

## *Retraction*

# **Retracted: Immunopathology and Immunogenetics of Allergic Bronchopulmonary Aspergillosis**

### **Journal of Allergy**

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The paper titled “Immunopathology and Immunogenetics of Allergic Bronchopulmonary Aspergillosis” [1], published in *Journal of Allergy*, has been retracted as it was found to contain a substantial amount of material from the following published article: “Allergic bronchopulmonary aspergillosis in asthma and cystic fibrosis” published in *Clinical and Developmental Immunology*, vol. 2011, Article ID 843763, 13 pages, 2011, DOI: 10.1155/2011/843763.

### **References**

- [1] A. P. Knutsen, “Immunopathology and immunogenetics of allergic bronchopulmonary aspergillosis,” *Journal of Allergy*, vol. 2011, Article ID 785983, 9 pages, 2011.

## Review Article

# Immunopathology and Immunogenetics of Allergic Bronchopulmonary Aspergillosis

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Allergic bronchopulmonary aspergillosis (ABPA) is a Th2 hypersensitivity lung disease in response to *Aspergillus fumigatus* that affects asthmatic and cystic fibrosis (CF) patients. Sensitization to *A. fumigatus* is common in both atopic asthmatic and CF patients, yet only 1%–2% of asthmatic and 7%–9% of CF patients develop ABPA. ABPA is characterized by wheezing and pulmonary infiltrates which may lead to pulmonary fibrosis and/or bronchiectasis. The inflammatory response is characterized by Th2 responses to *Aspergillus* allergens, increased serum IgE, and eosinophilia. A number of genetic risks have recently been identified in the development of ABPA. These include HLA-DR and HLA-DQ, IL-4 receptor alpha chain (*IL-4RA*) polymorphisms, *IL-10* –1082GA promoter polymorphisms, surfactant protein A2 (*SP-A2*) polymorphisms, and cystic fibrosis transmembrane conductance regulator gene (*CFTR*) mutations. The studies indicate that ABPA patients are genetically at risk to develop skewed and heightened Th2 responses to *A. fumigatus* antigens. These genetic risk studies and their consequences of elevated biologic markers may aid in identifying asthmatic and CF patients who are at risk to the development of ABPA. Furthermore, these studies suggest that immune modulation with medications such as anti-IgE, anti-IL-4, and/or IL-13 monoclonal antibodies may be helpful in the treatment of ABPA.

## 1. Introduction

Allergic bronchopulmonary aspergillosis (ABPA) is a hypersensitivity lung disease due to bronchial colonization by *Aspergillus fumigatus* that occurs in susceptible patients with asthma and cystic fibrosis (CF). The first published description of ABPA as an entity came from the United Kingdom in 1952 [1], while the first cases in the United States were reported a decade later [2, 3]. ABPA affects approximately 1%–2% of asthmatic patients and 7%–9% of CF patients [4–6]. If unrecognized or poorly treated, ABPA leads to airway destruction, bronchiectasis, and/or pulmonary fibrosis, resulting in significant morbidity and mortality.

The diagnosis of ABPA is based on clinical and immunologic reactivity to *A. fumigatus*. The minimal criteria required for the diagnosis of ABPA are: (1) asthma or cystic fibrosis with deterioration of lung function, for example, wheezing, (2) immediate *Aspergillus* skin test reactivity, (3) total serum IgE  $\geq$  1000 IU/mL, (4) elevated *Aspergillus* specific IgE and

IgG antibodies, and (5) chest radiographic infiltrates. Additional criteria may include peripheral blood eosinophilia, *Aspergillus* serum precipitating antibodies, central bronchiectasis, and *Aspergillus* containing mucus plug production [7–11]. The designation of ABPA-seropositive (ABPA-S) may be used to classify asthmatic patients who meet the required criteria but lack the proximal or central bronchiectasis (ABPA-CB). High-resolution computed tomography (HRCT) may demonstrate central bronchiectasis in the inner two thirds of the field even in the absence of chest radiograph lesions. The clinician should note that the development of ABPA is not dependent on asthma severity. The diagnosis of ABPA in CF is more complicated and disagreement exists in the literature regarding the diagnostic criteria. The difficulty lies in the fact that the usual criteria for ABPA and the common signs and symptoms of CF overlap. The most recent Cystic Fibrosis Foundation Consensus Conference proposed the following diagnostic criteria: (1) acute or subacute pulmonary deterioration not attributable to another etiology,

(2) total serum IgE >1000 IU/mL, (3) immediate cutaneous reactivity to *Aspergillus* or *in vitro* specific IgE antibodies to *Aspergillus*, and (4) one of the following: *Aspergillus* serum precipitins, elevated specific IgG anti-*Aspergillus* antibodies, new or recent chest radiographic, or chest CT abnormalities that have not cleared with antibiotics and chest physiotherapy [12].

