Immunogenetics of Chronic and Allergic Aspergillosis

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Abstract

Although invasive fungal diseases are often assumed to be the only form of fungal disease, fungi more commonly cause chronic disease in individuals with ostensibly normal immune systems, or allergic disease in those with hyperactive immune systems, such as atopic asthmatics. Invasive fungal diseases affect individuals with an underlying defect in the immune system and are rapid in onset with high mortality, whereas chronic and allergic diseases primarily affect individuals with normal immune systems and are long-term, even lifelong, conditions.

7.1 Introduction

Although invasive fungal diseases are often assumed to be the only form of fungal disease, fungi more commonly cause chronic disease in individuals with ostensibly normal immune systems, or allergic disease in those with hyperactive immune systems, such as atopic asthmatics. Invasive fungal diseases affect individuals with an underlying defect in the immune system and are rapid in onset with high mortality, whereas chronic and allergic diseases primarily affect individuals with normal immune systems and are long-term, even lifelong, conditions.

Chronic aspergillosis is a long-term disease occurring in those with a prior or underlying lung-damaging condition. At-risk individuals commonly have prior or current tuberculosis, COPD or sarcoidosis. The course of the disease can range from...
months to many years and is associated with significant mortality. Allergic forms of aspergillosis affect individuals with atopic asthma or severe asthma or may affect atopic individuals in the context of primary allergy or rhinitis.

Allergic and chronic forms of fungal disease are far more common than invasive disease, and it is therefore important to understand the immunology of these diseases and the potential defects in host defence against fungal infection that may lead to them. There is an increasing global awareness of these conditions, and since individuals with chronic or allergic fungal disease require long-term antifungal therapy, this results in a rapidly increasing healthcare cost. The issue of antifungal drug resistance is acute in these individuals as resistance frequently arises during their long-term therapy. This chapter will outline the features of allergic and chronic fungal diseases and our knowledge of the underlying immunology.

### 7.2 Disease Frequency

Allergic and chronic forms of fungal disease are far more common than invasive disease. This arises from the fact that these diseases are complications or sequelae of common underlying conditions such as asthma or tuberculosis, whereas the underlying condition for invasive disease and immune dysfunction is limited to small numbers of individuals with active HIV or those clinically immunocompromised for transplant or by chemotherapy. Complications of atopic asthma by fungal colonisation, such as allergic bronchopulmonary aspergillosis (ABPA) and severe asthma with fungal sensitisation (SAFS), represent the most severe and debilitating forms of asthma [1–3]. More than 4.8 million adult asthmatics have ABPA worldwide [4]; however, the disease is rare in children [5]. The exact numbers affected by ABPA is unclear, but ABPA is identified in 1–8% of asthmatics seen in hospital referral clinics [6, 7].

### 7.3 Description of Allergic and Chronic Fungal Disease Types

Several forms of allergic fungal disease have been described. These include fungal rhinosinusitis, allergic bronchopulmonary aspergillosis (ABPA), allergic bronchopulmonary mycosis (ABPM) and severe asthma with fungal sensitisation (SAFS). ABPA is the best studied disease in this group, and it is not known whether the other diseases are separate entities or form part of a disease continuum resulting from fungal persistence in the airway interacting with different underlying inflammatory diseases such as asthma or severe asthma.

Allergic bronchopulmonary aspergillosis (ABPA) is a hypersensitivity lung disease associated to airway colonisation by the pathogenic mould *A. fumigatus* [2, 8]. When other fungi are found to cause this condition, it is referred to as ABPM. Exposure to fungi is universal; every day we inhale litres of air that contains thousands of *Aspergillus* spores [9]. Due to the small size of spores, they can rapidly be deposited into the lower bronchial airways. In the healthy human host, spores are recognised and cleared by the innate immune system via a Th1 immune
response. ABPA is caused by an exuberant and distinctive host response to *Aspergillus* antigens [10–12]. It is frequently seen in asthma (2.5%) and cystic fibrosis patients (15%) [13, 14], who develop ABPA following inhalation and airway colonisation of *A. fumigatus* [14, 15]. In these patients, an allergic Th2 response develops on exposure to *A. fumigatus*.

