Immunopathogenesis of allergic bronchopulmonary aspergillosis in cystic fibrosis

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Abstract

Allergic bronchopulmonary aspergillosis (ABPA) is a hypersensitivity lung disease mediated by an allergic late-phase inflammatory response to Aspergillus fumigatus antigens. ABPA is characterized by markedly elevated Aspergillus-specific and total IgE levels and eosinophilia, and manifested by wheezing, pulmonary infiltrates, and bronchiectasis and fibrosis, which afflict asthmatic and cystic fibrosis (CF) patients. We propose that ABPA develops in genetically susceptible CF patients due to HLA-DR2 and DR5 restriction, increased sensitivity to IL-4 stimulation, and increased A. fumigatus allergen-specific Th2 CD4 T-cell-mediated responses. In addition, A. fumigatus proteases play a role in facilitation of antigen transport across the epithelial cell layer by damaging the epithelial integrity and by a direct interaction with epithelial cell surface receptors, resulting in pro-inflammatory cytokine production and corresponding inflammatory responses. © 2002 European Cystic Fibrosis Society. Published by Elsevier Science B.V. All rights reserved.

Keywords: Allergic bronchopulmonary aspergillosis (ABPA); Cystic fibrosis (CF); Aspergillus fumigatus

1. Introduction

Allergic bronchopulmonary aspergillosis (ABPA) is a hypersensitivity lung disease mediated by an allergic late-phase inflammatory response to certain Aspergillus fumigatus antigens that occurs in approximately 1–2% of asthmatic and 7–10% of cystic fibrosis patients [1–16]. ABPA is characterized by markedly elevated Aspergillus-specific IgE and total IgE levels and eosinophilia, and manifested by wheezing, pulmonary infiltrates, bronchiectasis and fibrosis. The immune response to Aspergillus antigens in ABPA, as well as in allergic asthmatic and cystic fibrosis patients, is characterized by a Th2 CD4+ T-cell response [1]. A central question then is how ABPA differs from Aspergillus-sensitive atopic asthmatic and CF patients. It is proposed that ABPA develops in genetically susceptible cystic fibrosis patients due to increased frequency and/or activity of Aspergillus-specific Th2 CD4+ T cells and to HLA-DR restriction. In addition, Aspergillus proteases damage the respiratory epithelial barrier, exposing the bronchoalveolar lymphoid tissue (BALT) to high concentrations of Aspergillus antigens that alter the immune response.

The allergic inflammatory response in ABPA appears to be quantitatively greater than in Aspergillus-sensitive cystic fibrosis patients. In the model proposed of the immunopathogenesis of ABPA, as illustrated in Fig. 1, A. fumigatus spores are inhaled into the bronchial airway, where they are trapped by the luminal mucus, germinate and form mycelia. A. fumigatus mycelia release allergens that are processed by antigen-presenting cells (APC) bearing HLA-DR2 or DR5 and presented to T-cells within the bronchoalveolar lymphoid tissue (BALT). The T-cell response to Aspergillus allergens becomes skewed toward a Th2 CD4+ T-cell response with IL-4, IL-5 and IL-13 cytokine synthesis and secretion.

2. Interaction of respiratory epithelium and Aspergillus

One of the characteristics features in patients with ABPA is the observations that A. fumigatus is found bound to the surface epithelium growing on and between the epithelial cells without being efficiently killed by mononuclear cells and eosinophils [17]. It has also been shown that spores of A. fumigatus are attached to...
epithelial surfaces cultured in vitro [18]. The physical presence of *A. fumigatus* is possibly of importance for the modulation of the immunological response towards a Th2-type response [19]. In order to evade the very effective natural defense system of the human airways against fungi, various virulence factors of *A. fumigatus* have been detected that have been shown to interfere with, or even block, normal functions of the humoral and cellular defense of the airways [20,21]. Some of these virulence factors are the proteolytic enzymes of *A. fumigatus*, which facilitate the immune response to the fungal antigens and play a role in the inflammatory response.