**1.1. Radiographic and Laboratory Investigations.** There are several characteristic radiographic abnormalities associated with ABPA [7–11]. The most common lesion is a large, homogeneous shadow in one of the upper lobes with no change in volume. The shadow may be triangular, lobar, or patchy, and it frequently moves to another site. “Tram-line” shadows are fine parallel lines radiating from the hila that represent inflammation of airway walls. Mucoïd impaction causes toothpaste shadows or gloved-finger shadows, which can be seen on plain radiograph.

Adult patients have been reported with normal chest radiographs so radiographic abnormalities are not invariably present. In these individuals, HRCT scan may reveal central cylindrical bronchiectasis even in the absence of chest radiograph abnormalities. Sometimes, “tree-in-bud pattern” may be seen on HRCT scan that indicates some degree of airway mucus plugging. It is more commonly considered evidence of atypical mycobacterial infection and may be seen in cystic fibrosis. However, central bronchiectasis is a common complication and finding in all CF patients.

Laboratory tests that support the diagnosis of ABPA are those that demonstrate allergy to the *A. fumigatus*, such as elevated specific IgE anti-*Aspergillus* antibodies and positive *Aspergillus* precipitins [7–11]. Culture of *A. fumigatus* from the sputum is only a secondary criterion for the diagnosis of ABPA, because a large proportion of individuals with CF without ABPA have *Aspergillus* on sputum cultures. Some normal individuals and many individuals with lung diseases have small numbers of spores in their sputum; these are probably present because of passive inhalation. The presence of hyphae is more specific, and the presence of eosinophils in association with hyphal elements is suggestive of the diagnosis. At the time of radiographic exacerbation, the presence of eosinophilia in sputum or blood is suggestive of ABPA in asthmatics and is a primary diagnostic criterion. The peripheral blood eosinophil count is usually greater than 1000/mm<sup>3</sup>, and values greater than 3000/mm<sup>3</sup> are common. Eosinophilia is not a diagnostic criteria of ABPA in CF patients. In the authors' experience, eosinophilia is an uncommon finding in CF ABPA patients.

An increased total serum IgE level is very characteristic of ABPA, and values may reach as high as 30,000 IU/mL. Usually, the level is greater than 1000 IU/mL. Much of the IgE is not specific to *Aspergillus* but is the result of polyclonal B-cell activation. The IgE level is a very useful marker of disease activity, and it can be used to follow outpatients for “flares”. The simple skin-prick test is a useful screening test, as ABPA is very unlikely in patients with a negative reaction. A dual-reaction skin test with an immediate (10–15 minutes) and a late (4–8 hours) reaction may occur but is uncommon in ABPA. Alternatively, serum may be

measured for the presence of specific IgE and IgG antibodies. Patients with *Aspergillus*-sensitive asthma will generally have elevated *Aspergillus*-specific IgE antibodies, but patients with ABPA will have much higher *Aspergillus*-specific IgE levels. Hemmann et al. [13] reported that ABPA and *Aspergillus*-sensitive patients have elevated IgE antibodies to recombinant *Aspergillus* Asp f1, Asp f3, Asp f4, and Asp f6 allergens and that IgE levels to Asp f4 and Asp f6 is highly specific for ABPA in CF patients.

Differentiating between a bacterial flare versus an ABPA flare in CF patients may be difficult. A useful serum biologic marker may be thymus and activation-regulated chemokine (TARC) or CCL17. Latzin et al. [14] and Hartl et al. [15] reported that TARC was elevated in CF patients with ABPA and was further elevated during acute flares of ABPA. TARC is a chemokine whose ligand is CCR4 receptor on CD4<sup>+</sup> Th2 cells.

