumigatus*-allergic patients ($p < 0.0005$), and 207 ± 426 EU/ml in CF control subjects ($p < 0.005$). Furthermore, *rAsp f* I/a-specific IgA was 470 ± 885 EU/ml in ABPA patients, 184 ± 202 EU/ml in *A. fumigatus*-allergic patients, and 151 ± 127 EU/ml in CF control subjects; these differences were not significant. There was no significant difference between *A. fumigatus*-allergic patients and CF control subjects for *rAsp f* I/a-specific IgE or, *rAsp f* I/a-specific IgG₁ or IgG₄ antibodies.

The sera from 11 CF patients from the Royal Brompton National Heart and Lung Hospital in London were analyzed in Davos without knowledge of their clinical state or diagnosis of ABPA (Table 5). The serology from these 11 patients suggested that 5 subjects had ABPA and 6 did not. Scrutiny of clinical records in England revealed that the testing had accurately identified all 4 patients with unequivocal ABPA (Patients 46, 49, 51, and 53, Tables 1 and 5), 3 with six and 1 with seven of Nelson's criteria (22). The patient falsely identified as an ABPA patient (Patient 54, Tables 2 and 5) was clinically atypical, having five Nelson's criteria but no wheezing or significant increase in pulmonary shadowing to suggest a clinical diagnosis of the condition. Among the 6 British children identified as not having ABPA, 3 had strongly positive skin tests and RAST to *A. fumigatus*. Although none of these patients had evidence of ABPA at the time of blood sampling, 1 developed unequivocal ABPA 6 mo later (Patient 55, Tables 2 and 5). Thus, overall, a correct diagnosis was made in 10 of 11 patients (91% prediction rate). Based on this small number of patients, the sensitivity of the serum testing was 100% and the specificity 86%.

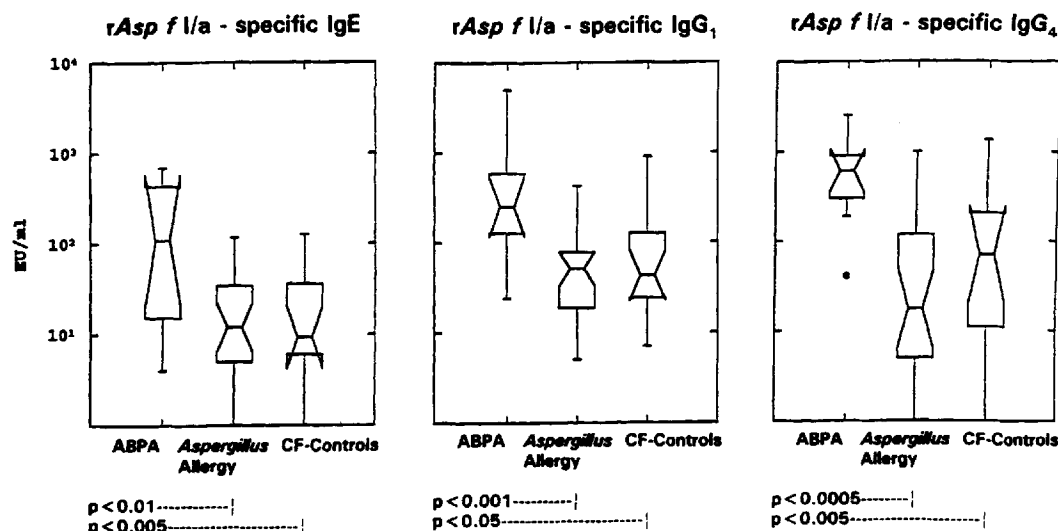


Figure 1. *rAsp f* I/a-specific IgE, IgG₁, and IgG₄ antibodies in the three groups. Diagrams are notched box plots in which the horizontal lines represent the 10th, 25th, 50th, 75th, and 90th percentiles. The boxes are notched at the median and return to full width at the 95% confidence intervals. *Outlying values. Significance level $p < 0.05$.

TABLE 5
RESULTS OF *rAsp f 1/a*-SPECIFIC SEROLOGY FROM THE 11 BRITISH CF PATIENTS ANALYZED
WITHOUT KNOWLEDGE OF THEIR CLINICAL STATE OR DIAGNOSIS OF ABPA

Patient	<i>rAsp f 1/a</i> -specific				Suspected Diagnosis	Final Diagnosis
	IgA (EU/ml)	IgE (EU/ml)	IgG ₁ (EU/ml)	IgG ₄ (EU/ml)		
45	36.2	6.0	122	24.6	Non-ABPA	CF control
46	83.7	15.3	126	658	ABPA	ABPA
47	15.9	< 5.0	13.5	< 5.0	Non-ABPA	CF control
48	36.8	< 5.0	52.0	< 5.0	Non-ABPA	CF control
49	115	15.0	264	310	ABPA	ABPA
50	30.1	6.6	65.8	32.1	Non-ABPA	<i>Aspergillus</i> allergy
51	140	24.2	234	572	ABPA	ABPA
52	39.9	7.4	50.7	19.2	Non-ABPA	<i>Aspergillus</i> allergy
53	2,940	217	5,000	2,560	ABPA	ABPA
54	90.4	15.4	152	785	ABPA	<i>Aspergillus</i> allergy
55	17.6	< 5.0	28.5	< 5.0	Non-ABPA	<i>Aspergillus</i> allergy