### 2.1. Aspergillus proteolytic enzymes

It has been found that certain strains of *A. fumigatus* release proteolytic enzymes with elastolytic and collagenolytic activity. The possible role of these proteolytic enzymes as a pathogenic factor in fatal invasive aspergillosis is still uncertain. However, findings in patients with ABPA or aspergilloma indicate that these proteases may be involved in the pathogenesis of these diseases. In studies of ABPA, culture filtrate extracts with marked elastase and collagenase activity were examined. In Western blot experiments, binding of IgG antibodies to a 32-kDa elastase protein was found with sera from patients with ABPA or aspergilloma [22]. The pronounced binding of IgG antibodies to the 32-kDa fungal elastase suggests that these proteases are produced in vivo in patients with ABPA and aspergilloma. Furthermore, during exacerbation of aspergilloma, antibody concentrations against different antigens, including the 32- and 40-kDa proteases, were markedly increased [23]. The observation that the antibody concentrations against the 32- and 40-kDa fungal proteases are increased during the exacerbation phase indicates that these proteases may play a role in the pathogenicity of the disease.

### 2.2. Aspergillus proteases and host cytokines

An important feature of pathogenic microorganisms is their capacity to interact with epithelial cells of the mucosal surface. Previously, it has been shown that products released in vitro by *A. fumigatus* are able to cause epithelial cell detachment [24,25]. This capacity to induce epithelial cell detachment is also characteristic of other proteases released by different fungi, e.g. *Alternaria* and *Cladosporium*, but Aspergillus proteases are more active at much lower concentrations [26]. Recent studies performed with proteases from various sources, e.g. Der p1 from house dust mite [27], have shown that degradation of epithelial cell structures results in facilitated transport of antigens and allergens across the epithelium, leading to enhanced exposure to
antigen-presenting cells with concurrent immune responses.

In addition to damaging the epithelial cell-layer integrity, recent studies have shown that human bronchial and alveolar epithelial cell lines produce pro-inflammatory cytokines, such as IL-8, IL-6 and MCP-1, after incubation with protease-containing culture filtrates of *A. fumigatus*. This cytokine-releasing activity could be ascribed to the proteolytic activity of these extracts [25]. These observations suggest that proteolytic enzymes released by *Aspergillus* growing on and between epithelial cells may be responsible for the induction of chemoattractive cytokines by epithelial cells and corresponding inflammatory responses. It has been proposed that induction of a severe inflammatory response by the proteolytic activity of these extracts is ascribed to the proteolytic activity of these extracts [25]. These observations suggest that proteolytic enzymes released by *Aspergillus* growing on and between epithelial cells may be responsible for the induction of chemoattractive cytokines by epithelial cells and corresponding inflammatory responses. It has been proposed that induction of a severe inflammatory response by the direct activation of epithelial cells may induce additional epithelial injury [20]. Destruction of the epithelial cell barrier by proteases from either the fungus, or from the eosinophilic and neutrophilic inflammation, is followed by repair mechanisms, resulting in the influx of serum proteins and extracellular matrix proteins to the luminal site of the epithelium [28]. Since spores and mycelia of *A. fumigatus* have surface structures that are able to interact with extracellular matrix molecules, damage and concurrent repair of the airway mucosa, with simultaneous release of repair molecules, may facilitate the binding of *Aspergillus* to the damaged sites of the airways. The interaction of enhanced release of proteolytic enzymes and allergens on the epithelial surface will induce a continuous inflammatory response and mast cell degranulation, resulting in severe and long-lasting periods of exacerbation of ABPA.

In addition to the induction of cytokine responses of epithelial cells, it was shown that proteases from *A. fumigatus* at higher concentrations also inhibit epithelial cell cytokine production [26]. This inhibition was specific for the elastase/collagenase-containing extracts of *A. fumigatus*, which may represent an additional virulence factor by preventing effective targeting by infiltrating phagocytic cells. This would lead to continuous release of antigens and allergens, and strong activation of the Th2-type immunological response, with very high production of total and specific IgE antibody, as well as an additional Th1 response, with the formation of IgG and IgA antibodies to antigens of *A. fumigatus*, as is observed in patients with ABPA.