In ABPA, immunoelectrophoresis generally shows one to three precipitin lines, often to only one extract [7–11]. Patients with aspergilloma will have multiple precipitin lines to all antigen extracts. Extracts of *A. fumigatus* contain a complex mixture of proteins that are mainly derived from the hyphae. Antigenic composition varies between batches according to the culture conditions even within the same laboratory. There is, therefore, a lack of standardization that makes it difficult to compare results between laboratories. There are 22 recognized recombinant allergens by the International Union of Immunological Societies. With purification of these major antigenic components, this may lead to improved diagnosis.

**1.2. Pulmonary Pathology of ABPA.** The gross pathology of ABPA demonstrates cylindrical bronchiectasis of the central airways, particularly those to the upper lobes [7–11]. These airways may be occluded by “mucoïd impaction,” a condition in which large airways are occluded by impacted mucus and hyphae. Airway occlusion may lead to atelectasis of a segment or lobe and, if the atelectasis is long-standing, saccular bronchiectasis may result. Typically, ABPA is worse in the upper lobes than in the lower lobes. Microscopic examination of the airways shows infiltration of the airway wall with eosinophils, lymphocytes, and plasma cells. The airway lumen may be occluded by mucus containing hyphal elements and inflammatory cells, especially eosinophils. Squamous metaplasia of the bronchial mucosa commonly develops, and granulomas may form. Rarely, bronchiolitis obliterans or bronchocentric granulomatosis develops.

## 2. Immunopathogenesis of ABPA

As seen in Figure 1, the pathogenesis of ABPA in susceptible persons begins with the inhalation of *A. fumigatus* spores that germinate into hyphae deep within the bronchi. Fragments of hyphae have also been found within the lung parenchyma, potentially resulting in high concentrations of *Aspergillus* allergens exposed to the respiratory epithelium and immune system [16–19]. These allergens are processed by HLA-DR2 or HLA-DR5 bearing antigen presenting cells (APCs) and presented to T cells within bronchoalveolar lymphoid tissue

