Chapter 7

Allergic bronchopulmonary aspergillosis and other fungal diseases

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Summary

Fungal spores are ubiquitously present in the air. Inhalation of these spores by humans causes disease in susceptible patients; most prevalent are invasive aspergillosis and allergic bronchopulmonary aspergillosis (ABPA). This chapter provides an overview of the pathogenicity, clinical appearance, diagnosis and treatment of ABPA.

ABPA is a hypersensitivity lung disease limited to patients with asthma or cystic fibrosis (CF) with a prevalence of 1–2% and 2–15%, respectively within these groups. It is triggered by the exposure to Aspergillus fumigatus. Although it is not clear what initiates this hypersensitivity response, polymorphisms in genes that drive innate and adaptive immune mechanisms as well as loss-of-function mutations in the CF transmembrane conductance regulator (CFTR) are associated with ABPA development. The chronic inflammatory conditions in ABPA eventually result in airway remodelling and functional impairment.

The diagnosis of ABPA is based both on clinical symptoms, laboratory testing and diagnostic imaging. Treatment consists of a two-tiered approach, glucocorticoids to control immunological activity and antifungal agents to suppress fungal load.

Keywords: ABPA, aspergillosis, Aspergillus fumigatus, CFTR, hypersensitivity

Humans continuously inhale fungal spores. Only some fungal species cause invasive, allergic or toxic disease, most prevalent of which are invasive aspergillosis in immunocompromised patients and allergic bronchopulmonary aspergillosis (ABPA) in asthmatics and patients with cystic fibrosis (CF). This chapter provides an overview of the current knowledge concerning the
role of fungi in the pathogenesis of bronchiectasis and describes the clinical appearance, immunological background, diagnosis and treatment of ABPA.

Centrally located, cylindrical bronchiectasis is a major characteristic of ABPA; however, 5–25% of patients with ABPA are diagnosed without the presence of bronchiectasis [1–3]. ABPA is predominantly observed in asthmatic and CF patients. Its prevalence among asthmatics and CF patients is 1–2% [4] and 2–15% [5–11], respectively.

In patients with ABPA, *Aspergillus fumigatus* antigens provoke a strong allergic reaction, characterised by the dominance of T-helper cell (Th) type 2 mediated responses, high numbers of eosinophils, a high total immunoglobulin (Ig)E level and high levels of *Aspergillus* specific IgE and IgG levels. Although it is not clear what initiates this hypersensitivity response, polymorphisms in genes that drive innate and adaptive immune mechanisms as well as loss-of-function mutations in the CF transmembrane conductance regulator (CFTR) are associated with ABPA development. The chronic inflammatory conditions in ABPA eventually result in airway remodelling, which is characterised by mucoid impaction, bronchial inflammation and obstruction. When left untreated fibrosis bronchiectasis and eventually respiratory insufficiency are the final pathophysiological stages in this remodelling process.

The diagnosis of ABPA is complex and difficult to discriminate from chronic inflammatory episodes already observed in patients with asthma or CF. It has been estimated that on average 10 years elapse between the onset of ABPA and its eventual diagnosis [12]. Criteria for the diagnosis ABPA in asthmatics include a history of asthma with immediate skin reactivity, elevated serum IgE, precipitating antibodies against *Aspergillus* sp., peripheral blood eosinophilia, current or previous infiltrates on chest radiographs and central bronchiectasis on high-resolution computed tomography (HRCT) scans. CF patients are chronically exposed to multiple microorganisms and discrimination of ABPA is difficult in these patients. The main diagnostic criteria are similar to those described above, except for higher total IgE levels.

ABPA treatment aims at reducing the fungal burden and dampening the immune response. Antifungal agents are effective in reducing IgE levels and improving clinical outcome within a 16-week period; however, their long-term clinical effects are unknown [13]. The role of antifungal agents in the eradication of *A. fumigatus* hyphae is limited. Immune suppression is mainly achieved by oral glucocorticoid therapy that reduces the total serum IgE levels and correlates with a reduction in symptoms and radiological findings. However, the long-term use of steroids is associated with serious side-effects. Therapy that targets individual components of the hypersensitivity reaction is being developed and tested. The identification of crucial immunological components and associated molecular targets is essential for the design of novel drugs.

Bronchiectasis due to other fungal disease is mainly limited to the immunocompromised host. Only limited studies are available on the role of fungi in otherwise healthy subjects. Both groups are briefly summarised in this chapter.

Figure 1 shows the structure and appearance of *A. fumagatis* under light microscopy.