### 3. Host immune responses to *Aspergillus*

The allergic inflammatory response in ABPA appears to be quantitatively greater than in *Aspergillus*-sensitive cystic fibrosis patients. In the model proposed of the immunopathogenesis of ABPA (Fig. 1), *A. fumigatus* spores are inhaled into the bronchial airway, where they are trapped by the luminal mucus, germinate and form mycelia. *A. fumigatus* mycelia release allergens that are processed by antigen-presenting cells (APC) bearing HLA-DR2 or DR5 and presented to T-cells within the bronchoalveolar lymphoid tissue (BALT). The T-cell response to *Aspergillus* allergens becomes skewed toward a Th2 CD4+ T-cell response, with IL-4, IL-5 and IL-13 cytokine synthesis and secretion.

#### 3.1. Th2 CD4+ T-cells in ABPA

Several groups have observed T-cell lymphoproliferative responses to crude *Aspergillus* extracts [29–32]. Subsequently, *Aspergillus*-specific T-cell responses were examined. In these studies, T-cells were stimulated with a crude *Aspergillus* extract for 48 h, and the *Aspergillus*-stimulated T-cell supernatant was then co-cultured with atopic control B-cells for 10 days. These supernatants obtained from *Aspergillus*-stimulated T-cells from ABPA patients enhanced B-cell IgE synthesis [33]. Subsequently, T-cell lines were generated from ABPA patients with an *Aspergillus* allergen, Asp f1 [34]. In these studies, T-cell phenotype was exclusively CD4+CD25+, HLA-DR+ T-cells (Table 1). The cytokine profile of these T-cells was IL-4+, IFN–γ–, i.e. Th2 CD4+ T-cells. Indeed, the lymphoproliferative stimulus for Asp f1 T-cell lines was predominantly IL-4-mediated, i.e. an autocrine pattern (Fig. 2). However, atopic *Aspergillus*-sensitive CF patients also developed Th2 CD4+ T-cells. Subsequent studies performed by Chauhan and co-workers [35,36] demonstrated that T-cell clones obtained from asthmatic ABPA patients were either Th2 (IL-4+, IFN–γ–) or Th0 (IL-4+, IFN–γ+) T-cells. Furthermore, tetanus toxoid-generated T-cell clones were the expected Th1 phenotype, namely IFN–γ+. Thus, the Th2 CD4+ T-cell response in ABPA is specific to *Aspergillus* antigens, and not a generalized Th2 cell response to all antigens.

#### 3.2. IL-4 cytokine in ABPA

IL-4 plays a critical role in the allergic inflammatory response in ABPA (Fig. 1). IL-4 up-regulates cellular activity via binding to IL-4 receptor (IL-4R) found on a variety of cells, including B-cells, NK-cells, mast cells, endothelial cells and a subpopulation of T-cells [37–42]. IL-4 and IL-13 induce IgE isotype switching of B-cells [43–48]. Although IL-4 is necessary for IgE isotype switching, it is not sufficient. In order for IgE secretion to occur, a second signal mediated by cell–cell T- and B-cell interactions via CD40L::CD40 and CD28::CD86 ligand–receptor interactions occurs [46–58]. IL-4 also induces the low-affinity IgE receptor CD23 and soluble CD23, which augments B-cell IgE synthesis [46,47,59–64]. In addition, T-cell CD23 and B-cell CD21 cognate ligation also augments B-cell IgE synthesis. CD86 expression on B-cells is also up-regulated by IL-4 in atopic patients [49–57]. CD86 on B-
cells is an important co-stimulatory molecule for augmentation of IgE synthesis. The ligand for CD86 is CD23 on B-cells. In addition, several studies have reported that the B-cells from ABP A patients were significantly more sensitive to IL-4 stimulation compared to non-ABP A CF and non-atopic patients, with up-regulation of CD23 and CD86, leading to a positive feedback amplification mechanism, which also increases Th2 CD4+ T-cell responses. In recent studies, it was observed that the B-cells from ABP A patients were significantly more sensitive to IL-4 stimulation compared to atopic and non-atopic patients, with up-regulation of CD23 and CD86 expression [65]. Recently, it was demonstrated that ABPA CF patients had significantly increased rates of CD23 expression per B-cell to IL-4, but not to IL-13 stimulation compared to non-ABPA CF patients and non-atopic controls (Figs. 3 and 4) [66]. Furthermore, ABPA CF patients also had significantly increased CD23 expression on CD86+ B-cells compared to non-ABPA CF and control patients (Fig. 3). At day 0 prior to culture, the number of CD23 molecules per B-cell and CD86+ B-cell were significantly elevated in vivo in ABPA CF patients compared to non-ABPA CF and to non-atopic patients (Fig. 3). Thus, ABPA patients had increased sensitivity to IL-4, but not IL-13 stimulation, with up-regulation of CD23 and CD86 expression compared to non-ABPA CF patients and non-atopic controls, such that ABPA CF > non-ABPA CF ≫ non-atopic patients. We propose that in ABPA there is a positive amplification feedback loop mechanism of B-cell CD86 and Th2 CD4+ T-cell IL-4 stimulation.