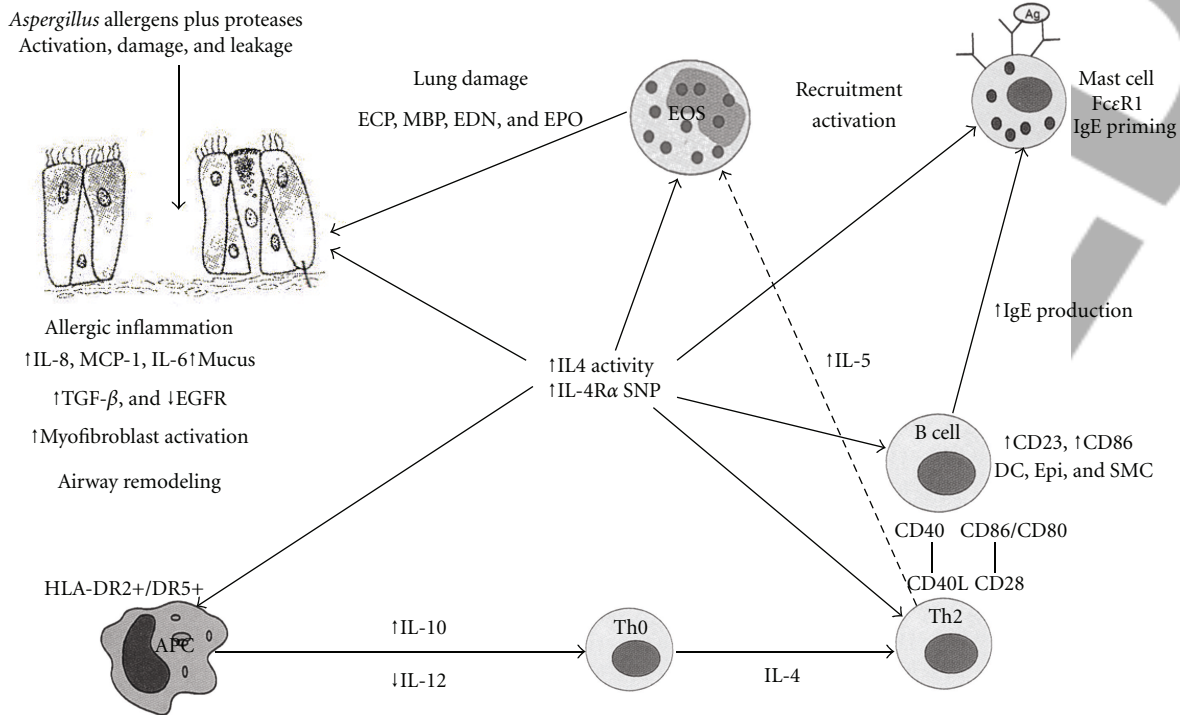


FIGURE 1: Proposed immunopathogenesis of ABPA. In the pathogenesis of ABPA, *A. fumigatus* proteases have a direct effect on bronchial epithelia causing epithelial cell damage with subsequent stimulation of cytokines and chemokines. *Aspergillus* proteins are processed via HLA-DR2/DR5 bearing dendritic cells that skew the Th0 response to a Th2 response. Th2 cytokines stimulate IgE synthesis and eosinophil activation. This leads to an eosinophilic inflammatory in the bronchial airways.

(BALT). The resulting CD4<sup>+</sup> T cell responses to *Aspergillus* are skewed toward Th2 response with the production of IL-4, IL-5, and IL-13 cytokines.

**2.1. Effect of *Aspergillus* on Bronchial Epithelium.** *A. fumigatus* spores 3 to 5  $\mu\text{m}$  in size are inhaled and germinate deep within the bronchi into hyphae [17]. In addition, fragments of the hyphae can be identified within the interstitial of the pulmonary parenchyma. The implication of this is that there is the potential for high concentrations of *A. fumigatus* allergens exposed to the respiratory epithelium and immune system. *A. fumigatus* releases a variety of proteins, including superoxide dismutases, catalases, proteases, ribotoxin, phospholipases, hemolysin, gliotoxin, phthioic acid, and other toxins. The first line of defense against *Aspergillus* colonization in the lungs is macrophage and neutrophil killing of the conidia and the hyphae. In the development of ABPA, Kauffman's group proposed that *Aspergillus* proteins have a direct effect on the pulmonary epithelia and macrophage inflammation [20, 21]. They demonstrated that *Aspergillus* proteases induce epithelial cell detachment. In addition, protease-containing culture filtrates of *Aspergillus* induce human bronchial cell lines to produce proinflammatory chemokines and cytokines, such as IL-8, IL-6, and MCP-1. Thus, various *Aspergillus* proteins have significant biologic activity that disrupts the epithelial integrity and induces a monokine inflammatory response. This protease activity is thought to allow for enhanced allergen exposure to the bronchoalveolar

lymphoid tissue immune system. This is evident by the bronchoalveolar lymphoid tissue synthesis of *Aspergillus*-specific IgE and IgA antibodies.

An important pathogenic feature of *Aspergillus* and other microbes is their ability to interact with epithelial cells on the mucosal surface. Macrophage and neutrophil killing of the conidia and hyphae is the first line of defense against colonization in the lungs [12, 22–24]. This is evidenced by an increased susceptibility to invasive pulmonary aspergillosis in patients with chronic granulomatous disease, a disorder of phagocyte killing. *A. fumigatus* has several virulence factors, including proteolytic enzymes that can interfere with humoral and cellular defense in the airways [25, 26]. Proteases from *Aspergillus* and other fungi, including *Alternaria* and *Cladosporium*, have been shown to cause epithelial cell detachment though *Aspergillus* proteases demonstrated more activity at lower concentrations [25–28].

In addition to damaging the integrity of the epithelial cell layer, Kauffman's group demonstrated that protease containing culture infiltrates of *A. fumigatus* induced human bronchial cell lines to produce proinflammatory chemokines and cytokines, such as monocyte chemoattractant protein (MCP)-1, IL-8, and IL-6 [20]. MCP-1 has been implicated in directly stimulating the development of Th2 cells [29]. The cytokine-release activity could be ascribed to the proteolytic activities of these extracts [20, 27]. These observations suggested that proteolytic enzymes released by *Aspergillus*, growing on and between epithelial cells, were responsible for



