

Development of Immune Responses to *Aspergillus* at an Early Age in Children with Cystic Fibrosis

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Although the ability of *Aspergillus* organisms to colonize the respiratory tract in patients with cystic fibrosis (CF) is well recognized, the contribution of *Aspergillus* to the disease process is poorly understood. Using sera from 147 CF patients (age 5 to 43 yr), we measured IgE antibody (ab) to *Aspergillus fumigatus* and five common inhalant allergens with a radioallergosorbent test (RAST). Total IgE levels and IgG ab to radio-labeled *Asp f I*, an allergen purified from *A. fumigatus* and a potent inhibitor of protein synthesis, were also measured. Thirty (20%) of the patients had IgE ab to *A. fumigatus*, and 22 (15%) of these patients had developed total IgE levels ≥ 400 IU/ml, raising the consideration of a diagnosis of allergic bronchopulmonary aspergillosis (ABPA). Five of the 22 patients developed these IgE responses by age 5 yr and 14 by age 10 yr. The proportion of patients with IgE ab to one or more of the other allergens tested was not significantly different from that of control subjects without respiratory symptoms. A striking proportion (84%) of CF sera contained IgG ab to *Asp f I*, compared with 6% of sera from control patients and 20% of sera from allergic children with asthma ($n = 25$), only one of whom had IgE ab to *A. fumigatus*. In an examination of additional sera from young CF patients, IgG anti-*Asp f I* ab was detected in 41% of these sera from patients 5 yr of age or older, increasing to 98% of 89 sera from patients older than age 10. The early development of immune responses to *Aspergillus* antigens, including the cytotoxic allergen *Asp f I*, raises the questions of whether *Aspergillus*, in addition to *Staphylococcus aureus* and *Pseudomonas aeruginosa*, contributes to progressive lung deterioration in patients with CF. El-Dahr JM, Fink R, Selden R, Arruda LK, Platts-Mills TAE, Heymann PW. Development of immune responses to *Aspergillus* at an early age in children with cystic fibrosis. *Am J Respir Crit Care Med* 1994;150:1513-8.

Previous studies of immediate hypersensitivity in patients with cystic fibrosis (CF) have consistently demonstrated an increased prevalence of sensitization to *Aspergillus* allergens (1-4). Positive skin tests to allergens produced by *Aspergillus* species, in particular *Aspergillus fumigatus*, have been reported to occur in as much as 31 to 59% of the CF population (2-6). There is more disagreement about whether CF patients are more generally atopic than normal children or those with asthma (5). The diagnosis of allergic bronchopulmonary aspergillosis (ABPA), an intense inflammatory reaction to *Aspergillus* in the lungs, is considered in patients with IgE anti-*Aspergillus* ab when serum total IgE levels exceed 400 IU/ml (or 1,000 ng/ml) in conjunction with deterioration in lung function (7). ABPA has been reported to affect as many as 10 to 12% of patients with CF (2, 4, 5), with up to 23% of CF patients meeting most but not all of the diagnostic criteria for the

disease (3, 6). The increased probability of the development of IgE antibody to *Aspergillus* in CF patients is presumed to be related to the ability of these fungi to grow at 37° C and therefore to colonize the respiratory tract. Nelson and colleagues grew *Aspergillus* species from sputum of 57% of patients with CF using multiple samples; however, in other studies, culture yields have been variable and have been reported to be as low as 9 to 11% (2, 4, 8, 9). Serologic responses to *Aspergillus* antigens generally indicate a high rate of exposure to the organism. Serum precipitins have been reported in 30 to 51% of patients with CF (2-5, 8, 9), and IgG anti-*Aspergillus* ab was demonstrated in 40 to 70% of CF sera by enzyme-linked immunosorbent assay (ELISA) (10, 11).

It has not been clear how early in life exposure and immune responses to *Aspergillus* antigens develop in CF patients, or whether chronic exposure to *Aspergillus* contributes significantly to the deterioration of their lung function if the criteria for ABPA are not present. Recently, a major allergen, *Asp f I*, has been purified from *A. fumigatus* (12). This allergen shows extensive sequence homology (95%) with a cytotoxin (mitogillin) produced by *A. restrictus*, and both proteins were shown to be potent inhibitors of protein synthesis.