**History and epidemiology of ABPA**

In 1952, Hinson *et al.* [14] provided the term ABPA for the description of three patients who suffered from pulmonary eosinophilia in the UK, and in 1969 ABPA was first reported in the USA. In 1971, immunoserological features were discovered that supported hypersensitive immune reactivity as a disease mechanism in ABPA. From that time onwards the diagnostic possibilities rapidly improved and in the early 1980s ABPA was reported throughout the world.

Still, the true population prevalence of ABPA remains highly speculative: ABPA was not acknowledged by the World Health Organization (WHO) as a disease entity in their 2007 International Classification of Disease (ICD-10) [15] and the diagnostic criteria for ABPA vary greatly within international medical societies. It has been generally assumed that there is an
estimated population ABPA prevalence of 1–2% in asthmatics [16, 17]. Overviews on the prevalence of ABPA show a spectre of 1% prevalence in the general population of asthmatics to 38.6% in patients with acute severe asthma [18].

In patients with CF the prevalence is estimated to be 1–15% [16, 19]. NOVEY [16] found an average of 7% among a total of 1,096 patients, taken from eight studies. Despite the differences in diagnostic criteria, laboratory methodology, demographical and geographical features, the range of prevalence was narrow in these studies ranging from 3–11%. MASTELLA et al. [20], on behalf of the European Registry of Cystic Fibrosis, reported data for 12,447 patients with CF in nine European countries. The overall prevalence among the European CF patients was found to be 7.8%, with a range of 2.1% in Sweden and 13.8% in Belgium. Age was found to be an important factor; in the group aged <6 years the prevalence was 6% and a stable 10% thereafter [20].

**Bronchiectasis and ABPA**

Bronchiectasis is a morphological disorder, defined as the irreversible dilatation of the cartilage containing airways or bronchi. Approximately half of the patients with bronchiectasis is classified as having idiopathic bronchiectasis. In 7–8% of patients with bronchiectasis, ABPA is the causative factor [21, 22]. ABPA can be subclassified into three groups based upon radiological features indicating the presence or absence of central bronchiectasis and other radiological features. Approximately 75–95% of ABPA patients display both centrally located, cylindrical bronchiectasis (ABPA-CB) with or without other radiological features (ABPA-CB-ORF). The remaining 5–25% of the patients with ABPA are diagnosed without the presence of bronchiectasis, in these patients the diagnosis is based on seropositivity (ABPA-S) [1–3].

The presence of central bronchiectasis is associated with disease severity. The small group of patients with ABPA-S appear to suffer from a less aggressive form of the disease when compared with ABPA-CB and ABPA-CB-ORF patients. Whether ABPA-S is able to progress into ABPA-CB or whether it is a pathogenetically different form of the disease is unclear. In a 3-year prospective cohort study in 11 patients, KUMAR and CHOPRA [23] described better lung function and a lower number of exacerbations in the ABPA-S group compared with an ABPA-CB control group. GREENBERGER et al. [24] included 28 patients in a 2-year prospective cohort study and found different immunological parameters in the ABPA-S group compared with the ABPA-CB group. The study found significantly lower serum specific anti-*A. fumigatus* IgG subclasses in patients with ABPA-S, and a trend towards lower levels of total serum IgE and specific anti-*Aspergillus* IgE and IgA [24]. The radiological differences between the groups are, therefore, also reflected by clinical and immunological differences. The question remains whether early recognition and treatment of ABPA-S can prevent progression into ABPA-CB [23].

**Pathogenesis of ABPA**

The pathophysiological mechanisms underlying the development of ABPA are still poorly understood, but clearly share important immunological mechanisms with other hypersensitivity diseases. ABPA primarily develops in patients with asthma or CF, and is caused by an *Aspergillus*-driven strong hypersensitivity response [25]. Immunological features include highly elevated levels
of total IgE and Aspergillus-specific IgE and IgG, increased eosinophil numbers, and a Th2-dominated antigen-specific CD4+ T-cell response. Hypersensitivity to Aspergillus and colonisation by Aspergillus appear to be required but are not sufficient to develop ABPA alone. Between 31 and 59% of CF patients display sensitisation towards Aspergillus and up to 40% are colonised; however, only 1–10% of CF patients develop ABPA [26]. It appears that unique characteristics within Aspergillus itself in combination with patient-specific environmental and genetic factors facilitate the chronic colonisation and development of a deteriorating immune response, which ultimately induces the irreversible airway remodelling associated with fibrosis, pulmonary obstruction and bronchiectasis.