Studies have attempted to elucidate the underlying mechanism for this Th2 response in ABPA. Some have focused on the response of the bronchial epithelium, while others have focussed on specific cells such as macrophages [16–21]. The numerous *A. fumigatus* antigens promote an IgE-mediated and eosinophilic response, which is thought to be responsible for the disease features and symptoms. IgG and cellular responses are implicated as well, but tissue invasion does not occur [22]. This unusual reaction induces the production of Th2 pro-inflammatory cytokines responsible for IgE production, mast cell degranulation and allergic airway response activation [10, 11, 23]. Stimulation of PBMCs with *Aspergillus* results in production of Th2 cytokines IL5 and IL13, and ABPA patients show increased *Aspergillus*-induced IL5 and IL13, and decreased IFNγ production, compared to healthy controls [24]. IgE production, eosinophil recruitment and production of an abnormal host inflammatory response in the bronchi and bronchioles of the lungs is observed [25]. This is followed by excessive mucin production, eosinophil infiltration of the bronchial mucin and development of the features of ABPA [15]. Moreover, certain secreted *Aspergillus* proteins can induce toxicity in the bronchial epithelium causing cell detachment and death.

### 7.4 Clinical Symptoms of ABPA

Although ABPA caused by *A. fumigatus* is the most common form of allergic bronchopulmonary mycosis, other fungi can cause the disease [4]. Patients have poorly controlled asthma, wheezing, haemoptysis and expectoration of mucus plugs [22]. Other symptoms include fever, malaise and fatigue. Recurrent pulmonary infiltrates with or without bronchiectasis is frequent [4]. Elevated total blood IgE levels and IgE reactivity to *A. fumigatus* is observed in patients, and *A. fumigatus* is often isolated from sputum. Often central bronchiectasis and mucoid impaction of bronchi with distal atelectasis occurs. Untreated ABPA can result in pulmonary fibrosis and eventually respiratory failure [22].

Differential diagnosis of ABPA used to be difficult as clinical signs are unspecific and frequently misdiagnosed as pulmonary tuberculosis. However, in 2013, the ABPA complicating asthma group, from the International Society for Human and Animal Mycology (ISHAM), proposed the new diagnostic and classification criteria for ABPA [22]. They agreed on the need for one of the predisposing conditions of bronchial asthma and cystic fibrosis and two obligatory criteria. Patients should have a Type I *Aspergillus* skin test positive or elevated IgE levels specific against *A. fumigatus*, and the total IgE levels should be >1000 IU/ml. Moreover, at least two out three of the next criteria should be met: the presence of precipitating of IgG antibodies against *A. fumigatus* in serum, radiographic pulmonary opacities consistent with ABPA or total eosinophil counts >500 cells/ml in patients that have never been treated with steroids before [22].
Severe asthma with fungal sensitisation (SAFS) (also known as fungal-associated severe asthma) is a complication of severe asthma caused by sensitisation to one or many fungi including *A. fumigatus*, *Penicillium chrysogenum*, *Cladosporium herbarum*, *Alternaria alternata*, *Candida albicans* and *Trichophyton* spp. The underlying condition, severe asthma, is defined by the 2009 ATS/ERS guidelines or earlier BTS guidelines. In practice this means a low FEV1 or peak flow (usually persistently), high-dose inhaled steroids and/or frequent courses of oral steroids. SAFS is indicated when total IgE to fungi is <1000 KIU/L or when skin test or specific IgE test is positive for any fungus. Severe asthma affects 5–20% of those with asthma; of these, 35–50% have SAFS, depending on how extensively they are tested. It is conservatively estimated that SAFS might affect 6 million people worldwide. Treatments for SAFS include treatment for severe asthma with additional antifungal treatment for asthma. Itraconazole treatment benefits ~60% patients in terms of quality of life, although not necessarily improved lung function. Severe asthma is a very debilitating disorder, with frequent medical contacts and multiple treatments. Poor or late treatment of severe asthma results in a number of intensive care admissions or deaths each year, but it is not known how many of these are in SAFS patients.

7.5  **Fungal Sinusitis**

The terms allergic fungal rhinosinusitis (AFRS), eosinophilic fungal rhinosinusitis (EFRS), allergic *Aspergillus* sinusitis and eosinophilic mucin rhinosinusitis encompass several disease entities, with unclear boundaries between them. The predominant fungus responsible varies geographically but includes *A. fumigatus*, *A. flavus*, *Bipolaris spicifera*, *Curvularia lunata* and *Alternaria alternata*. *Alternaria alternata* is the most common causative agent in the USA, while *A. flavus* is the most common in the Middle East, India and Pakistan. Fungal sinusitis is usually a chronic condition causing nasal obstruction, loss of smell, nasal discharge (productive sneezing and/or postnasal mucus) and a pressure sensation over the sinus area. Patients often have asthma. AFRS and EFRS are estimated to affect ~12 million people at any time.