In this study we examined the frequency of immune responses to *Aspergillus* as well as hypersensitivity to common aeroallergens in patients with CF. The results were compared with similar analyses in a control group and in allergic children with asthma. Particular attention was focused on analyzing the antibody re-

(Received in original form May 19, 1993 and in revised form December 29, 1993)

Supported by Grants Nos. AI 24261 and AI 30840 from the National Institutes of Health and in part by research funds from the Children's Medical Center, University of Virginia Health Sciences Center, Charlottesville, Virginia.

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Am J Respir Crit Care Med Vol 150, pp 1513-1518, 1994

TABLE 1
ALLERGEN-SPECIFIC IgE ANTIBODY RESPONSES*

	Cystic Fibrosis		Control Patients
	Skin Test (n = 50) [†]	RAST (n = 147) [‡]	RAST (n = 32) [§]
Allergens Tested			
<i>Aspergillus</i> species	15 (30%)	30 (20%)	1 (3%)
Mite (<i>D. farinae</i>)	5 (10%)	14 (10%)	4 (13%)
Cat	4 (8%)	20 (14%)	1 (3%)
Ragweed	8 (16%)	7 (5%)	1 (3%)
Rye grass	5 (10%)	10 (7%)	3 (9%)
Cockroach	0	0	0
Patients with IgE ab to:			
At least one allergen tested	22 (44%)	49 (33%)	5 (16%)
Inhalant allergens and <i>Aspergillus</i> [†]	5 (10%)	13 (9%)	1 (3%)
<i>Aspergillus</i> only	10 (20%)	17 (12%)	0
Inhalant allergens only**	7 (14%)	19 (13%)	4 (12.5%)

* The number of positive tests, followed by the percentage of tests positive in parentheses, is shown for each allergen tested.

[†] Skin-prick tests were done on 50 patients with cystic fibrosis ($\geq$ age 5 yr) enrolled consecutively at the University of Virginia Children's Medical Center. A wheal diameter of at least 3 mm greater than the negative control was regarded as positive.

[‡] Sera for RAST analyses were obtained from 97 patients seen at the Children's Hospital National Medical Center, Washington, DC, and 50 patients seen at the University of Virginia Children's Medical Center. All patients were $\geq$ age 5 yr.

[§] Sera for control patients were obtained from children ($\geq$ age 5 yr) seen in the University of Virginia Pediatric Emergency Room for a chief complaint that did not involve the respiratory tract.

^{||} An extract of mixed *Aspergillus* species (*fumigatus*, *flavus*, *glaucus*, *nidulans*, and *niger*) was used for skin testing. An extract of *A. fumigatus* was used for the RAST analyses.

[†] Patients in this group had IgE ab to one or more of the five inhalant allergens tested and also had IgE ab to *Aspergillus*.

** Patients in this group had IgE ab to one or more of the five allergens tested, but did not have IgE ab to *Aspergillus*.

sponses to *A. fumigatus* and *Asp f I* in CF patients with respect to age.

METHODS

Population

Sera from 147 patients 5 to 43 yr (mean, 14.4 yr) were analyzed. In all CF patients the diagnosis was established from sweat chloride levels of > 60 mEq/L measured by quantitative iontophoresis. Fifty of these patients (ages 5 to 43 yr; mean, 18.2 yr) were enrolled consecutively during routine visits to the CF clinic at the University of Virginia Children's Medical Center (Charlottesville, VA), and had skin testing together with serum analysis. Informed written consent was obtained from these patients or their parents. In addition, 25 patients evaluated consecutively for asthma (ages 5 to 18 yr; mean, 10.3 yr) in the pediatric allergy clinic and 32 children (ages 5 to 19 yr; mean, 11.1 yr) seen in the emergency room of the medical center for nonpulmonary diagnoses (primarily for trauma or possible appendicitis) and who required venipuncture for management were enrolled as control patients. The protocol for this study was approved by the Investigational Review Board of the University of Virginia Health Sciences Center. Sera stored at -20° C were available from the additional 97 CF patients who were age 5 yr or older (ages 5 to 26 yr; mean, 13.0 yr) and from 25 younger patients (ages 12 mo to 4 yr 11 mo) seen in CF clinics at the Children's Hospital National Medical Center (Washington, DC) and the University of Virginia. Two or more samples, collected at different ages, were also available from 30 of these patients, primarily from children in the younger age group.