A. fumigatus virulence

Fungal spores (fig. 2) or conidia are ubiquitously present in our environment. A cubic meter of air typically contains approximately $10^4$–$10^5$ conidia, predominantly of the Cladosporium and Alternaria genera, and of a lesser amount Aspergillus and Penicillium. The genus Aspergillus consists of 250 subspecies of which A. fumigatus is considered the most prevalent airborne fungal human pathogen, its conidia are present at approximately 1–100 m$^{-3}$ of air [27]. A. fumigatus causes life threatening, invasive disease in immunocompromised patients and is associated with multiple hypersensitivity responses including allergic asthma, hypersensitivity pneumonitis and ABPA [28]. Many molecular subtypes of A. fumigatus exist, 85% of analysed A. fumigatus in air samples were unique; however, in general none of these subtypes were found to be selectively enriched in patients suggesting that most subtypes are equally pathogenic [29, 30]. The development of novel antifungal reagents may, however, select for some subtypes [31, 32]. The presence of specific subtypes of A. fumigatus in ABPA remains unknown, but these may be prime candidates to study ABPA-related disease mechanisms.

In recent years insights into the mechanisms by which A. fumigatus regulates its pathogenic potential or virulence have progressed significantly. These mechanisms regulate the rapid growth characteristics of A. fumigatus at 37°C (A. fumigatus conidia germinate within 4–5 hours on nutrient rich media in vitro), the overall mechanical fitness of conidia to withstand environmental pressure, and its capacity to extract nutrients of dead organic matter for growth. Selective mechanisms have also co-evolved. These selective mechanisms directly impair the epithelial barrier function and host immune defence, facilitating its infection.

The particularly small size of a A. fumigatus conidia range between 1 μm and 3 μm, thereby facilitating its ability to be airborne and allowing it to reach the alveolar spaces upon inhalation. The cell wall of A. fumigatus conidia consists of a thick internal layer of structural polysaccharides enriched for branched β(1,3)/(1,6) glucans linked to chitin as observed in most fungi [33, 34]. Additional bonds to this backbone are species specific, in the case of A. fumigatus this core polysaccharide backbone is further linked to galactomannan and linear β(1,3)/(1,4)-glucans. This large polysaccharide complex is embedded in a cement-like mixture consisting of α1,3-glucan, galactomannan and polygalactosamine. A thin hydrophobic protein layer, termed surface hydrophobin, is composed of cross-linked proteins (including RodA) that form a regular pattern of rodlet structures and melanin that confers pigment, which further shields and protects the polysaccharide shell.

Germination initiates an asexual developmental growth programme. It starts with conidial swelling followed by a polar growth programme that results in the protrusion of an elongating germ tube, termed hyphae, from
the conidium cell [35]. Hyphae are covered by a newly synthesised polysaccharide coat without the typical protein coat present on conidia [35]. Simultaneously with polar growth comes nuclear division by mitosis, resulting in further elongating hyphae with each cycle. In immunocompromised patients, *Aspergillus* grows into large hyphal networks termed mycelia and forms extracellular matrices termed biofilms that contain α1,3-glucan, galactomannan and galactosaminogalactan, and possibly other components that promote growth [36]. Interestingly, germination of *A. fumigatus* conidia is increased compared with *Aspergillus flavus* and *Aspergillus niger* at 37°C but not at 20°C [37]. This increased growth rate at 37°C likely contributes to the prevalence of *A. fumigatus* in fungal diseases, such as ABPA.

*A. fumigatus* expresses multiple factors to evade host immune defence mechanisms, which in total contribute to the virulence of *A. fumigatus* in humans. These factors may be part of the growth cycle of *A. fumigatus*, but may also be uniquely expressed as secondary metabolites during specific phases of growth. For example, the binding of conidia to various extracellular matrix (ECM) proteins prevents its mucociliary clearance and the oxidative mechanisms of phagocytes are counteracted by the production of superoxide dismutases, mannitol and three types of catalases. A range of other toxins and proteases further inhibit immune responses and promote epithelial cell penetration including ribotoxin [38], phospholipases [39], haemolysins, gliotoxins, metalloproteinase, alkaline proteinase and elastase [40].

Interestingly, when comparing fungal proteases of *A. fumigatus* with those of *Alternaria alternata* and *Cladosporium herbarum*, KAUFFMAN et al. [41] reported an increase in the activity of *A. fumigatus*-derived proteases, as indicated by the shrinking and desquamation of epithelial cells and pro-inflammatory cytokine production. Although the role of isolated components from *A. fumigatus* in conferring virulence as a human pathogen remains difficult to establish, it is clear that their combined activity contributes to the strong association of *A. fumigatus* with fatal human diseases.

