
Case report

Allergic bronchopulmonary aspergillosis in a patient with cystic fibrosis: diagnostic criteria when the IgE level is less than 500 IU/mL

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Background: Recently, the Cystic Fibrosis Foundation developed a consensus report recommending diagnostic criteria for allergic bronchopulmonary aspergillosis (ABPA) in patients with cystic fibrosis that includes a serum IgE level greater than 500 IU/mL as the “minimal diagnostic criterion.”

Objective: To describe a 7-year-old girl with ABPA whose serum IgE level increased to only 398 IU/mL.

Methods: Total IgE and anti-*Aspergillus* serologic measurements were performed using enzyme-linked immunosorbent assay and standard laboratory techniques; HLA analysis was performed; interleukin 4 receptor α single nucleotide polymorphisms were performed using polymerase chain reaction and DNA sequencing; CD23⁺ B cells were measured using flow cytometry; and cytokine synthesis to *Aspergillus* purified antigens was assessed using flow cytometry.

Results: A 7-year-old girl with cystic fibrosis who had mild pulmonary disease and well-controlled asthma developed pulmonary infiltrates, increased wheezing, and decreased pulmonary function. Additional studies demonstrated peripheral blood eosinophilia (eosinophil count, 1,807 cells/mm³ [19%]) and an increase in IgE and IgG anti-*Aspergillus* serology; bronchoalveolar lavage revealed septate hyphae with 45° branching subsequently identified as *A fumigatus* and pulmonary eosinophilia. Previous HLA typing revealed that the patient was HLA-DR2+, DRB*1501, HLA-DQ2–, a pattern associated with increased risk of ABPA. In addition, there was increased up-regulation of CD23 molecules by interleukin 4 stimulation on the patient's B cells, as observed in ABPA. The patient was treated with corticosteroids and itraconazole with resolution of symptoms and pulmonary infiltrates.

Conclusions: Examination of the pulmonary inflammatory response using bronchoalveolar lavage, genetic risk with HLA-DR2+DQ2– typing, and increased interleukin 4 sensitivity are useful adjunctive studies in the diagnosis of ABPA.

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INTRODUCTION

Allergic bronchopulmonary aspergillosis (ABPA) occurs in approximately 1% to 2% of patients with asthma and in 7% to 9% of patients with cystic fibrosis (CF).^{1–3} However, the prevalence of ABPA in patients with CF may vary among different medical centers and has been reported to be as low as 1% to 2% (personal communication). In ABPA, *Aspergillus fumigatus* spores are inhaled and germinate in the bronchial airway, eliciting an inflammatory response that is characterized by an eosinophilic and T_H2 T-cell response to *A fumigatus* allergens. In addition, IgE, IgA, and IgG anti-*A fumigatus* antibodies contribute to the inflammatory reaction

via mast cell degranulation and probably natural killer cell activity.⁴ Skin test reactivity to *Aspergillus* is found in 20% to 25% of patients with asthma^{2–6} and in 31% to 59% of patients with CF.^{7,8} Although sensitization is common in these populations, only a small percentage of patients develop ABPA. A central question then is how patients with ABPA differ from *Aspergillus*-sensitive atopic patients with asthma and CF. We hypothesize that ABPA develops in genetically susceptible individuals with asthma and CF because of increased frequency or activity of *A fumigatus*-specific T_H2 CD4⁺ cells. We further propose that polymorphisms of the interleukin 4 receptor α (IL-4R α) subunit and HLA-DR2/DR5 are genetic susceptibility risk factors responsible for the development of ABPA.

The diagnosis of ABPA can be difficult in patients with CF because of overlapping diagnostic criteria for ABPA and pulmonary complications of CF. Although sensitization is common in these populations, only a small percentage of patients develop ABPA. Pulmonary exacerbations with radiographic infiltrates due to bacterial endobronchitis are a

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major problem in CF resulting in pulmonary fibrosis and bronchiectasis. Because colonization and sensitization to *A fumigatus* is a major problem in patients with CF that may lead to the development of ABPA, which also results in pulmonary fibrosis or bronchiectasis, the diagnosis of ABPA is of high importance. Recently, the Cystic Fibrosis Foundation⁹ developed a consensus report that recommended the following diagnostic criteria for ABPA in patients with CF:

1. Acute or subacute clinical deterioration not attributable to another cause: cough, wheeze, exercise intolerance, exercise-induced asthma, decline in pulmonary function, or increased sputum.