Immunoassays

Allergen-specific IgE antibodies in sera were measured with a quantitative radioallergosorbent test (RAST) using cyanogen bromide-activated filter disks coated with either a crude extract of *A. fumigatus* (Greer Laboratories, Lenoir, NC) or one of five other common inhalant allergens. Ten micrograms of protein from a 100% ammonium sulfate cut of the *A. fumigatus* extract, prepared as previously described (13), was coupled to each disc. The other allergens included *Dermatophagoides farinae* mite allergen, cat epithelium, short ragweed pollen, rye grass pollen, and mixed cockroach extracts (Hollister-Stier Laboratories, Spokane, WA). Approxi-

mately 0.1 to 0.5 μ g of major allergen (e.g., *Der f I*, *Fel d I*, *Amb a I*, and *Lol p I* from mite, cat, ragweed, and grass extracts, respectively) were coupled to each disk. The allergen-coated disks were incubated with dilutions (1/4 and 1/10) of patient serum, and IgE ab bound to allergen was detected with 2 ng of affinity-purified ^{125} I-labeled goat anti-human IgE (14). A reference control curve for each assay was established using *D. farinae*-coated disks together with serial twofold dilutions of serum pooled from eight mite-allergic patients. This serum pool was allotted an activity of 1,000 RAST U/ml by reference to a serum pool established at the National Institute of Biological Standards and Control in London (NIBSC 82/528), which contains 1,800 RAST units of IgE antibody to *D. farinae*. Dilutions of sera were done in 50% horse serum in PBS/TWEEN-20, pH 7.5. Two sera from nonallergic patients and two sera from patients who were known to have IgE ab specific for the allergen being tested were run in parallel with each assay. RAST scores of ≥ 40 RAST U/ml (equivalent to 4 ng/ml of allergen-specific IgE ab) were considered positive (14).

Serum IgG and IgE antibodies to the radiolabeled *Asp f I* were measured in an antigen-binding radioimmunoassay (RIA) described previously (12, 15). Precipitins to *Aspergillus* antigens were identified with the standard gel double immunodiffusion template technique, using unconcentrated serum (16). Each serum was tested against four sources of antigen: *A. fumigatus* Antigen #1 (20 mg protein/ml), aspergillin (10 mg protein/ml), and a 1:20 wt/vol *Aspergillus* mix (spp. *fumigatus*, *flavus*, *glaucus*, *nidulans*, and *niger*), each obtained from Greer Laboratories, Lenoir, NC, and a 1:20 wt/vol *Aspergillus* mix from Hollister-Stier Laboratories, Spokane, WA. Measurements of total serum IgE were performed by ELISA as previously described (14).

Skin Testing

Skin-prick testing was done on 50 CF patients and 25 allergic asthmatic patients enrolled at the University of Virginia. The extracts used were a 1:20 wt/vol *Aspergillus* mix, ragweed mix, nine southern grass mix, cat epithelium, and American cockroach extract from Greer Laboratories, Lenoir, NC, and a 1:50 wt/vol *D. farinae* extract from Hollister-Stier Laboratories, Spokane, WA. Histamine (0.25 mg/ml) and saline prick tests were included as positive and negative controls, respectively. The patients had not taken an antihistamine for > 72 h prior to testing. Results were assessed after 15 min. A wheal response with a diameter at least 3 mm greater than that of the negative control was read as positive.