The model proposes that Aspergillus growing within the airways releases high levels of allergens in the airway and lung parenchyma, which in turn produces a heightened and prolonged late-phase allergic inflammatory response. Furthermore, ABPA patients develop IgE antibodies to specific Aspergillus proteins, Asp f2, Asp f4 and/or Asp f6, whereas atopic patients develop IgE antibodies to Asp f1 and/or Asp f3 [67–72]. It is hypothesized that mycelial formation and secretion of proteins in ABPA is necessary, suggesting that the colonization in ABPA is greater than in Aspergillus-sensitive atopic patients. This increased exposure to Aspergillus allergens occurring in a genetically susceptible host then drives the skewed Th2 CD4+ T-cell and hyper-IgE responses observed in ABPA. Recently, studies to determine whether there is increased frequency of Th2 CD4+ T-cells in patients with ABPA have been

Table 1
Asp f1 T-cell lines from patients with ABPA

<table>
<thead>
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<th>Asthmatic patients</th>
</tr>
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</tr>
<tr>
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<td>+</td>
</tr>
<tr>
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</tr>
<tr>
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</tr>
<tr>
<td>CD3+</td>
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<td>99</td>
</tr>
<tr>
<td>CD4+</td>
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</tr>
<tr>
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</tr>
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</tr>
<tr>
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<td>91</td>
<td>100</td>
</tr>
<tr>
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</tr>
<tr>
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</tr>
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Lymphoproliferation<sup>b</sup>

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<tr>
<td>(SI)</td>
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<tr>
<td>Tetanus toxoid (cpm)</td>
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<td>(SI)</td>
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Cytokine synthesis supernatant<sup>c</sup>

<table>
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</tr>
<tr>
<td>IFN-γ (pg/ml)</td>
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<td>1200</td>
</tr>
<tr>
<td>IL-2 (U/ml)</td>
<td>ND</td>
<td>ND</td>
</tr>
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<sup>a</sup> Analyzed by flow cytometry using FITC or PE labeled monoclonal antibodies.
<sup>b</sup> For lymphoproliferation assay of Asp f1 T-cell lines, the cell lines were rested, then incubated with media, Asp f1 or tetanus toxoid for 72 h. Counts per minute (cpm) are net cpm calculated as stimulated minus media.
<sup>c</sup> For cytokine synthesis of Asp f1-stimulated T-cell lines, the cell lines were rested, then incubated with Asp f1 for 48 h.

From Knutsen et al. [34].

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Fig. 2. Asp f1-specific T-cell line was rested for 2–17 days after IL-2 and Asp f1 stimulation (top panel). Supernatant IL-4 concentration was measured serially. Significant amounts of IL-4 were synthesized for up to 10–14 days of rest. Monoclonal anti-IL-4 antibody inhibited Asp f1-stimulated lymphoproliferation of antigen-specific T-cell lines in a dose–response manner (bottom panel). Maximum inhibition was 84% at 5% anti-IL-4 antibody concentration (v/v) in this experiment and 78% in a second experiment. Anti-IL-2 had little inhibition of proliferation, indicating an IL-4-dependent autocrine lymphoproliferative response (from Knutsen et al. [34]).