7.6  **Chronic Pulmonary Aspergillosis (CPA, Aspergilloma, Chronic Necrotising Pulmonary Aspergillosis, Chronic Cavitary Aspergillosis)**

The term chronic pulmonary aspergillosis embraces several closely related disease entities including simple aspergilloma, chronic cavitary pulmonary aspergillosis and chronic fibrosing pulmonary aspergillosis. CPA is a slowly progressive and destructive disease of the lungs, usually of one or both upper lobes, with cavity formation the most common radiological feature. It is arbitrarily defined as being present for at least 3 months. It occurs in non-immunocompromised or minimally immunocompromised patients. Some patients have nodules, which probably
represent early disease, may be mistaken for lung cancer and have a positive PET scan. Common symptoms are fatigue, weight loss, breathlessness, productive cough and haemoptysis (coughing up blood). The disease is often mistaken for pulmonary tuberculosis and both diseases can coexist. About 25% of patients have an aspergilloma (fungal ball) present; the remainder have one or more cavities and/or nodules. Chronic pulmonary aspergillosis is estimated to affect over 3 million people worldwide, of whom ~1.2 million have had tuberculosis.

7.7 Clinical Symptoms of Chronic Pulmonary Aspergillosis

7.7.1 Aspergilloma

Aspergillomas (or fungal balls) are a gelatinous mass of fungus, usually *A. fumigatus*. Diagnosis is normally from an x-ray or CT scan showing an approximately spherical shadow with surrounding air in a pulmonary cavity, with serological or microbiological evidence that *Aspergillus* spp. is present in the material. Patients are normally not immunocompromised, and aspergillomas can remain stable for many months without progression or obvious symptoms. Multiple aspergillomas or those that are complicated by cavities may be known as complex aspergillomas [26].

7.7.2 Chronic Cavitary Pulmonary Aspergillosis (CCPA)

CCPA is defined as the presence of one or more pulmonary cavities, which may or may not contain a fungal ball, with serological or microbiological evidence implicating *Aspergillus* spp. in a non-immunocompromised patient (or one whose immunocompromising condition has remitted or is trivial) with significant pulmonary or systemic symptoms and overt radiological progression (new cavities, increasing pericavity infiltrates or increasing fibrosis) over at least 3 months of observation [27]. If untreated, cavities can continue to form and/or expand over a period of months or years, with progressive lung fibrosis and chronic inflammation [27]. *Aspergillus* growth on the cavity surface, without tissue invasion, may lead to fungal balls (aspergillomas). The mechanisms underlying the observed pathology are largely unknown, but development of aspergilloma(s) represents a later phase of CCPA [28]. If biopsy of the affected area is performed, it demonstrates hyphae with surrounding chronic inflammation and fibrosis but not tissue invasion.

7.7.3 Chronic Fibrosing Pulmonary Aspergillosis (CFPA)

CFPA patients have severe fibrotic destruction of at least two lobes of lung complicating chronic cavitary pulmonary aspergillosis, leading to a major loss of lung function. Usually the fibrosis is in the form of consolidation, but it may be large cavities with surrounding fibrosis. Severe fibrotic destruction of one lobe with a
A cavity is simply referred to as chronic cavitory pulmonary aspergillosis affecting that lobe.

### 7.7.4 Subacute Invasive Aspergillosis or Chronic Necrotising Pulmonary Aspergillosis (CNPA)

More mild than true invasive aspergillosis, subacute invasive aspergillosis usually occurs in mildly immunocompromised patients, occurring over 1–3 months, with marked pleiotropic radiological features (cavitation, nodules and progressive consolidation with “abscess formation”) and hyphae visible in destroyed lung tissue or inferred from microbiological investigations (i.e. positive Aspergillus antigen).

### 7.8 Predisposing Conditions for CPA

Individuals affected with chronic cavitory pulmonary aspergillosis (CCPA) almost invariably have some prior lung disease (e.g. chronic obstructive pulmonary disorder [COPD] or pulmonary tuberculosis [TB]) but are overtly immunocompetent and do not generally have clinical history of recurrent infection [29, 30]. The commonest underlying lung diseases are tuberculosis, chronic obstructive pulmonary disease, sarcoidosis, ABPA, prior pneumothorax, prior lung cancer (sometimes with lung radiotherapy or surgery) and asthma (including SAFS). Most patients are not taking corticosteroids or other immunosuppressant drugs, but many are on inhaled corticosteroids or take small doses of oral corticosteroid. Many patients have low IFN-γ responses to standard stimuli.