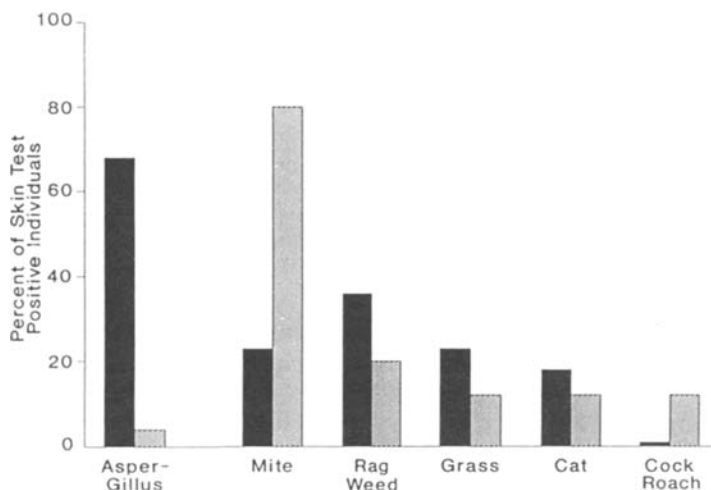


Figure 1. Distribution of skin test results in allergic patients with CF (solid bars) and asthma (hatched bars). Skin-prick tests were positive to one or more allergens tested in 22 of 50 CF patients ($\geq$ age 5 yr) enrolled consecutively at the University of Virginia Health Sciences Center. Results were compared with skin responses in 25 allergic asthmatic children ($\geq$ age 5 yr) tested in the Pediatric Allergy Clinic at the University of Virginia. Allergen extracts used were *Aspergillus*, dust mite (*D. farinae*), ragweed, rye grass, cat, and cockroach.

Sputum

At the time of the patients' enrollment at the University of Virginia, one sample was obtained from all CF patients able to produce sputum. The sample was cultured on Sabouraud's dextrose agar with 100 μ g/ml of gentamicin and 50 μ g/ml of chloramphenicol added to inhibit the growth of *Pseudomonas* (2). The culture plates were incubated at room temperature, and positive fungal cultures were identified in the University of Virginia Hospital microbiology laboratory after 6 wk. A complete blood count was also obtained from each patient to determine the absolute eosinophil count. Counts > 500 cells/mm³ were considered elevated (7).

Pulmonary Function Testing

For the 50 patients enrolled at the University of Virginia, FEV₁ and FVC were measured without bronchodilation on an S-model Vitalograph Spirometer (Kansas City, MO). Results were expressed as a percentage of predicted values for age, sex, race, and height based on the standards of Hsu and colleagues (17). Four patients were unable to undergo spirometry because of acute pulmonary exacerbations, and for these, spirometry values obtained from the previous routine visit were used.

Statistical Analysis

Chi-square analysis was used to compare the difference between percentages of CF patients and asthma patients with IgE ab to *A. fumigatus* and dust mite allergens. A two sample *t* test on the log of geometric means was used to compare the difference between CF patients with and without IgE ab to *A. fumigatus* with respect to titers of IgG ab to *Asp f* I.

RESULTS

Sensitization to *Aspergillus* and Common Inhalant Allergens

In the CF patients, IgE ab specific to *Aspergillus* allergens was detected more often than was IgE ab specific to the other allergens tested (Table 1). Of the 49 patients in whom RAST revealed IgE ab to one or more of the six allergens used, 30 had IgE ab to *Aspergillus*, 17 of whom had IgE ab to *Aspergillus* alone. If one excludes those patients sensitized to *Aspergillus* alone, the prevalence of IgE ab to the six allergens was similar in the CF population and the control group (21% and 16%, respectively). By skin-

test analysis, positive prick tests to *Aspergillus* were observed in 68% of allergic CF patients compared with 4% of patients with allergic asthma (Figure 1). By contrast, 80% of the asthmatic subjects had IgE antibody to *D. farinae* mite allergen, as compared with 23% of the skin-test-positive CF patients. The difference between CF patients and asthma patients with respect to the percentage of individuals with either *Aspergillus* or dust mite sensitivity was highly significant ($p < 0.001$).

Total IgE Levels and Suspected ABPA

Sera from 17 of the 30 patients who had anti-*Aspergillus* IgE ab by RAST had total IgE levels ≥ 400 IU/ml (Table 2). Eight of these patients had IgE ab to *Aspergillus* only. In the absence of IgE to *Aspergillus*, CF patients with inhalant allergy tended to have lower IgE levels. In addition to the 17 patients with total IgE levels ≥ 400 IU/ml, 5 other patients with anti-*Aspergillus* IgE antibody had previous records of total IgE levels over 400 IU/ml. Three of these five patients had never taken steroids. Thus, 22 (73%) of the patients with IgE to *Aspergillus*, or 15% of the 147 patients age 5 yr or older, had had or did have immune responses characteristic of ABPA. Fourteen of these patients were $\leq$ age 10 yr when this first occurred, and five of the patients were age 5 yr or younger. However, the diagnosis of ABPA had been considered clinically by physicians in only eight of the 22 patients.