initiated [66]. As observed in Fig. 5, the frequency of cytoplasmic IFN-γ+ and IL-4+ CD3+ T cells in PMA- and ionomycin-stimulated cultures was comparable in CF patients with ABPA compared to non-ABPA CF patients, indicating that there was not a skewing of Th2 CD4+ T-cell responses (Fig. 5a). Interestingly, IFN-γ+ CD3+ T-cells were significantly decreased in both ABPA and non-ABPA CF patients compared to non-atopic controls. When antigen-specific frequency of Th1 and Th2 responses were evaluated, a different picture emerged. The frequency of IFN-γ+ CD3+ T-cells in tetanus toxoid-stimulated cultures was similar in ABPA and non-ABPA CF patients and was decreased compared to non-atopic controls. However, in Asp f2/ f3/f4-stimulated cultures, the frequency of IL-4+ CD3+ T-cells was significantly increased in ABPA CF patients compared to non-ABPA CF patients (Fig. 5b). This suggests that there is an increased frequency of *Aspergillus*-specific Th2 CD4+ T-cells in ABPA compared to non-ABPA CF patients.

3.3. BALT immune response in ABPA

Since the immune response to *Aspergillus* antigens originates in the bronchoalveolar lymphoid-associated tissue (BALT), investigators have examined bronchoalveolar immunity. Analysis of the cells obtained from bronchoalveolar lavage fluid (BALF) in ABPA reveals an admixture of alveolar macrophages, eosinophils and lymphocytes similar to that found in asthma [44,45,73,74]. Eosinophil infiltration predominates, both in BALF and lung tissue, as is evident on lung biopsy [75]. In addition, eosinophils are activated and have released their mediators, such as major basic protein. Thus, eosinophils are a major effector cell causing inflammation. Lymphocytes found in BALF are composed of T-, B- and NK cells. The T-cells are an admixture of CD4+ and CD8+ T-cells, approximately in a 2:1 ratio. Interestingly, we have observed increased CD23+ NK cells and CD23+CD4+ T-cells obtained from BALF of ABPA patients, indicating in vivo IL-4 stimulation [1]. Recently, in preliminary studies, increased in vivo CD23+ expression (~10%) on CD4+ T-cells in CF ABPA patients has been observed. The significance of CD23+ T-cells is probably T-cell CD23 and B-cell CD21 T::B ligand–counterligand interaction and augmentation of IgE synthesis. Similarly, CD23+ NK cells are an important source of sCD23 and/or NK CD23::B cell CD21 interaction, increasing immunoblast IgE secretion. Recently, we have observed induction of CD23+CD69+CD4+ T-cells in *Aspergillus*-stimulated cultures of CF ABPA patients (unpublished results). In addition, the expression of CD23 is extremely elevated on addition of IL-4. The role of this CD4+ T-cell subpopulation in ABPA is unknown, but is under study.