2. Total serum IgE level greater than 1,000 IU/mL in a classic case, and greater than 500 IU/mL meets the "minimal diagnostic criteria." If ABPA is suspected and the total IgE level is 200 to 500 IU/mL, repeated testing in 1 to 3 months is recommended.

3. Immediate cutaneous reactivity to *A fumigatus* or serum IgE anti-*A fumigatus* antibody.

4. Precipitating antibodies to *A fumigatus* or serum IgG anti-*A fumigatus* antibody.

5. New or recent abnormalities on chest radiography (infiltrates or mucus plugging) or chest computed tomography (bronchiectasis) that have not cleared with antibiotic drug therapy and standard physiotherapy.

Noted in these guidelines is that ABPA should be considered when the total serum IgE level is greater than 500 IU/mL and that ABPA is diagnostic when the serum IgE level is greater than 1,000 IU/mL along with pulmonary disease and evidence of IgE sensitization to *A fumigatus*. When the serum IgE level is 200 to 500 IU/mL, the panel recommended repeated testing. However, we contend, as other researchers have, that ABPA may develop when the IgE level is less than 500 IU/mL.

The following case report illustrates a young patient with CF who developed ABPA but whose serum IgE level was less than 500 IU/mL. The report demonstrates the utility of identifying the pulmonary eosinophilic inflammatory reaction and the genetic risk factors in the development of ABPA. Specifically, Chauhan et al¹⁰⁻¹² reported that patients with

ABPA and either asthma or CF were HLA-DR2+ or HLA-DR5+ (or to a lesser extent HLA-DR4+ and HLA-DR7+) and were HLA-DQ2-. In addition, it was recently reported that patients with ABPA have increased sensitivity to IL-4 stimulation, measured by the up-regulation of CD23 expression on B cells.^{13,14} Recently, it was reported that this increased sensitivity is due to single nucleotide polymorphisms (SNPs) of the IL-4R α , identified in 92% of patients with ABPA and either asthma or CF.^{15,16} Specifically, IL-4R α SNPs were present in 92% (12/13) of the patients with CF compared with 54% of the patients without CF. The ile75val SNP was the principal polymorphism identified. This patient had genetic risk factors for the development of ABPA, namely, HLA-DR2+ DQ2- and IL-4R α SNPs, which aided in the diagnosis of ABPA.

CASE REPORT

The patient is a 7-year-old girl with CF who had mild pulmonary disease. She developed episodes of wheezing approximately 2 years earlier that were well controlled with inhaled fluticasone-salmeterol inhalations (Advair; GlaxoSmith-Kline, Research Triangle Park, NC). Beginning in June 2003, she developed a right-sided lower lobe infiltrate and increased wheezing, but her pulmonary function remained normal (Table 1). In July 2003, *Aspergillus* but no bacterium was obtained from a forced sputum culture. Symptom cultures before and after the development of ABPA only occasionally isolated *Serratia marcescens*. By August 2003, there were increased pulmonary infiltrates, increased wheezing, and decreased forced expiratory flow between 25% and 75%. A bronchoalveolar lavage performed on July 30, 2003, showed a predominantly eosinophilic inflammatory response (Table 2). In addition, peripheral blood eosinophilia, 1,807 cells/mm³ (19%), was also noted. Septate hyphae with 45° branching were demonstrated by periodic acid-Schiff and Gomori methenamine silver stains and were subsequently identified as *A fumigatus*.

A diagnosis of ABPA was suspected; however, her serum IgE level increased only from 58 IU/mL in November 2002 to 398 IU/mL in August 2003 (Table 2). In November 2002,

Table 1. Findings From Pulmonary Function Studies, Chest Radiographs, and Sputum Cultures in a Patient With ABPA

	Study Date						
	6-23-03	7-21-03	8-15-03	10-27-03	11-24-03	12-30-03	1-19-04
FVC, %	NM	109	NM	101	109	100	117
FEV ₁ , %	NM	104	NM	86	98	86	110
FEF ₂₅₋₇₅ , %	NM	79	NM	47	65	52	93
Chest radiograph	↑ RLL	↓ RLL	↑ RML, RLL	NM	NM	Infiltrates cleared	NM
Sputum cultures	NM	<i>Aspergillus</i> , no bacteria	NM	<i>Serratia marcescens</i>	NM	NM	NM
Corticosteroids	NM	NM	+ (8-22-03*)	—	—	+	—
Itraconazole	NM	NM	+	+	+	+	+