Of the 50 patients screened with skin-prick tests and assay of their total IgE levels, 15 had a positive test for *Aspergillus* (Table 1) and eight were suspected of having ABPA based on their total IgE levels, while one additional patient had the diagnosis of ABPA made at the age of 2 yr. Only two of the screened patients had a total blood eosinophil count > 500 mm³, and only one of these two had a positive *Aspergillus* skin test and an elevated total IgE titer (i.e., 690 IU/ml). Measurements of FEV₁, FVC, and the FEV₁/FVC ratio did not differentiate the seven patients with suspected ABPA from the other patients.

IgG Antibodies to *A. fumigatus* and Sputum Cultures

A large proportion (84%) of the CF patients had serum IgG antibodies to the allergen *Asp f* I (Figure 2). All 30 CF patients with IgE ab to *Aspergillus* by RAST had IgG anti-*Asp f* I ab. By contrast, only 20% of the allergic patients with asthma and 6% of the children enrolled as healthy control subjects had detectable IgG ab to *Asp f* I. Titers of IgG ab to *Asp f* I were significantly higher in patients with IgE ab to *A. fumigatus* (geometric mean = 164.8 U/ml; 95% confidence limits = 77.5 to 352.1 U/ml) than in CF patients who did not have IgE to *Aspergillus* (geometric mean = 47.8 U/ml; 95% confidence limits = 34.9 to 67.0 U/ml), $p < 0.01$ (Figure 2). Among 21 sera from CF patients who had developed immune

TABLE 2
CORRELATION BETWEEN SERUM IgE ANTIBODY TO INHALANT ALLERGENS AND TOTAL IgE LEVELS IN CYSTIC FIBROSIS

	Total IgE (IU/ml)*		
	> 400	100–400	< 100
Patients with IgE ab to:	(n = 19)	(n = 24)	(n = 104)
One or more allergens tested	18 (95%)	13 (54%)	18 (17%)
Inhalant allergens and			
<i>A. fumigatus</i>	9 (47%)	2 (8%)	2 (2%)
<i>A. fumigatus</i> only	8 (42%)	5 (21%)	4 (4%)
Inhalant allergens only	1 (5%)	6 (25%)	12 (12%)

* Total serum IgE levels were measured using a two-site monoclonal-antibody-based RIA specific for Fc-epsilon (12).

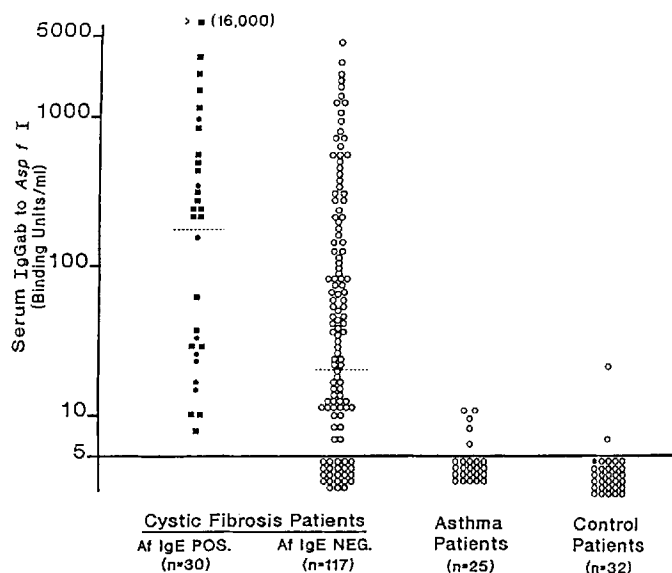


Figure 2. Measurements of IgG antibody to *Asp f I* in sera from 147 patients with CF, 25 patients with asthma, and 32 control subjects. IgG antibodies were measured by radioimmunoprecipitation. Sera with IgE antibodies to *A. fumigatus* by RAST are indicated by solid symbols (i.e., Af IgE POS). Solid squares indicate patients who also developed a total IgE level ≥ 400 IU/ml. Open circles represent patients who were RAST negative to *A. fumigatus* (i.e., Af IgE NEG). Sera with ≤ 5 U/ml of IgG ab to *Asp f I* (i.e., less than 2 SD above the mean IgG binding to *Asp f I* in control sera) were regarded as negative. Geometric means (hatched lines) for CF patients who were Af IgE POS and Af IgE NEG were 164.8 U/ml and 47.8 U/ml, respectively, and were significantly different, at $p < 0.01$.

responses suggesting ABPA, 57% also showed IgE ab binding to *Asp f I* by antigen-binding RIA.