#### 7.8.1 Tuberculosis

A recent study identified tuberculosis (either classical TB or non-tuberculous mycobacterial infection, atypical TB) as an underlying condition in 32.5% (41/126) of patients [30]. This finding is lower than in other earlier studies, which identified tuberculosis as an underlying condition in 50–72% of CPA patients [27, 31, 32]. The importance of tuberculosis in the development of aspergillosis is supported by various studies of tuberculosis patients. Of 544 patients who had cured tuberculosis but who had been left with a residual cavity of ≥2.5 cm 1 year after recovery, 36% were found to have positive Aspergillus antibodies, and 22% were found to have radiological aspergillomas after 3 years [33, 34]. As these reports are historical and are from a time when the various forms of CPA were not recognised as separate entities, they are likely to contain cases of what we now recognise as CCPA, SAIA and CFPA, as well as simple aspergilloma cases. As 21–35% of patients who survive pulmonary tuberculosis have residual cavities [35, 36], it is likely that 8–12%
patients who recover from classical tuberculosis develop CPA over 4 years. In addition, CPA can occur simultaneously to tuberculosis; one study has identified *Aspergillus* coinfection in 14/136 (10%) cases of *Mycobacterium xenopi* pulmonary infection [37], while another found that in 4% of 302 patients with *Mycobacterium kansasii* infections developed aspergillosis [38].

### 7.8.2 ABPA, SAFS and Asthma

Case reports of coexistent ABPA and CPA exist, and studies of aspergilloma and CPA patients have identified ABPA as an underlying condition in 12–14% (10/85) of the cases [30, 39–42]. In one recent study, ABPA was found to be the most common primary underlying condition [30]. Additionally, this study identified SAFS [43] as an underlying condition in 2.4% of CPA patients, and SAFS was found to be the primary underlying condition for 1.6% of cases [30]. Asthma has also been identified in 6–12% of CPA patients [27, 30, 31, 42].

### 7.8.3 COPD and/or Emphysema

COPD and/or emphysema is one of the most common underlying conditions in CPA, identified in over a third of patients in various studies [27, 30, 31, 42]. In addition, recent data indicates a substantial rise in acute invasive pulmonary aspergillosis (IPA) diagnosed in COPD patients, and this disease has a 95% mortality [44, 45]. It is possible that transformation from CPA to acute IPA can occur following an exacerbation of COPD and treatment with corticosteroids.

### 7.8.4 Pneumonia

Various studies have identified pneumonia as a predisposing factor for aspergilloma and CPA. One identified *Pneumocystis carinii* pneumonia in 12% of aspergilloma cases, while another identified pneumonia and lung abscess in 9.4% of aspergilloma cases [32, 41]. A more recent study found that 22.2% (28/126) of the CPA patients analysed have pneumonia as an underlying condition [30].

### 7.8.5 Sarcoidosis

Sarcoidosis has been identified as a predisposing factor for CPA, in a number of published case reports and studies [30, 46, 47]. Cohort studies have identified sarcoidosis as an underlying condition in 11.8–17% of CPA patients [31, 32, 41]. In addition, a study that followed 100 sarcoidosis patients over a 10 year period found that 10% (10/100) developed aspergillomas [48].
7.8.6 Pneumothorax

History of pneumothorax has been identified by various studies, in 11.1–17% of CPA cases [27, 30, 31], and, where a primary underlying disease is identified, previous pneumothorax (±bullae) is also common [30].

7.8.7 Lung Cancer and Thoracic Surgery

In a recent study, prior treated lung cancer was identified in 10.3% of CPA cases, and thoracic surgery was identified in 14.3% [30]. The relationship of cancer to CPA is complex as chemotherapy, chest radiotherapy and/or thoracic surgery are normal treatments for lung cancer and may themselves predispose for the disease [27, 31, 42].

7.8.8 Rheumatoid Arthritis

Rheumatoid arthritis (RA) (with little or no immunosuppressive treatment) was identified as an underlying condition in 4% of CPA patients in a recent study [30], and upper lobe fibrosis and/or cavitation associated with RA has also been identified as an underlying disease in 2.4% of aspergilloma cases [41].

7.8.9 General Comments on Pathogenesis of CPA

The studies discussed here demonstrate the importance of underlying conditions in the development of CPA. Many of these underlying conditions have effects on the physical structure of the lung. Pneumothoraces, lung cancer and thoracic surgery, by their nature and by their treatment, result in lung damage, while tuberculosis can leave cavities in the lung [33–35]. RA can lead to the development of pulmonary fibrosis, pneumonia can cause extensive damage and scarring, and sarcoidosis, particularly the late stage fibrotic form, results in lung fibrosis and cavities [41, 48]. Lung fibrosis can also occur in ABPA [49]. These sequelae result in areas of damaged lung, which Aspergillus can colonise. Once inhaled, the fungus can grow and either forms a simple fungal ball or go on to invade the lung parenchyma and cause/expand cavities. Other conditions, such as ABPA or SAFS, have a strong fungal component themselves, and the presence of the fungus in these patients may precipitate colonisation and development of CPA. Additionally, genetic factors may be important in the development of CPA, and some have been identified, but this work is in its infancy, as it is for most respiratory conditions [50–53].