3.4. B-cell responses in ABPA

In ABPA, extremely elevated total serum IgE concentrations and elevated IgE anti-*Aspergillus* antibodies are characteristic of ABPA. There appear to be quantitative,
Fig. 3. IL-4 induction of CD23+ molecules on CD20+ and on CD86+ CD20+ B-cells. ABPA CF patients had significantly increased number of CD23 molecules (a) per B-cell and (b) per CD86+ B-cell on day 0 compared to non-ABPA CF patients (P < 0.01) and non-atopic control patients (P < 0.01). Following IL-4 stimulation, ABPA CF patients had a significantly increased rate of CD23+ expression (a) per B-cell and (b) per CD86+ B-cell compared to non-ABPA CF (P < 0.01) and non-atopic control patients (P < 0.01). Data presented as mean ± S.E. (from Knutsen et al. [66]).
Fig. 4. IL-13 induction of CD23+ molecules on CD20+ and on CD86+ CD20+ B-cells. Following IL-13 stimulation, ABPA CF patients had no significantly increased rate of CD23+ expression (a) per B-cell and (b) per CD86+ B-cell compared to non-ABPA CF and non-atopic control patients ($P < 0.01$). Data presented as mean ± S.E. (from Knutsen et al. [66]).
Fig. 5. Th1 and Th2 CD3+ T cells. (a) Following PMA and ionomycin stimulation, ABPA CF patients had comparable percentages of IFN-γ and IL-4 CD3+ T-cells compared to non-ABPA CF patients. However, both ABPA and non-ABPA CF patients had significantly decreased percentages of IFN-γ CD3+ T-cells compared to normal control subjects (\(P<0.001\), \(P<0.001\) respectively). (b) Following tetanus toxoid and Asp f2/3/4 stimulation, both ABPA and non-ABPA CF patients had significantly decreased IFN-γ CD3+ T-cells compared to normal control subjects (\(P<0.05\), \(P<0.05\), respectively) but not different from each other. However, ABPA CF patients had significantly increased Asp f2/3/4-stimulated IL-4 CD3+ T-cells compared to non-ABPA CF patients and normal control subjects (\(P<0.01\), \(P<0.01\), respectively). Data presented as mean±S.E. (from Knutsen et al. [66]).
and perhaps qualitative, differences of the B-cell IgE antibody responses in ABPA compared to Aspergillus-sensitized non-ABPA atopic patients. The heightened total and specific anti-Aspergillus IgE antibody responses have been described by several groups [4, 10, 76–80]. In ABPA, there are also increased amounts of IgG and IgA anti-Aspergillus antibodies, reflective of the Th2 humoral vs. Th1 cellular response to Aspergillus antigens [81–88]. Although other Aspergillus-exposed groups develop IgE, IgG and IgA anti-Aspergillus antibodies, there is a quantitative increase in IgE anti-Aspergillus antibodies in ABPA patients. As observed in Table 2, it was previously reported that B-cells obtained from patients with ABPA spontaneously synthesize increased amounts of IgE in vitro compared to Aspergillus-sensitized non-ABPA patients groups, indicating in vivo activation of IgE immunoblasts [33]. In recent studies [66], we have observed increased CD23+CD86+ B-cells in ABPA that probably accounts for this previous observation and for this hyper-IgE state [65]. Furthermore, Greenberger and Patterson [77] demonstrated that specific anti-Aspergillus fumigatus IgE and IgA antibodies are produced within BALT. In contrast, IgG anti-Aspergillus antibodies obtained from BAL fluid were predominantly exudative, derived from the peripheral systemic lymphoid system. Slavin et al. [89] demonstrated lymphoid follicles that stained with anti-IgE in a lung biopsy of a patient with ABPA, indicating in vivo IgE-bearing B-cells and immunoblasts. Greenberger and Patterson [77] further demonstrated that anti-Aspergillus IgE antibodies comprised only a small fraction of the total IgE in the peripheral lymphoid system. This implies that the CD4+Th2 cells have traveled to the systemic immune system and have activated other clones of B-cells, in addition to those with Aspergillus specificity.

3.5. IL-4 receptor (IL-4R)

As a potential mechanism for increased B-cell IgE synthesis and secretion, mutations of IL-4 receptor alpha chain (IL-4Rα) were evaluated in collaboration with Dr Talal Chatila. Mutations or polymorphism of IL-4Rα have been identified in atopic individuals with elevated IgE levels [40–42]. These polymorphisms increase IL-4 and IL-4R interactions, resulting in a gain-of-function of IL-4Rα that promotes B-cell IgE isotype switching. Subsequently, seven mutations have been identified that result in increased IL-4R activity [40, 90, 91]. In addition, increased IL-4 activity would result in increased expression of other receptors, including CD23 and CD86 on B-cells, eosinophils and NK cells, VLA-4 on eosinophils and T-cells, VCAM on endothelial cells, CCR-3, CCR-4 and eotaxin secretion (Fig. 1). In preliminary studies, homozygous mutations of IL-4Rα chain in 2/2 ABPA patients and heterozygous mutations in 3/5 atopic patients and 2/5 non-atopic control patients have been reported. However, increased sensitivity to IL-4 stimulation was observed in ABPA and atopic patients with IL-4Rα chain mutations and wild type. IL-4R is a heterodimer, consisting of IL-4Rα chain and the common gamma chain (Cγ) [46–48]. The IL-13R is also a heterodimer, consisting of IL-4Rα and IL-13Rα chains. IL-4 stimulates both the IL-4R and the IL-13R receptors, whereas both IL-4 and IL-13 stimulate the IL-4α/IL-13Rα receptor. IL-13, like IL-4, increases CD23 expression, IgE isotype switching and IgE synthesis. However, IL-13 does not activate and skew Th2 responses. There is evidence that IL-4Rα and IL-13Rα interact with the Janus kinase 1 (Jak-1), whereas the Cγ chain interacts with Jak-3. Upon IL-4 stimulation, IL-4α and Cγ chains undergo phosphorylation by Jak-1 and Jak-3. After IL-13 stimulation, the IL-13Rα chain is phosphorylated by Jak-1. Phosphotyrosines in the IL-4Rα and IL-13Rα serve as docking sites for the SH2 domains of the signal transducer and activator of transcription (STAT6) molecule. STAT6 is subsequently phosphorylated by the Jak kinases, when it is then released as a dimer and translocates to the nucleus activating IL-4 and IL-13 responsive transcription. Our studies demonstrating increased CD23 expression with IL-4 stimulation sug-