Precipitins were detected in 15 of the 147 sera tested, and 12 of these reacted with antigens present in the *A. fumigatus* Antigen #1 extract. All sera with precipitins also had IgG ab to *Asp f I*. Of these sera, eight had IgE to *A. fumigatus* by RAST, and six of these had total IgE levels > 400 IU/ml. Forty of the 50 CF patients enrolled for skin testing were able to produce sputum. *Aspergillus* species were grown in 18 specimens (45%), but only three of these came from patients who had total IgE ab ≥ 400 IU/ml. *A. fumigatus* was identified in 50% of the positive cultures but was found only in patients who had high titers (> 100 U) of IgG ab to *Asp f I*.

Antibody Responses to *Aspergillus* in Young Patients

Prior to age 3, the prevalence of IgG ab to the allergen *Asp f I* was low (13%) (Figure 3). After age 3, a marked increase occurred in the percentage of patients with IgG anti-*Asp f I* ab, such that after age 10, 98% of patients had detectable antibody responses. A progressive increase with age in titers of IgE ab to *Aspergillus* by RAST was also evident in sera from 58 CF patients $\leq$ age 10 (Figure 4A). Fifteen of these patients had developed total IgE levels ≥ 400 IU/ml, 14 of whom had IgE ab to *Aspergillus* (Figure 4B). Titers of IgE ab to *Aspergillus* in serial serum samples were available from nine of these patients (Figure 4A). Six of these patients developed titers > 200 RAST U/ml, as well as total IgE levels ≥ 400 IU/ml. Five of these patients developed these levels by age 5 (Figure 4B).

DISCUSSION

Allergens produced by *A. fumigatus*, as well as others by *Aspergillus* species, have been shown to be potent stimuli for IgE ab

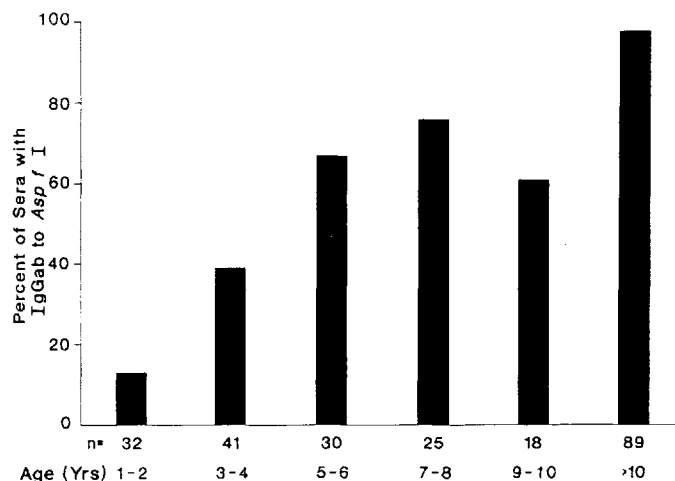


Figure 3. Prevalence with respect to age of serum IgG ab to *Asp f I* in patients with CF. Sera from 147 patients $\geq$ age 5 yr together with sera from 25 patients $<$ age 5 yr were analyzed. Two or more samples, obtained at different ages, were available from 30 of the patients. The age ranges indicated along the abscissa span periods of 2 yr (e.g., 1-2 is the same as 12 mo through 2 yr, 11 mo).

production in patients with CF. Our results demonstrate further that these vigorous IgE responses can develop at an early age, as do IgG ab to the *A. fumigatus* allergen and cytotoxic protein *Asp f I*. Twenty-two (15%) of our patients older than age 5 developed IgE ab to *Aspergillus* in association with high total levels of IgE ab (≥ 400 IU/ml) and IgG ab, suggesting the diagnosis of ABPA. Five of these patients developed these immune responses by the age of 5 yr, and 14 patients had them by the age of 10 yr.