7.8.9.1 Diagnosis

The key diagnostic tests are serum Aspergillus IgG testing, also known as Aspergillus precipitins, and radiology showing one or more cavities or nodules.
A. *fumigatus* IgG antibodies are detectable in ~90% of patients. Alternative approaches to diagnosis include detectable *A. flavus* or *A. niger* IgG antibodies, *A. fumigatus* IgE antibodies and biopsy/excision of lesions showing hyphae consistent with *Aspergillus* within a cavity. Sputum culture positive rates are ~25%, and *Aspergillus* PCR is more sensitive, but many patients are still negative.

### 7.8.9.2 Outlook and Prognosis

CPA progresses at a variable rate and is often diagnosed late. Severe disease carries a 15–30% mortality in the first 6 months after diagnosis. Death is mainly due to pneumonia and lung bleeding. Those with minor involvement will do well for many years, if progressive lung destruction can be halted. Azole resistance in *A. fumigatus* is becoming an increasing problem, especially in patients with aspergillomas and those with low levels of itraconazole.

### 7.9 Routes of Infection in Chronic and Allergic Fungal Disease

It is presumed that fungi are able to grow and persist in the airways of individuals with the discussed underlying diseases, giving rise to a dynamic, responsive and potent source of allergens as well as secreted fungal proteins such as proteases that may damage the airway epithelium. The combination of tissue damage and a dynamic source of allergens is thought to be important in triggering allergic fungal disease. Other evidence suggests that submicroscopic fragments of dead fungi are present in air and that these allergen-bearing particles may also trigger allergic responses in the lung. The dynamics of particle deposition in the lung means that the smallest particles or spores penetrate furthest. Thus, fungi with small spores can penetrate into the alveolae, whereas other fungi with larger spores are restricted to the nose and upper airway (Fig. 7.2). Lung epithelium also differs in structure and complexity depending on its position in the lung. For example, alveolar epithelium is a relatively simple cell monolayer, whereas bronchial epithelium cells are more substantial and differentiated into several cell types. The combination of this difference in epithelium composition with the different penetration of fungal spores means that different fungi are likely to encounter different epithelium structures and that the differences in the interaction may mean that immune responses could differ dramatically depending on fungal species.

It seems clear that neither live fungus nor hyphal fragments penetrate the epithelium to invade lung tissue to any great extent. Exacerbated symptoms of asthma arise from formation of mucus plugs in the airways that restrict air flow and from potentially continuous allergen exposure with heightened immune responses (Table 7.1 and Fig. 7.1).

Deposition of fungal spores in the lung is dependent on spore size, branching and tubule diameter. Different spore sizes are predicted to penetrate the lung to varying
degrees with only the smallest spores penetrating to the alveolus. The structure of the airway lining differs considerably from the upper airway through to the alveolae with the higher airways having the most complex epithelial structure. Thus, spores of *Alternaria* will not penetrate further than the bronchus and will only encounter the highly differentiated epithelium consisting of epithelial cells, goblet cells and Clara cells, whereas *Aspergillus* spores will be deposited throughout the airway but predominantly in the alveolus.

### Table 7.1 Prevalence and annual burden of fungal disease in the EU

<table>
<thead>
<tr>
<th>Predominant risk groups</th>
<th>At-risk population</th>
<th>Prevalence</th>
<th>Annual burden</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABPA Asthma</td>
<td>34,700,000</td>
<td>2.1%</td>
<td>729,000</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(243–1215)</td>
</tr>
<tr>
<td>ABPA Cystic fibrosis</td>
<td>29,000</td>
<td>15%</td>
<td>4300</td>
</tr>
<tr>
<td>SAFS Severe asthma</td>
<td>3,470,000</td>
<td>33%</td>
<td>1,145,000</td>
</tr>
<tr>
<td>CPA COPD, sarcoidosis, TB, ABPA</td>
<td>&gt;13,600,000</td>
<td>1–10%</td>
<td>204,000</td>
</tr>
<tr>
<td>IPA HSCT, neutropenia, corticosteroids</td>
<td>~5–50,000,000</td>
<td>1–10%</td>
<td>~50,000</td>
</tr>
</tbody>
</table>

**Fig. 7.1** Deposition of fungal spores in the lung
The fact that only 2–4% of atopic asthmatics acquire ABPA in an environment providing constant exposure to fungi suggests that susceptibility may be genetic. Two to four percent of the atopic population is allergic to fungi; however, most