<table>
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<td></td>
<td></td>
<td>B cells alone</td>
<td>Preformed</td>
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<tr>
<td>ABPA (5)</td>
<td>4870±836</td>
<td>1754±595</td>
<td>1980±617</td>
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<tr>
<td>ST/PPT positive (9)</td>
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<tr>
<td>ST/PPT negative (13)</td>
<td>58±22</td>
<td>167±66</td>
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<tr>
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<td>355±58</td>
<td>76±34</td>
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<tr>
<td>P (ANOVA)</td>
<td>&lt;0.01</td>
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From Knutsen et al. [33]. Abbreviations: ST, Aspergillus prick skin test; PPT, Aspergillus precipitins. The number of individuals studied is in parentheses. Purified-B cells were cultured for 10 days. The net B-cell IgE synthesis was calculated from IgE concentrations of day 10 minus preformed day 0 cultures. Preformed day 0 IgE concentration determined by lysis of day 0 B-cells. Data are presented as mean ± S.E.M. P values were determined by ANOVA. P < 0.05 comparing ABPA vs. ST/PPT positive and ST/PPT negative using the post hoc Sheffe test.
gest that other mutations or polymorphisms of the IL-4Rα, IL-13α, Jak-1 or STAT6 pathway may exist.

3.6. Eosinophils and mast cells in ABPA

The effector cells responsible for the allergic inflammatory responses in ABPA are predominantly mast cells and eosinophils. In our model, when Aspergillus antigens cross-link IgE bound to mast cells, mast cells release a variety of mediators, such as histamine, leukotrienes and PAF, which induce bronchial smooth-muscle contraction and vascular permeability [1]. A number of mast cell cytokines, such as leukotriene B4 and PAF, are chemoattractant for eosinophils. In addition, chemokines, such as eotaxin, RANTES and MCP-3, derived from a variety of cell types, such as epithelial and phagocytic cells, induce eosinophil chemotaxis and activation [43–45,92]. Basophil hyperreactivity with increased histamine release also has been reported in ABPA, probably due to IL-4 stimulation [93]. Th2 CD4+ T-cells secrete the cytokines IL-3 and IL-5 that promote bone marrow maturation of eosinophils and activation of eosinophils [74,94–98]. Furthermore, IL-4 induces expression of VCAM-1 on vascular endothelial cells and its ligand VLA-4 on T-cells and eosinophils. Recent studies have demonstrated selective expression of the eotaxin receptor, chemokine receptor 3 (CCR3), on eosinophils, basophils, and Th2 T-cells, and CCR3 expression is up-regulated by IL-4 polarizing conditions [99,100]. Thus, both chemotactic and cell-surface adhesion molecules promote recruitment of Th2 T-cells and eosinophils within the allergic inflammatory site [44,45,101,102]. Eosinophils possess Fc receptors for IgE, IgG and IgA, and IL-4 also induces increased expression of the low-affinity IgE receptor CD23 on eosinophils [74]. In addition, IL-4 and IL-5 induce Fc receptors for IgA [74,103]. In ABPA, significant amounts of Aspergillus-specific IgA antibodies are produced within BALT and are present within the bronchial mucus. Thus, both IgE and IgA anti-Aspergillus antibodies bind to their Fc receptors on eosinophils and trigger mediator release when they engage allergen [74,104]. When eosinophil-bound IgE, IgA and IgG are cross-linked by Aspergillus antigens, the eosinophils are triggered to secrete inflammatory mediators, such as major basic protein and eosinophil-derived neurotoxin [74].