**Innate mechanisms underlying ABPA**

The innate defence mechanisms involved in the clearance and inflammatory response to *A. fumigatus*, and how these may impact on the development of ABPA will be discussed here. The majority of conidia are cleared without inflicting a strong inflammatory response associated with tissue destruction. Most inhaled conidia are efficiently trapped by mucus and removed by mucociliary clearance systems that are affected in CF and asthma patients. Nevertheless, ABPA is generally not observed in patients with primary ciliary dyskinesia in which impaired mucociliary clearance leads to the accumulation of mucus and primarily bacterial infections, suggesting additional mechanisms contribute to the development of ABPA [42].

Beyond the mucociliary system, resident cells of the lungs, such as alveolar macrophages (AM) and type II pneumocytes, destroy conidia by phagocytosis and the production of reactive oxygen species (ROS) upon activation of the membrane-bound NADPH-oxidase complex. It was recently shown that RodA in the protein coat surrounding conidia inhibits the inflammatory response to conidia by masking the highly immunogenic polysaccharide cell wall [43]. This may promote survival of conidia by escaping host immunity, but may also be beneficial to the host by limiting inflammatory responses upon inhalation of conidia.

However, during germination the extending hyphae expose their polysaccharide wall and start to produce metabolites that trigger a strong inflammatory response. Pattern recognition receptors, e.g. Toll-like receptors (TLRs) and carbohydrate-binding proteins termed C-type lectins, are expressed by lung epithelial and resident immune cells, such as AM and dendritic cells (DCs), which recognise the β-glucans, chitin and galactomannan of the cell wall. Controversy still exists over the exact functional role of individual TLRs in the recognition of fungi, but it appears that TLR2, TLR4 and TLR9 do signal in a fungal morphotype-specific manner [44]. Activation of TLR2 and inhibition of TLR4 signalling during hyphal growth has been proposed to promote the development of a Th2 response [45, 46].
In addition to TLRs, members of the C-type lectin family, e.g. the mannose receptor, DC-specific intercellular adhesion molecule-grabbing nonintegrin (DC-SIGN), Dectin-1, and Dectin-2, recognize carbohydrate structures of the fungal wall and play an important role in fungal recognition, killing, and inflammatory signalling. Dectin-1 binds \( \beta-(1,3)\)-glucan and is prevalent on neutrophils, AM and DC. Neutrophils are the first cells to enter an inflammatory site and are short-lived phagocytic effector cells. Neutrophils produce ROS and release proteolytic enzymes, upon apoptosis their DNA traps pathogens but also increase mucus viscosity [47, 48]. Mice lacking Dectin-1 are highly susceptible to \( A. fumigatus \) infection; their macrophages and DC produce low levels of inflammatory cytokines and have limited recruitment of neutrophils to the site of infection with reduced killing capacity [49].

Triggering these pattern-recognition receptors induces the release of multiple inflammatory networks that recruit cells from the blood to the infected area, and play a crucial role in shaping the adaptive immune response at later stages [50–52]. Human polymorphisms in these systems can affect fungal load, growth properties and the balance of inflammatory mediators produced by innate cells that can impact on the quality and quantity of the adaptive response. ABPA is correlated with polymorphisms in TLR9 [53]. The mechanism by which TLR9 predisposes to ABPA in humans remains uncertain; however, pulmonary hypersensitivity induced by \( A. fumigatus \) in TLR9 \(-/-\) mice is significantly reduced [54]. DCs of these mice have lower Dectin-1 levels and produce low amounts of interleukin (IL)-17, which was associated with pulmonary infection of \( A. fumigatus \). Multiple polymorphisms in other innate recognition systems including TLR2 and TLR4 and humoral pattern recognition factors, such as mannose binding lectin and surfactant protein A, have also been associated with ABPA and other different types of fungal diseases [55–59].

Collectively, it is clear that a complex multi-layered innate response to \( A. fumigatus \) has evolved to prevent infection and subsequent invasive disease. Genetic variations in innate systems that impact on pathogen recognition, fungal infection and induction of hypersensitivity responses have been associated with fungal diseases including ABPA. The extent to which genetic variation within these systems affects the development of ABPA in subgroups of CF or asthmatic patients requires further attention and may have prognostic value for patient subgroups.