Abbreviations: ABPA, allergic bronchopulmonary aspergillosis; FEF₂₅₋₇₅, forced expiratory flow between 25% and 75%; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; NM, not measured; RLL, right-sided lower lobe infiltrates; RML, right-sided middle lobe infiltrates; +, treated with medication; —, not treated with medication; ↑, increased; ↓, decreased.

* The date that corticosteroid drug therapy was initiated.

Table 2. Immunologic Studies in a Patient With CF Who Developed ABPA

	Study Date					Profile in	
	11-19-02	7-30-03	8-11-03	10-27-03	1-19-04	ABPA	Non-ABPA
IgE, IU/mL	58	NM	398	185	354	>1,000 (classic) >500 (minimal)	<500 200-500 repeat
<i>Aspergillus</i> skin test	+++	NM	+++	NM	NM	+	+ or -
<i>Aspergillus</i> precipitin	0	NM	++++	NM	NM	+	-
IgE anti- <i>Aspergillus</i> , μg Ab/mL	1.6	NM	4.2	NM	NM	44.6 ± 10.8*	14.0 ± 12.3*
IgG anti- <i>Aspergillus</i> , μg Ab/mL	37.9	NM	126	NM	NM	75.3 ± 15.7*	35.6 ± 4.4*
HLA typing							
HLA-DR	DRB*1501/1300	NM	NM	NM	NM	HLA-DR2+/DR5+	NM
HLA-DQ	DRB*0602/0603	NM	NM	NM	NM	HLA-DQ2-	HLA-DQ2+
CD23 molecules/10 ng/mL of IL-4	8,521	NM	NM	10,465	NM	9,835 ± 1,437*	5,627 ± 1,075*
IL-4Rα polymorphisms	NM	NM	NM	NM	asn98thr	75val, 400ala, 503pro, 576arg	NM
BALF	NM	<i>Aspergillus</i> 71% eos 20% neut 7% lymph 2% mac	NM	NM	NM	Eos, mac, lymph	NM
WBC count/mm ³	NM	NM	9,510	NM	NM	NM	NM
Eos, cells/mm ³ (%)	NM	NM	1,807 (19)	NM	NM	>500	NM

Abbreviations: Ab, antibody; ABPA, allergic bronchopulmonary aspergillosis; BALF, bronchoalveolar lavage fluid; CF, cystic fibrosis; eos, eosinophils; IL-4Rα, interleukin 4 α chain receptor; lymph, lymphocytes; mac, macrophages; neut, neutrophils; NM, not measured; WBC, white blood cell; 0 to +++++, a range from negative to strongly positive.

* Data are given as mean ± SE.

her skin prick test reaction to *A fumigatus* was positive, and the serum IgE and IgG anti-*A fumigatus* levels were low, and *A fumigatus* precipitins were negative. At the time ABPA was suspected, the *A fumigatus* precipitins had become strongly positive and the specific IgE and IgG anti-*A fumigatus* antibody levels had increased (Table 2).

Previously, HLA-DR and HLA-DQ typing had revealed that the patient was HLA-DR2+, DRB*1501, and HLA-DQ2-, DRB*602/603, a pattern that placed her at risk for ABPA (Table 2). In addition, we evaluated whether there was increased up-regulation of CD23 on her B cells by IL-4 stimulation, which has previously been reported in patients with ABPA. There was increased sensitivity to IL-4 stimulation of CD23 expression in B cells in the patient before (data not shown) and after (Figure 1) the development of ABPA. Subsequently, the extracellular ile75val, glu400ala, cys431arg, ser503leu, and gln576arg SNPs of the IL-4Rα were examined and were normal. We then identified a new SNP in the IL-4 binding region, 468 A→C, resulting in an asn98thr SNP. Although this SNP has not been previously identified, this patient demonstrated increased up-regulation of CD23 expression by IL-4 stimulation, eg, gain-of-function, as observed in other patients with ABPA. The patient was treated with 2 courses of 21 days of prednisone and itraconazole. The pulmonary infiltrates cleared, pulmonary function test results returned to normal baseline, and wheezing resolved (Table 1).