**Fig. 7.2** Immunology of the ABPA response. Glucan and other surface components of *Aspergillus fumigatus* germinating spores and hyphae activate resident macrophages, dendritic and respiratory epithelial cells via innate recognition receptors, such as dectin-1 or toll-like receptor (TLR2/4) to produce T-helper cell (Th2) promoting cytokines; chemokines and co-stimulatory molecules that include thymic stromal lymphopoetin (TSLP), interleukin (IL)-25, IL-33 and CCL17. These stimulate differentiation, chemotaxis and activation of CD4+ Th2 cells. CCL17 also attracts regulatory T cells (Treg) capable of suppressing protective Th1. Th2 cells produce IL-4 and IL-5 that attract and activate eosinophils. Fungal antigens are captured and presented to the adaptive immune system by professional antigen-presenting cells such as macrophages and dendritic cells leading to the presence of B cells that produce IgG specific to fungal proteins. On further exposure to these proteins and with co-stimulation by IL4, these B cells differentiate into IgE-producing B cells or plasmacytes. IgE antibodies bind to the FcεR receptor of mast cells; these receptors dimerise in the presence of fungal antigen triggering an immediate hypersensitivity reaction. Further inflammatory responses are caused by fungal protease-mediated degradation of the epithelial cell tight junction typically leading to release of the broad spectrum IL6 and IL8 cytokines. Combination of the Th2 cell response and mast cell degranulation leads to the typical mucosal inflammation and mucus over secretion seen in ABPA

### 7.10 Immunology of Allergic and Chronic Fungal Disease

#### 7.10.1 Immunopathogenesis of ABPA

The fact that only 2–4% of atopic asthmatics acquire ABPA in an environment providing constant exposure to fungi suggests that susceptibility may be genetic. Two to four percent of the atopic population is allergic to fungi; however, most
individuals with fungal allergy do not have ABPA. These observations suggest that susceptibility to ABPA is not simply a matter of atopic asthma complicated by fungal allergy but that it must depend on some more profound susceptibility to fungal colonisation. Atopic asthma is not a strict requirement for ABPA; however, almost all individuals with ABPA have this underlying condition. This poses difficulties in attempting to define genetic susceptibility factors for ABPA as affected individuals already have a genetically and immunologically complex disease. Furthermore, the use of animal models to discover genes involved in the disease is hindered by the lack of refined models for either ABPA or atopic asthma.

In a normal, healthy immune response, lung epithelial cells, dendritic cells and macrophages regulate the Th1/Th17/Th2/Treg balance, which is critical for pathogen clearance [54]. For most fungal exposure, this response is biased towards Th1 cell proliferation which is thought to lead to effective killing and clearance of fungi by neutrophils, macrophages and T cells [55].

In ABPA or other allergic fungal diseases, cells respond to fungal exposure with a Th2 response. *A. fumigatus* germinating spores and hyphae activate dendritic and respiratory epithelial cells via components of the innate immune system [9, 56, 57]. Toll-like receptor (TLR) 2/4, dectin 1 and 2 and other pattern-associated molecular pattern receptors located on epithelial cells, dendritic cells and macrophages resident in the lung produce cytokines, chemokines and co-stimulatory molecules such as thymic stromal lymphopoetin (TSLP), interleukin (IL)-25, IL-33, OX40 ligand (OX40L) and CCL17 (thymus-activated and thymus-regulated chemokine), which stimulate differentiation and activation of CD4+ Th2 cells. CCL17 also attracts regulatory T cells (Treg) capable of suppressing protective Th1 responses.

The Th2 cell response releases IL-4 and IL-5 that attract eosinophils and drive differentiation of B cells to IgE-secreting plasmacytes. Since the lungs may contain a substantial amount of fungus, many B cells will carry fungus-specific IgG that is converted to fungus-specific IgE by chain switching. Fungus-specific IgE binds a specific receptor, FCER, on mast cells and basophils, and these cells react to the binding of fungal allergen and antigen proteins by degranulation and release of histamines and other factors that trigger an immediate hypersensitivity reaction. This results in bronchoconstriction and overproduction of mucus in the airway leading to mucus plugging. The inflammatory response can lead to bronchiectasis that may progress to fibrosis if untreated. Recent work by Becker et al. suggested that the Th2 stimulatory signalling observed in ABPA arises through CR3 receptor-mediated detection of fungi, specifically *A. fumigatus* [24].

7.10.1.1 SAFS

It is not clear why the proportion of individuals sensitised to fungi is so high in severe asthmatics. The tenfold higher rate of fungal allergy in severe asthma compared to normal allergic individuals suggests a role for fungal colonisation in driving asthma to its most extreme form; however, no mechanisms have yet been proven to support this hypothesis.
7.10.2 Immunology of Chronic Pulmonary Aspergillosis

It is thought that the scar or cavity left by the predisposing disease or lung injury forms a weak point in the lung that can be exploited by the infecting fungus. Few immunological features have been associated with the disease; however, many individuals with CPA have high levels of *Aspergillus*-specific IgG, which may arise from the very high fungal burden observed in this condition. The field has also been hampered by lack of an animal model for chronic fungal infection. One recent promising advance has been the use of agarose bead protected fungal hyphae in mouse models of long-term fungal colonisation [58]. In this model system, fungi can persist in mouse airways for up to 28 days. Pro-inflammatory cytokines and chemokines increased early in infection with raised levels of interleukin-4 (IL-4), elevated IgE levels in serum and a mild increase in airway responsiveness. T-cell analysis suggested a Th2-type response, followed by a rise in IL-17 and Foxp3 (+) cells by day 14. A high proportion of patients with chronic pulmonary aspergillosis are poor producers of IFN-γ in response to multiple stimuli, and this is currently being explored as a possible therapeutic tool for CPA [59].