the induction of chemoattractive cytokines by epithelial cells and the corresponding inflammation. It was proposed that the induction of the severe inflammatory responses by the direct activation of epithelial cells may cause additional harm to the epithelial cell layer [25]. Destruction of the epithelial cell barrier either by fungal proteases or eosinophilic and neutrophilic inflammation was followed by repair mechanisms, resulting in the influx of serum proteins and extracellular matrix proteins to the luminal side of the epithelium [30]. Because spores and mycelium of *A. fumigatus* have surface structures that are able to interact with extracellular matrix molecules, damage and repair mechanisms of the airway mucosa may facilitate the binding of *Aspergillus* to the damaged sites of the airways. The enhanced release of proteolytic enzymes and allergens on the epithelial surface would induce a continuous inflammatory response and mast cell degranulation, resulting in severe and long-lasting periods of exacerbations of ABPA.

**2.2. *Aspergillus*-Specific Th2 Cells.** The immune response to *Aspergillus* antigens in ABPA patients, as well as allergic asthmatic and CF patients, is characterized by a Th2 CD4<sup>+</sup> T lymphocyte response [17, 31–35]. Skin test reactivity to *Aspergillus* is found in 20%–25% of asthmatic patients [5, 36, 37] and 31%–59% of CF patients [13, 38]. Although sensitization is common in these populations, only a small percentage of patients develop ABPA.

Several groups have observed T cell lymphoproliferative responses to crude *Aspergillus* extracts [31, 39–41]. Subsequently, *Aspergillus*-specific T cell responses were examined and shown to enhance B cell IgE synthesis [41]. In addition, Asp fl T cell lines were generated, and the phenotypes were found to be CD4<sup>+</sup> CD25<sup>+</sup> T cells with the cytokine profile IL-4<sup>+</sup> and IFN $\gamma$ <sup>-</sup>, indicating Th2 CD4<sup>+</sup> T cells [4]. Chauhan et al. [42] subsequently developed T cell clones from asthmatic ABPA patients and demonstrated either Th2 (IL-4<sup>+</sup>, IFN- $\gamma$ <sup>-</sup>) or Th0 (IL-4<sup>+</sup>, IFN- $\gamma$ <sup>+</sup>) patterns. We demonstrated that ABPA subjects have increased frequency of IL-4<sup>+</sup> CD3<sup>+</sup> T cells from Asp f2/f3/f4-stimulated peripheral blood lymphocytes compared to *Aspergillus* sensitive non-ABPA subjects [4]. IL-4 produced by T lymphocytes binds to the IL-4 receptor (IL-4R) on B cells and in association with the CD40L/CD40 signals, results in IgE isotype switching and B cell proliferation [43]. IL-4 also increases the expression of CD86, which has been linked to eosinophilic airway inflammation and airway hyperresponsiveness after allergen challenge. A central question then is how ABPA patients differ from *Aspergillus*-sensitive atopic asthmatic and CF patients. We hypothesize that ABPA develops in genetically susceptible individuals with asthma and CF because of increased frequency and/or activity of *A. fumigatus*-specific Th2 CD4<sup>+</sup> cells. We further propose that polymorphisms of the interleukin-4 receptor alpha chain (*IL-4RA*) subunit and HLA-DR2/DR5 are the genetic susceptibility risk factors responsible for the development of ABPA.

**2.3. IL-4 Responses in ABPA.** Human studies and murine models have shown that CD4<sup>+</sup> Th2 cells and their cytokines are central to the development of ABPA [4, 32–35, 39, 44].