DISCUSSION

Our study clearly demonstrates the value of a serologic test to detect ABPA in CF and to distinguish ABPA from patients sensitized to *A. fumigatus* who do not fulfill the clinical criteria of Nelson for assignment to ABPA. There was a 10-fold increase in *rAsp f 1/a*-specific IgE, a 5-fold increase in *rAsp f 1/a*-specific IgG₁, and a 4-fold increase in *rAsp f 1/a*-specific IgG₄ antibodies compared with the CF control groups. By these three antibody classes we were able to distinguish ABPA patients from *A. fumigatus*-allergic patients and CF patients not allergic to *A. fumigatus*. This was not the case for the IgG antibodies to *Aspergillus* using DPC antigens nor for RAST, which detects IgE antibodies. These results indicate that *rAsp f 1/a*-specific serology is superior to other serum tests commonly used to diagnose ABPA. In adult asthmatic subjects, the diagnostic value of *rAsp f 1/a*-specific serology has already been shown (29). Here we have demonstrated the same for CF patients and confirmed the results by blind testing of the sera from the British patients.

Allergy to *A. fumigatus* is common in CF but must be clearly distinguished from ABPA, which has been reported to occur with a prevalence rate of 0.5 to 11% (2, 22, 30, 31). The wide range of prevalences might be explained by differences in defining the condition. We have chosen Nelson's criteria (22), which include positive cultures for *Aspergillus*, rather than Laufer's criteria (31), which favor central bronchiectasis. Laufer and coworkers have reported that classic radiographic findings of ABPA, such as centrally located bronchiectasis with a predilection for the upper lobes, could be detected not only in 71% of CF patients with ABPA but also in 71% of non-ABPA CF patients irrespective of whether they had asthma (31). The authors conclude that radiographic distinction between patients with CF alone and those with superimposed ABPA seems to be impossible. On the other hand, sputum cultures for *Aspergillus* have been shown to be positive in 80 to 100% of CF patients with ABPA but in only 22 to 50% of CF patients without ABPA (2, 22). For this reason we have chosen Nelson's criteria, which include *Aspergillus* cultures but not bronchiectasis. The wide range in reported prevalences of ABPA may also be a result of the quality of *A. fumigatus* allergens used for testing (32). This emphasizes the need for standardized extracts. Our study substantiates the diagnostic value of *rAsp f 1/a*, which has functional characteristics similar to those of the native 18 kD ribotoxin of *A. fumigatus* (20).

It is well known that corticosteroid treatment can suppress allergic immune responses to *A. fumigatus* (4, 7). In our study, three ABPA patients had low maintenance doses of steroids. All three patients still had clinical symptoms of exacerbation of ABPA as well as serologic evidence of ABPA (22). Longitudinal

measurements of *rAsp f 1/a*-specific serology would establish whether it will be useful as a marker of disease severity. ABPA Patient 4 (Tables 1 and 4) had low immune responses to *rAsp f 1/a* despite having never had any steroid medication at the time of blood sampling. Several other major antigens of *A. fumigatus* have been described that can be released and provoke immunologic reactions (32, 33). Although it is a major allergen, *rAsp f 1/a* is not sufficient to recognize all *A. fumigatus*-allergic patients (29). Positive RAST and prick test results to commercially available preparations of *A. fumigatus* indicate that the patient just described was reacting to other allergens that are present in crude extracts (33). One *Aspergillus*-allergic patient who went on to develop full-blown ABPA 6 mo later was not picked up by the *rAsp f 1/a*-specific serology at the time the blood was taken (Patient 55, Tables 2 and 5). This suggests that the antibody to *rAsp f 1/a* generates very acutely with the onset of the disease. Probably both a proliferation of the organism within the airways and an alteration in the host response account for the acute change. *In vitro* hyphal growth of *A. fumigatus* is inhibited by *P. aeruginosa*, leading to ultrastructural abnormalities, such as plasmolysis, vacuolation, and degeneration of organelles (34, 35). These ultrastructural abnormalities may increase the release of 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* (2).

A few patients with very raised *rAsp f 1/a*-specific IgE and IgG₁/IgG₄ antibodies but without ABPA were observed (Table 4). One could speculate that raised *rAsp f 1/a*-specific immune responses in these patients indicate an early stage of ABPA that is established only by a follow-up study. Nevertheless, we suggest that *rAsp f 1/a*-specific serology should be used in addition to the criteria outlined by Nelson to establish the diagnosis of ABPA in CF patients. In the future, a panel of major recombinant *A. fumigatus* allergens might allow reliable diagnosis of ABPA solely on the basis of serology. Early and reliable diagnosis of ABPA will facilitate appropriate treatment and might help to prevent irreversible lung damage.

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