3.7. HLA-DR restriction in ABPA

Chauhan et al. [35] investigated whether there is unique TCR recognition (T-cell epitopes), TCR-Vβ restriction or HLA-class II restriction that would promote enhanced Th2 responses. Analysis of T-cell epitope mapping has revealed that there are three immunodominant regions of the Asp fl protein in ABPA patients that are recognized by TCR [35]. Their findings were similar to that found in other allergen models. O’Hehir and Lamb’s group has evaluated T-cell responses to purified house dust mite allergens. In their model, T-cell clones were generated from atopic and non-atopic individuals [105]. Significantly, the T-cell clones from non-atopic individuals proliferated in response to allergen stimulation, but did not support IgE synthesis, whereas the T-cell clone from atopic patients did. Furthermore, TCR epitope mapping studies revealed a limited number of epitopes reacting with TCR [106,107], TCR Vβ restriction or usage [108,109], and HLA class II restriction [109]. Four major Vβ chains, Vβ 3, 6, 13 and 14, react to Asp fl. This will allow for evaluation of whether mutations of the epitope might alter the T-cell cytokine and/or lymphoproliferative responses for potential immunotherapy of ABPA. Recently, Chauhan and co-workers [35,36] identified that there is HLA-DR2 (split into HLA-DR15 and DR16) 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 1503 were reported to provide high relative risk. On the other hand, 40–44% of non-ABPA atopic Aspergillus-sensitive individuals have the HLA-DR2 and/or DR5. 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 [110–112]. Thus, certain genotypes of HLA-DR2 and DR5 may be necessary, but not sufficient to cause ABPA. Furthermore, Chauhan et al. [36] demonstrated that Asp fl allergen has 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 favors a Th1 cellular response and low-affinity binding favors a Th2 humoral response [111–115].

3.8. CFTR mutations in ABPA

Since ABPA is found in highest incidence in atopic CF patients, Miller et al. [116] examined CFTR mutations in asthmatic ABPA patients. Six of 11 patients had mutations of the CFTR gene. Clearly, there was increased frequency of heterozygous mutations of the CFTR gene in these asthmatic patients. It has been hypothesized that, in CF, the abnormal mucus promotes the trapping of Aspergillus spores within the bronchial airway, permitting and perhaps promoting Aspergillus mycelia growth. The significance of the heterozygous CFTR mutation in asthmatic patients is unclear as to the mucus properties of these asthmatics. Thus, the abnormal mucus may allow increased Aspergillus colonization within the bronchial airway of CF and asthmatics, and, in a genetically susceptible individual, stimulate a Th2 T-cell response and subsequent ABPA.
In summary, a quantitative increased Th2 CD4+ T-cell response to *Aspergillus fumigatus* in both the BALT and systemic immune systems characterize ABPA (Table 3). Perhaps key in the immunopathogenesis is that the BALT is exposed to high levels of *Aspergillus* allergens, perhaps because of abnormal mucus properties due to CFTR mutations. Antigen presentation to T-cells is characterized by HLA-DR2 and DR5 restriction of low-affinity antigen binding. In addition, there is restricted TCR-Vβ usage. Thus, there is an immunogenetic susceptibility to develop ABPA, which resides within the HLA-DR-Ag-TCR signaling of the T-cells toward a Th2 CD4+ T-cell response. In addition, there may increase sensitivity of T-cells, B-cells, NK cells and eosinophils to IL-4 stimulation due to mutations of IL-4R with or without CD86. The results of these studies have significance for the atopic state in general: they suggest that the airway changes observed in ABPA are an example of allergen-induced allergic inflammation airway remodeling observed in asthmatic patients.

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