In particular, IL-4 has a key role in the allergic inflammatory response with effects on various cell populations. Its functions include increasing VCAM-1 expression on endothelial cells, which enhances the recruitment of other immune cells, particularly eosinophils, stimulating proliferation of fibroblasts, important in airway remodeling, and increasing Th2 differentiation while decreasing Th1 differentiation and the production of IFN- $\gamma$  [45, 46]. IL-4 also has a myriad of effects on B lymphocytes including the stimulation of growth and activation, increasing HLA-DR class II expression important for antigen presentation and inducing cell surface expression of CD23 and soluble CD23. This cell surface molecule is the low affinity IgE receptor (Fc $\epsilon$ RII) and an activation marker present on a number of cells including B cells, activated T cells, monocytes, and eosinophils. CD23 plays a role in augmenting B cell IgE synthesis through its interactions with CD21 [47, 48]. Recently, in 2003, anti-CD23 monoclonal antibody was administered to atopic asthmatic subjects and resulted in decreased serum IgE levels [49]. In addition, IL-4 has a more direct role in IgE isotype switching by B-cells. It should be noted that IL-13 may also stimulate the synthesis of IgE and is the only other cytokine that has this capability [50–52]. Recently, in 2000 and 2004, increased sensitivity to *in vitro* IL-4 stimulation as measured by enhanced expression of the low-affinity IgE receptor (CD23) on B cells was observed in ABPA patients [32, 33]. This was associated with single-nucleotide polymorphisms of the IL-4 receptor alpha chain (*IL-4RA*) in 92% of ABPA subjects, principally the IL-4-binding single-nucleotide polymorphism ile75val [19, 34, 35]. This increased sensitivity to IL-4 is demonstrated by increased expression of CD23 and CD86 on B cells of ABPA subjects and increased CD23 expression during flares of ABPA [19]. CD23 is expressed on a variety of cells, including B cells, natural killer cells, subpopulations of T cells, and a subpopulation of dendritic cells. T-cell CD23 and B-cell CD21 form a costimulatory pathway. T-cell CD28, B-cells CD80, and CD86 costimulatory pathways activate both T and B cells, and CD28:CD86 is important in IgE synthesis. CD86 is also found on dendritic cells that have the histamine receptor 2, which skews antigen-specific T cells to a Th2 response. We have also observed increased CD86 expression on monocyte-derived dendritic cells of ABPA subjects. Thus, antigen-presenting cells such as monocytes and dendritic cells bearing HLA-DR2 and/or HLA-DR5 and increased sensitivity to IL-4 stimulation probably play a critical role in skewing *A. fumigatus*-specific Th2 responses in ABPA.

### 3. Immunogenetics of ABPA

**3.1. HLA-DR and HLA-DQ.** HLA-DR restriction has been shown to be a risk factor for the development of ABPA (Table 1). Chauhan et al. [42, 53] observed that asthmatic and CF patients who expressed HLA-DR2 and/or DR5 but lacked HLA-DQ2 were at increased risk for ABPA after exposure to *A. fumigatus*. Within HLA-DR2 and HLA-DR5, there are restricted genotypes. In particular, HLA-DR2 HLA-DRB1\*1501 and HLA-DRB1\*1503 genotypes were reported to provide high relative risk. On the other hand, 40% to 44% of non-ABPA atopic *Aspergillus*-sensitive individuals have

TABLE 1: Genetic risk factors in the development of allergic bronchopulmonary aspergillosis.

(i) HLA-DR restriction and HLA-DQ protection
(a) HLA-DR2 restriction
HLA-DRB1*1501 and *HLA-DRB1*1503
(b) HLA-DR5
HLA-DRB1*1104
(c) HLA-DQ2 protective, decreased in ABPA
DQB1*0201
(ii) <i>IL-4RA</i> polymorphisms
(a) <i>IL-4RA</i> ile75val
(iii) <i>IL-10</i> polymorphisms
(a) Promoter –1082 GG genotype
(iv) Surfactant protein A2 ( <i>SP-A2</i> ) polymorphisms
(a) <i>SP-A2</i> ala91pro
(v) Cystic fibrosis transmembrane conductance regulator ( <i>CFTR</i> ) mutations
(a) Heterozygous <i>CFTR</i> mutations in asthmatic patients with ABPA
(vi) Toll-like receptor ( <i>TLR</i> ) polymorphisms
(a) <i>TLR9</i> T-1237C polymorphism

the HLA-DR2 and/or DR5 type. Further studies indicated that the presence of HLA-DQ2, especially DQB1\*0201, provided protection from the development of ABPA. Furthermore, Chauhan et al. [42] demonstrated that Asp f1 allergen has a low-affinity of binding to HLA-DR. This is consistent with Th2 T cell response previously reported by others in that strong antigen HLA-DR-Ag-TCR affinity binding favored a Th1 cellular response, whereas low affinity binding favored a Th2 humoral response [54–58]. Four major V $\beta$  chains, V $\beta$  3, 6, 13, and 14, reacted to Asp f1.