As in previous reports, the CF patients were not sensitized to the inhalant allergens tested (mite, cat, cockroach, rye grass, and ragweed) more often than were control subjects representing the general population (5, 8). The proportion of CF patients with IgE antibody to dust mite allergen was significantly less than that of allergic children with asthma, while a much larger percentage of the CF patients had IgE antibody to *A. fumigatus*. While the CF patients were not tested for hypersensitivity to other mold spores that could also be inhaled, previous studies have shown that the high frequency of IgE antibodies to fungi in CF patients can be attributed primarily to sensitization to *Aspergillus* (2, 5).

In patients with asthma, ABPA is considered when IgE ab to *Aspergillus* and high total IgE ab titers occur together with other clinical and immunologic manifestations of the disease (e.g., wheezing, recurrent pulmonary infiltrates, central bronchiectasis, peripheral eosinophilia, and serum precipitins) (7). In patients with CF, however, the diagnosis is more challenging. First, recurrent pneumonic infiltrates and bronchiectasis occur frequently, making it difficult to judge the pulmonary manifestations of ABPA with standard X-ray techniques (4). Second, airway hyperreactivity requiring treatment with bronchodilators and sometimes steroids is also common in CF, and is often associated with infectious processes (19). Third, CF patients with serologic evidence of ABPA can show variable immune responses to *Aspergillus* (e.g., loss of precipitins and fluctuations in the levels of serum IgE and IgG ab to *Aspergillus*), as well as a marked decline in total IgE levels over time, without having been treated with steroids (20). Furthermore, elevated total IgE levels and peripheral blood eosinophilia are not always a marked feature in CF patients with suspected ABPA (3-5). In keeping with these diagnostic problems, 22 patients in our study developed the antibody characteristics of ABPA,