DISCUSSION

In the immunopathogenesis of ABPA, *Aspergillus* spores measuring 3 to 5 μm are inhaled and germinate deep within the bronchi into hyphae.⁴ The bronchoalveolar-associated lymphoid tissue initiates and maintains an immune response to *A fumigatus*, with subsequent trafficking to the peripheral lymphoid system.¹⁷⁻²⁰ Analysis of cells obtained from bronchoalveolar lavage fluid (BALF) in ABPA reveals that they are an admixture of alveolar macrophages, eosinophils, and lymphocytes similar to that found in asthma. Eosinophil infiltration is predominant in BALF and lung tissue, as is evident in lung biopsy samples.²¹ In addition, eosinophils are activated and have released their mediators, such as major basic protein. In our patient, BALF demonstrated the presence of a predominantly eosinophilic inflammatory reaction and hyphae of *A fumigatus*. In contrast, bacterial endobronchitis causing a CF pulmonary flare would have demonstrated a neutrophilic response.

In human and murine models, T_H2 CD4⁺ T cells and their cytokines are central to the development of ABPA.^{1,22,23} Both IL-4 and IL-13 induce an IgE isotype switch of B cells to IgE synthesis. Also, IL-4 induces CD23⁺ expression, the low-affinity IgE receptor, and soluble CD23, which augments B-cell IgE synthesis. In Asp f1 T-cell lines, the phenotypes were CD4⁺CD25⁺ T cells and had the cytokine profiles IL-4+ and interferon γ-, indicating T_H2 CD4⁺ T cells.¹ In

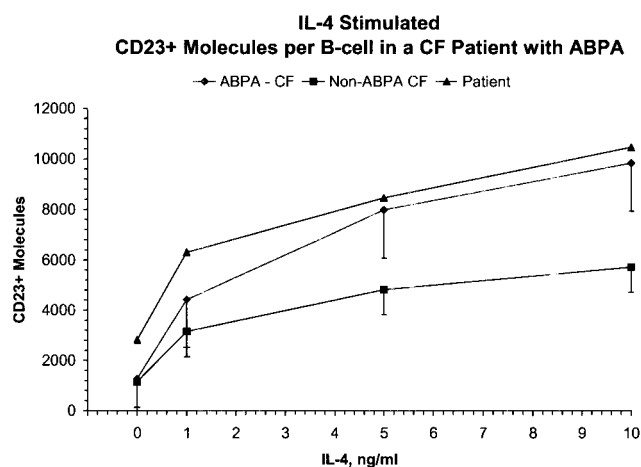


Figure 1. Mean up-regulation of CD23⁺ molecules by interleukin 4 (IL-4). After IL-4 stimulation, patients with allergic bronchopulmonary aspergillosis (ABPA) and cystic fibrosis (CF) had a significantly increased rate of CD23⁺ expression per B cell compared with non-ABPA CF ($P < .01$) and nonatopic control patients (data not shown). The patient before and after the development of ABPA demonstrated increased sensitivity to IL-4 stimulation similar to patients with ABPA. Error bars represent SD.

patients with ABPA and CF, the cytokine pattern to Asp f2-, Asp f3-, and Asp f4-stimulated 7-day mononuclear cell cultures demonstrated significantly increased IL-5 synthesis compared with non-ABPA and nonatopic controls. Interleukin 10 synthesis was increased in all patient groups to Asp f2, Asp f3, and Asp f4 stimulation but not to tetanus toxoid stimulation. Interleukin 10 is an anti-inflammatory cytokine synthesized by a variety of cell types, including monocytes, T_H1 and T_H2 cells, and bronchial epithelial cells. It regulates T_H1 and T_H2 lymphocytes, although there is a preferential inhibition of T_H1 responses.²⁴