**3.2. *IL-4 Alpha Chain Receptor (IL-4RA) Polymorphisms.*** The *IL-4* receptor is a type I cytokine receptor and exists as a heterodimer that shares a subunit, *IL-4* receptor alpha chain (*IL-4RA*), with the *IL-13* receptor alpha (*IL-13RA*) [59]. There are two types of *IL-4* receptors. Type I receptors, found on all lymphohematopoietic cells, are composed of the *IL-4RA* and the common gamma chain ( $\gamma$ C), which is also a component of *IL-2*, *IL-7*, *IL-9*, *IL-15*, and *IL-21* cytokine receptors [60]. *IL-4* receptor type II, also known as the *IL-13* receptor, is formed by the association of *IL-4RA* with the *IL-13RA* subunits and is located on immune cells, bronchial epithelium, and vascular endothelium. *IL-4* stimulates both type I and type II receptors, while *IL-13* signals through type II receptors.

A potential gain-of-function in the *IL-4RA* subunit may be responsible for B cell hyperreactivity in ABPA. As a consequence of increased *IL-4R* activity, proinflammatory cytokines skew T cell responses to a dominant Th2 pattern which ultimately contributes to the pathophysiology and progression of ABPA. There are eight naturally occurring single nucleotide polymorphisms (SNPs) of the *IL4RA* gene: ile75val, glu400ala, cys431arg, ser436leu, ser503pro,

glu576arg, ser752ala, and ser786pro reported thus far [61–71]. Chromosome 16, which has been associated with asthma, contains the *IL-4RA* gene [66]. Studies have identified a number of these SNPs to be associated with atopy prevalence and asthma severity. In 1997, Hershey et al. [62] initially reported on a high prevalence of atopy and a gain-of-function in the *IL-4RA* as measured by increased CD23 expression in patients with glu576arg and a later study found that this allele correlated with asthma severity [68]. Hershey's group found that the presence of two variants, val75 and arg576 together, resulted in elevated *IL-4* dependent CD23 expression which was not observed when these SNPs were present alone [71]. In our studies, the presence of the val75 allele, located within the *IL-4* binding region, was found in 87.5% of ABPA subjects examined, while the cytoplasmic SNPs were present much less frequently at 27.3% for ala400, 27.3% pro503, 27.3% arg576, and 9.1% arg431. Although these alleles, particularly val75, appear to be common in the general population, their high prevalence in ABPA suggests that they may be a risk factor in the development of the disease (Table 1).

**3.3. *IL-10 Polymorphisms.*** Brouard and coworkers [72] recently in 2005 reported another genetic risk, the association of the –1082GG genotype of the *IL-10* promoter with colonization with *A. fumigatus* and the development of ABPA in CF (Table 1). The –1082GG polymorphism has been associated with increased *IL-10* synthesis, whereas the –1082A allele has lower *IL-10* synthesis. Thus, dendritic cells expressing HLA-DR2/DR5, increased *IL-10* synthesis and increased sensitivity to *IL-4* stimulation due to *IL-4RA* polymorphisms, may be responsible for skewing *Aspergillus*-specific Th2 responses in ABPA.

**3.4. *Surfactant Protein A2 (SP-A2) Polymorphisms.*** Recently, in 2003, Saxena et al. [73] reported that ABPA patients with polymorphisms (ala91pro and arg94arg) in the collagen region of pulmonary surfactant protein A2 (*SP-A2*) had more elevated total IgE levels and higher percentages of eosinophilia than observed in those patients who lacked the SNPs (Table 1). They also found that 80% of patients carrying both alleles had ABPA ( $P = 0.0079$ , OR = 10.4), while only 50% and 60% of patients carrying each allele, individually, were ABPA subjects, suggesting an additive effect. How these SNPs affect SP-A has not yet been elucidated, but the collagen region spanning both SNPs has been shown to associate with receptors of alveolar macrophages [74], which are important in protecting against *Aspergillus* colonization [22]. It is theorized that changes in conformation or affinity of SP-A2 may decrease these interactions and compromise host defense.