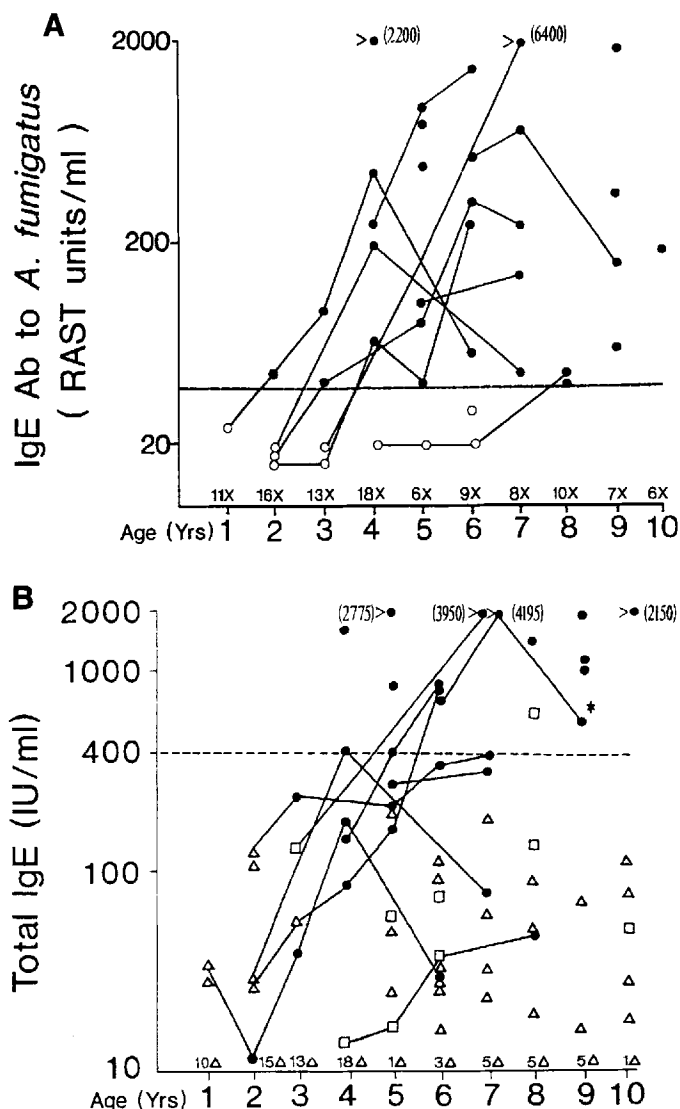


Figure 4. (A) Development of IgE antibodies to *A. fumigatus* in patients with CF. Results are shown for all sera tested from children 10 yr of age or younger. Results of serial serum samples available from nine patients are shown by connected circles. Closed circles represent RAST measurements ≥ 40 U/ml (i.e., above hatched line), and were regarded as positive. Numbers along the abscissa followed by an X represent sera tested at each age with measurements < 20 RAST U/ml. (B) Development of total IgE levels in patients with CF. Results are shown for all sera tested in (A), with serial samples represented by connected symbols. Sera that were positive by RAST to *A. fumigatus* are shown as closed circles. Those that were RAST negative to *A. fumigatus* but positive to one or more of the five other allergens tested are indicated by open squares, and those negative to all six allergens are indicated by open triangles. Asterisk denotes a decline in total IgE observed after treatment of one of the patients with steroids. Numbers along the abscissa followed by a triangle represent sera tested at each age with < 10 IU/ml of total IgE.

but only eight were clinically suspected of having the disorder, and three had a spontaneous decline in their total IgE levels without steroid treatment.

Although it can be difficult to culture *Aspergillus* fungi from sputum obtained from CF patients, *Aspergillus* is reported to colonize and persist in the respiratory tract (3, 5). The ability of this fungus to survive and grow at human body temperature makes exposure to *Aspergillus* in the respiratory tract the most likely explanation for the high incidence of IgE and IgG ab to *Aspergillus*

allergen in CF patients. In accord with a high incidence of exposure, 89% of patients in our study had serum IgG ab to *Asp f* I allergen which was very high compared with those in previous reports of serum IgG ab responses to *Aspergillus* in CF patients as measured by precipitins or ELISA (2–5, 7–10). Furthermore, the detection of IgG ab to *Asp f* I in the sera from 39% of our patients aged 5 yr or younger strongly suggests that exposure to *Aspergillus* fungi begins at an early age. It is possible that genetic factors could predispose CF patients to respond to *Aspergillus*. However, the CF gene is localized on chromosome 7 (21, 22), whereas allergen-specific IgE ab responses to ragweed and rye grass allergens have been reported to be associated with HLA-DR loci located on chromosome 6 (23). A gene locus associated with increased IgE ab responses to inhaled allergens has been reported on chromosome 11 (24). Thus, a linkage disequilibrium between CF and allergen-specific immune responses appears to be unlikely on the basis of current information.

The predilection of *Pseudomonas aeruginosa* for the lungs of patients with CF is well recognized (25). The same might be proposed for *Aspergillus* fungi, considering the high rate of immune responses to *Asp f* I and growth of *Aspergillus* in 45% of sputum cultures in our study. Recently, *Asp f* I has been shown to be an 18 kD secreted protein (15). It is also a potent inhibitor of protein synthesis (26). Other toxic agents have also been reported in *A. fumigatus* extracts, including metabolites with antiphagocytic properties (27–29) and a proteinase able to induce epithelial-cell detachment from basement membranes (30). Although local factors in the lungs of CF patients may create a suitable growth environment for *Aspergillus*, the secretion of toxins by this organism, including *Asp f* I, may further enhance the ability of *Aspergillus* to colonize and persist in the respiratory tract. Thus far, the potential pathologic consequences of *Aspergillus* infection have focused on CF patients who develop vigorous IgE antibody responses to the organism. The data in the current study raise the question of whether exposure to *Aspergillus* and its secreted products may contribute to chronic and gradual pulmonary deterioration in CF.

In practice, the diagnosis of ABPA is often suspected in CF patients who have a positive skin test and/or serum IgE ab to *Aspergillus*, together with a high total IgE level, particularly when a lung infiltrate is present and the patient's respiratory status fails to improve with appropriate antibiotic and supportive therapy. Oral steroids still represent the basic approach to management in these patients, even though long-term courses of treatment with these drugs have well-known complications. As yet there is little information about the efficacy of oral antifungal agents, such as ketoconazole and itraconazole, in treating CF patients with suspected ABPA. Additionally, *Aspergillus* may have pathogenic consequences in the lungs of patients with CF who do not have findings characteristic of ABPA, since the organism is able to survive in the respiratory tract and secrete cytotoxic metabolites such as *Asp f* I. In the future, the use of antifungal medications may help clarify the pathophysiologic role of *Aspergillus* in CF patients who develop immune responses to *A. fumigatus* at an early age.

Acknowledgment: We are very grateful to Eliza T. Platts-Mills for technical assistance and to Steven J. Savitt and Marcella A. Wise for help in preparing this manuscript.

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