7.10.3 Gene Variants Associated with Chronic and Allergic Fungal Disease

The study of genetic variants associated with chronic and allergic fungal disease has been hampered by the complexity of both the disease and by the associated underlying conditions. For instance, it is not clear whether genetic factors discovered for CPA are somehow related to underlying tuberculosis or COPD.

7.10.3.1 CPA

Little is known about the factors affecting susceptibility, continuing inflammation or disease pathogenesis in CCPA. The most commonly cited susceptibility factor is underlying disease; however, the proportion of patients with any one disease is small, and only a small percentage of individuals with any one underlying disease develop CCPA [30]. Previous genetic association studies involving small numbers of patients have identified associations between CPA and TNF, MBL2, *TGFB1*, *IL15*, *TLR4* and *IL10* [51–53, 60] but do not explain all cases of CPA and have proved difficult to replicate.

Recent studies [29, 61] using larger CPA populations suggested several SNPs associated with CCPA including three intronic mutations in *IL15* (rs6842735, rs12508866, rs1519551), an intronic insertion-deletion mutation in *IL1B* (rs3917354), a missense non-synonymous coding SNP in *TLRI* (rs4833095), a 3’UTR SNP in *ILRN* (rs4252041), a *CLEC7A* SNP (rs7309123) previously associated with IA [62] and further SNPs in *DENND1B* (rs2477077), *PLAT* (rs8178890)
and VEGFA (rs10434) (Table 7.2) [61]. Additional SNPs in TLR1, CLEC7A, IL17A and IL1B showed trends towards significance but failed to pass correction for multiple testing (FDR corrected p-values 0.050–0.051) (Table 7.2) [29].

### 7.10.3.2 ABPA

Why some asthmatic individuals develop ABPA upon exposure to *A. fumigatus* while others are unaffected remains unclear, despite studies. Chronic intranasal administration of mould spores or extracts to unsensitised mice can lead to allergic lung inflammation, hyperreactivity and lung remodelling [63], but the effect in humans is unclear. There are reports of ABPA within families, suggesting a common genetic basis with low penetrance [64, 65], and familiar occurrence of ABPA has been reported in 4.9% of ABPA patients in India, where the disease is common [66].

Although our knowledge of genetic risk factors that might be involved in ABPA is limited, polymorphisms in genes that might play a crucial role for the ABPA immune response (IL4R, IL10, TLR9, SFTPA2 and HLA-DR) have been described in various small genetic association studies (involving ≤38 patients) [51, 60, 67–69]. In addition, the structural gene CFTR has been previously associated with ABPA [70], and a more recent, larger genetic association study has found associations with other genes [71]. This study identified 17 ABPA-associated polymorphisms, three of which remained significantly associated after correction for multiple testing. These were in the immune genes IL13, IL4R and TLR3 [71] (Table 7.3).

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**Table 7.2** SNPs associated with CCPA