**3.5. *Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) Gene Mutations.*** Because ABPA is found in highest incidence among atopic patients with CF, Miller et al. [75] examined mutations in the cystic fibrosis transmembrane conductance regulator gene (*CFTR*) in subjects without CF (Table 1). Their group reported that mutations were present at a higher frequency in asthmatic patients who developed

ABPA, 6 of 21 (28.5%), versus control asthmatics, 2 of 43 (4.6%). These ABPA patients were heterozygous for the mutations (1 patient was compound heterozygote and reclassified as atypical CF), did not have a clinical diagnosis of CF, and had sweat chlorides <60 mEq/L. Although the abnormal airway mucus in CF is thought to be a susceptibility factor for ABPA due to enhanced trapping of *Aspergillus* spores, it is unclear what effect heterozygous CFTR mutations may have on mucus quality in asthmatic airways.

**3.6. Toll-Like Receptor (TLR) Polymorphisms.** Carvalho et al. [76] examined Toll-like receptor (TLR) polymorphisms of *TLR2*, *TLR4*, and *TLR9* in cavitory pulmonary aspergillosis (CCPA) and severe asthma associated with fungal sensitization (SAFS). TLR-4 is among the major receptors for *Aspergillus* hyphae and plays an important part in innate host defense as TLR-4-deficient mice have increased susceptibility to invasive aspergillosis [77]. In CCPA patients, there was significantly increased frequency of the G allele of *TLR4* on asp299gly. ABPA patients had increased frequency of allele C for the *TLR9* T-1237C polymorphism compared to control patients. However, in SAFS patients who are predominantly *Aspergillus* sensitive, there was no association of polymorphisms of *TLR2*, *TLR4*, or *TLR9*. TLR-9 is a receptor that recognizes CpG motifs prevalent in bacterial and viral DNA. *Aspergillus* hyphae and conidia do signal through TLR-9 on murine neutrophils [78]. TLR-9-deficient mice demonstrate greater conidial and hyphal damage. In addition, Lazarus et al. [79] reported that TLR9 polymorphisms have been associated with increased risk of asthma. Novak et al. [80] reported that the *TLR9* C allele of T-1237C decreases expression. Thus, decreased TLR-9 protective function may be an underlying susceptibility in the development of ABPA.

#### 4. Conclusions

The prognosis of ABPA is good if the disease is detected early and treatment started promptly. It is important that the diagnosis is made and treatment commenced before there is permanent lung damage from bronchiectasis. In such patients, there should be no progression of the disease, although relapses can occur many years later, and long-term followup is recommended. In children with CF, the relapses seem to be more frequent than they are in patients with asthma, and careful surveillance is necessary to ensure resolution of the disease process. In some CF patients, it is difficult to wean the steroids without an increase in symptoms, such as dyspnea and wheezing, whether this is due to the underlying CF lung disease or due to patients going from stage II to stage III ABPA on withdrawal of steroids is unclear. Adjunctive treatment with antifungal therapy to *Aspergillus* should be considered. Symptoms are not a reliable guide to therapy; therefore, it is important to reevaluate the chest radiograph and the serum IgE at regular intervals until a long-term remission is established.

ABPA occurs with a worldwide distribution in a significant number of patients with CF and less frequently in those with asthma. Early diagnosis and treatment are essential in

preventing end-stage progression. The development of ABPA is probably the combination of many genetic susceptibility factors, gene-gene interactions, and environmental exposure which work together. Understanding of the genetic risks and immunopathogenesis of ABPA hopefully will lead to improved early diagnosis and improved treatment of ABPA.

#### Abbreviations

Af:	<i>Aspergillus fumigatus</i>
Asp fx:	<i>Aspergillus fumigatus</i> proteins
APC:	Antigen presenting cell
MBP:	Major basic protein
ECP:	Eosinophil cationic protein
EDN:	Eosinophil derived neurotoxin
VLA:	Very late activation antigen
VCAM:	Vascular cell adhesion molecule
CxCR and CCR:	Chemokines receptors
MCP:	Monocyte chemotactic protein
sCD23:	Soluble CD23
cyst-LT:	Cysteinyl leukotriene
ABPA:	Allergic bronchopulmonary aspergillosis
CFTR:	Cystic fibrosis transmembrane conductance regulator
IL-4RA:	IL-4 receptor alpha chain
TARC:	Thymus and activation-regulated chemokine
SP-A2:	Surfactant protein A2 polymorphisms.

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