In addition to up-regulating CD23 expression, IL-4 seems to up-regulate CD86 expression in B cells in atopic patients.^{25–28} This is important because CD86 in B cells is an important co-stimulatory molecule for the augmentation of IgE synthesis. The ligand of CD86 is CD28 on T cells. CD86 and CD28 colligation also stimulates T cells, promoting T_H2 CD4⁺ T-cell responses and cytokine synthesis, eosinophil airway inflammation, and airway hyperresponsiveness after allergen challenge. Recently, we examined the effects of IL-4 stimulation of CD23 and CD86 expression on the B cells of patients with ABPA. In previous studies,²⁹ purified B cells from ABPA were observed to spontaneously synthesize elevated amounts of IgE compared with *A fumigatus*-sensitive non-ABPA patients, indicating in vivo stimulation into IgE-secreting immunoblasts. In recent studies, CD23 and CD86 expression on freshly isolated B cells of patients with ABPA and this patient were increased compared with non-ABPA *A fumigatus*-sensitive patients, suggesting in vivo IL-4 or IL-13 stimulation. After IL-4 stimulation, patients with ABPA had significantly increased rates of CD23 expression per B cell

compared with atopic and nonatopic individuals, as seen in this patient. Thus, patients with ABPA had increased sensitivity to IL-4 stimulation with up-regulation of CD23 and CD86 expression compared with other atopic individuals such that ABPA > atopic >> nonatopic patients. The consequences of repeated allergic inflammation in ABPA are central bronchiectasis and fibrosis.

The IL-4R is a type I cytokine receptor and exists as a heterodimer that shares a subunit, IL-4R α , with the IL-13R α .³⁰ There are 2 types of IL-4Rs. Type I receptors, found on all lymphohematopoietic cells, are composed of the IL-4R α and the common γ chain, which is also a component of IL-2, IL-7, IL-9, IL-15, and IL-21 cytokine receptors. The type II receptor, also known as IL-13R, is formed by the association of IL-4R α with the IL-13R α subunit and is located on immune cells, bronchial epithelium, and vascular endothelium. Interleukin 4 stimulates type I and type II receptors, whereas IL-13 signals only through type II receptors. In 1997, Hershey et al³¹ initially reported on the high prevalence of atopy and a gain-of-function in the IL-4R as measured by increased CD23 expression in patients with the gln576arg SNPs of the IL-4R α . Eight naturally occurring SNPs of the IL-4R α gene have been reported thus far: ile75val, glu400ala, cys431arg, ser436leu, ser503pro, gln576arg, ser752ala, and ser786pro (numbering system including the 25-amino acid signal peptide).^{31–39} A subsequent study³⁹ found that the presence of these 2 variants (val75 and arg576) together resulted in elevated IL-4-dependent CD23 expression that was not observed when these SNPs were present alone. In preliminary studies, the ile75val SNP was observed in 87% of patients with ABPA, and cytoplasmic SNPs were observed in 22%.¹⁵ Thus, IL-4R α SNPs resulting in increased sensitivity to IL-4 stimulation may be a genetic risk factor in the development of ABPA. Indeed, we identified a new IL-4R α SNP in the IL-4 binding region, asn98thr, that was associated with the up-regulation of IL-4 stimulation.

However, SNPs of the IL-4R α do not explain the specificity to *Aspergillus* in ABPA. Chauhan et al^{10–12} identified that there is HLA-DR2 and HLA-DR5 restriction in patients with ABPA. Furthermore, within HLA-DR2 and HLA-DR5, there are restricted genotypes. In particular, HLA-DRB1*1501 and HLA-DRB1*1503 was reported to provide high relative risk. On the other hand, 40% to 44% of non-ABPA atopic *A fumigatus*-sensitive individuals have the HLA-DR2 or HLA-DR5 type. Further studies indicated that the presence of HLA-DQ2 (especially DQB1*0201) provided protection from the development of ABPA. These results are similar to those found with purified house dust mite allergens.^{40–42} Thus, certain genotypes of HLA-DR2 and HLA-DR5 may be necessary but not sufficient to cause ABPA. This patient was identified to be HLA-DR2+DQ2- before the development of ABPA and was known to be at risk for ABPA.

In summary, we hypothesize that patients with ABPA have genetic risk factors—HLA-DR restriction and IL-4R α

SNPs—that, when colonized with *A. fumigatus*, are prone to develop ABPA. The bronchial pathologic process is characterized by a T_H2 eosinophilic IgE-mediated pulmonary inflammatory reaction. All of these mechanisms were demonstrated in this patient. Thus, an understanding of the immunopathogenesis of ABPA allows for additional criteria in the diagnosis and perhaps treatment of ABPA in questionable cases.

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