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Alleles</th>
<th>Model for association</th>
<th>Odds ratio (95% CI)</th>
<th>FDR p-value</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL1RN</td>
<td>rs4252041</td>
<td>C/T</td>
<td>TT+TC vs. CC</td>
<td>0.23 (0.07, 0.76)</td>
<td><strong>0.039</strong></td>
<td>3' UTR</td>
</tr>
<tr>
<td>IL1B</td>
<td>rs3136558</td>
<td>A/G</td>
<td>AG+GG vs. AA</td>
<td>0.57 (0.35, 0.92)</td>
<td>0.051</td>
<td>Intronic</td>
</tr>
<tr>
<td></td>
<td>rs3917354</td>
<td>T/−</td>
<td>T−+− vs. TT</td>
<td>0.55 (0.33, 0.91)</td>
<td><strong>0.046</strong></td>
<td>Intronic</td>
</tr>
<tr>
<td>IL15</td>
<td>rs1519551</td>
<td>A/G</td>
<td>AA+AG vs. GG</td>
<td>0.48 (0.29, 0.79)</td>
<td><strong>0.011</strong></td>
<td>Intronic</td>
</tr>
<tr>
<td></td>
<td>rs6842735</td>
<td>G/T</td>
<td>TT+GT vs. GG</td>
<td>1.79 (1.12, 2.88)</td>
<td><strong>0.038</strong></td>
<td>Intronic</td>
</tr>
<tr>
<td></td>
<td>rs12508866</td>
<td>T/C</td>
<td>CC+TC vs. TT</td>
<td>1.70 (1.09, 2.66)</td>
<td><strong>0.046</strong></td>
<td>Intronic</td>
</tr>
<tr>
<td>IL17A</td>
<td>rs3748067</td>
<td>G/A</td>
<td>AA+GA vs. GG</td>
<td>1.90 (1.10, 3.28)</td>
<td>0.050</td>
<td>3' UTR</td>
</tr>
<tr>
<td>TLR1</td>
<td>rs4833095</td>
<td>A/G</td>
<td>AG+GG vs. AA</td>
<td>0.58 (0.36, 0.95)</td>
<td>0.065</td>
<td>Exonic (Asn/Ser)</td>
</tr>
<tr>
<td>CLEC7A</td>
<td>rs7309123</td>
<td>C/G</td>
<td>CC+GC vs. GG</td>
<td>0.59 (0.35, 0.99)</td>
<td>0.099</td>
<td>Intronic</td>
</tr>
<tr>
<td>PLAT</td>
<td>rs8178890</td>
<td>G/A</td>
<td>AA+GA vs. GG</td>
<td>0.38 (0.16, 0.86)</td>
<td><strong>0.049</strong></td>
<td>Intronic</td>
</tr>
<tr>
<td></td>
<td>rs879293</td>
<td>G/A</td>
<td>AA+GA vs. GG</td>
<td>1.65 (1.01, 2.69)</td>
<td>0.097</td>
<td>Intronic</td>
</tr>
<tr>
<td>DENND1B</td>
<td>rs2477077</td>
<td>C/T</td>
<td>CC+CT vs. TT</td>
<td>0.34 (0.14, 0.83)</td>
<td><strong>0.041</strong></td>
<td>Intronic</td>
</tr>
<tr>
<td>VEGFA</td>
<td>rs10434</td>
<td>G/A</td>
<td>GG+AG vs. AA</td>
<td>2.12 (1.16, 3.90)</td>
<td><strong>0.036</strong></td>
<td>3' UTR</td>
</tr>
</tbody>
</table>

From Smith et al. [29, 61]

Risk allele shown in bold. All SNPs are associated with CCPA before correction for multiple testing (*p* < 0.05). SNPs in bold remain significant after Benjamini-Hochberg adjustment for false discovery rate (FDR adjusted p-values shown)

CI confidence interval
Recent work in our laboratory suggests that there are some genetic susceptibility factors for SAFS. Additional and broader genetic association studies in SAFS, combined with experimental work, are likely to contribute to our understanding of different phenotypes of problematic asthma.

**7.11 Discussion**

Chronic and allergic fungal diseases are both common and complex. They are almost exclusively secondary infections that complicate an existing condition or display a specialised opportunistic mode of infection utilising lung damage caused by a
previous disease. The manner in which allergic disease can progress to chronic disease suggests that there may be shared susceptibility for both allergic and chronic fungal disease, but the symptoms and features of the two types of disease are quite distinct.

Study of genetic factors underlying chronic and allergic fungal disease has been hampered by a number of factors: until recently clinical guidelines and descriptions for either disease were not available, so few cases were diagnosed, symptoms for either disease are diffuse and easily misdiagnosed so strict phenotyping of patients for genetic typing has proved problematic, and, finally, the nature of the underlying disease needs to be considered, and this has posed problems in choosing the right control group for genetic comparisons.

The gene variants associated with either disease can be grouped into those affecting antigen presentation for the adaptive immune response (HLA genes), genes involved in the innate immune response particularly pattern recognition receptors (e.g. dectin-1, TLR genes, CLEC7A, MBL and SP-A) and genes involved in immune cell attraction, maturation and migration (e.g. PLAT, CCL2). The genes selected for analysis were chosen from our existing knowledge of the innate immune response to fungi, and so these results are not unexpected. Although these known variants are significant, they are all present at low frequency in the affected population. No variant yet studied is capable of explaining the full range or frequency of genetic susceptibility to fungal infection.

Future research in this area is likely to include more wide-ranging genetic techniques such as whole genome and exome sequencing that will shed light on the true range of factors that underlie these diseases.

References

7 Immunogenetics of Chronic and Allergic Aspergillosis


63. Denis O, van den Brule S, Heymans J, et al. Chronic intranasal administration of mould spores or extracts to unsensitized mice leads to lung allergic inflammation, hyper-reactivity and remodelling. Immunology. 2007;122(2):268–78.