**Adaptive immunity in ABPA**

DCs are specialised cells that take up antigens at local inflammatory sites and then migrate to draining lymph nodes or bronchus associated lymphoid tissue (BALT) where they activate naïve T-cells by presentation of antigenic peptides in the context of major histocompatibility complex (MHC) [50]. Upon activation of naïve Th cells, these cells acquire distinct cytokine-secreting properties that impact on the developing immune response. Multiple subsets of committed antigen-experienced Th cells are recognised including Th1, Th2, Th17 and induced T-regulatory (T-reg) cells [60]. In general, interferon-\( \gamma \) producing Th1 and IL-17 producing Th17 subsets are important inflammatory cells associated with cell-mediated immunity against viral infections and intracellular bacteria, and are associated with multiple autoimmune diseases. IL-4 producing Th2 cells are typically associated with strong immune responses against large extracellular organisms that cannot be cleared through phagocytosis, such as intestinal parasites, and are associated with allergic diseases and ABPA in humans. Induced T-reg cells and natural T-reg cells are important to dampen immunological responses by the production of IL-10 and transforming growth factor (TGF)-\( \beta \) [61].

Th responses are skewed towards Th2 in ABPA as indicated by *in vitro* lymphocyte responses against secreted proteins from \( A. fumigatus \) and animal models [26, 62–66]. Th1 and Th17 responses against \( A. fumigatus \) appear protective against hypersensitivity and are associated with clearance of *Aspergillus* [67–69]. Why ABPA patients mount such a vigorous Th2-response is not known and remains a key question. Activation of specific pattern recognition receptors and cytokine receptors at the site of inflammation induces DCs to express surface molecules and
cytokines, which help to commit naïve Th cells; however, T-cell intrinsic factors, such as T-cell receptor (TCR) avidity for its antigen, also appear important.

Recently, epithelial products such as IL-25 and thymic stromal lymphopoietin (TSLP) have been shown to alter DCs function and subsequent Th responses [70–72]. TSLP stimulated DCs from ABPA patients use ligand OX40 to potently induce Th2 responses [71]. Other ABPA-associated polymorphisms in genes, e.g. TLR9, IL-4Rα subunit and the IL-10 promoter, may all affect DC maturation and or induction of Th differentiation, but these proteins are expressed by many cells and thus it remains difficult to pinpoint at which level these polymorphisms affect disease [73].

TCRs that bind with low affinity to their cognate antigen may also confer Th2 properties in ABPA. Variants of human MHC class II, such as HLA-DR2 and HLA-DR5 alleles, are associated with ABPA and promote the expansion of T-cells with selective αβ TCR chains. Although expression of these MHC class II variants is not sufficient for ABPA disease, peptides of a dominant allergen of *A. fumigatus*, termed Asp f1, are presented by these molecules and are recognised by low-affinity, TCR-expressing Th2-skewed cells [74]. Other MHC class II alleles also appear to protect against ABPA.

Th2 cells and their cytokines play a crucial role in B-cell class switching and the recruitment of IgE-responding innate cells such as eosinophils, basophils and mast cells. Early studies indicate that supernatants of lymphocytes incubated with *A. fumigatus* antigens regulate IgE production by B-cells [74]. Cytokines, such as IL-4, IL-5 and IL-13, by Th2 cells facilitates B-cell class switching to IgA and IgE in BALT, and induce the production and recruitment of eosinophils to the inflammatory site [19]. IgE levels are quantitatively higher in ABPA compared with other atopic conditions, though little is known about the width of the antibody response against *A. fumigatus* and possible bystander antigens including self antigens. However, recent evidence indicates the existence of a Th2-mediated immune response without the presence of IgE [75]. To place this contradiction into perspective, data from a recent study indicated that out of 66 proteins present in the cytosol of *A. fumigatus*, which were recognised by pooled serum of ABPA patients, 63 were targeted by IgE and only three by IgG antibodies [76]. The prevalence of *A. fumigatus*-specific IgE over IgG antibodies suggests BALT to be a primary site for development of high-specific *Aspergillus* IgE and not the peripheral lymphoid system.

Upon comparison of atopic and ABPA patients, ABPA B-cells were found that expressed higher levels of the low-affinity IgE receptor CD23 and the co-stimulatory molecule CD86 that is crucial for positive reinforcement by Th2 cells, a phenotype associated with *in vitro* IL-4 responsiveness [77]. Indeed, polymorphisms in IL-4Ra have been found to be enriched within ABPA patients in comparison with non-ABPA patients. Furthermore, CF patients with ABPA are more sensitive to IL-4 than CF patients without ABPA, a finding that was not observed for IL-13 [78, 79].

These antibodies trigger hypersensitivity responses by interacting with specialised innate immune cells. IgA and IgE-responsive granulocytes, such as eosinophils, basophils, and mast cells are activated by the Th2 response and recruited to the inflammatory site by a network of soluble mediators and cell-surface molecules [80]. Ligation of IgE on mast cells releases histamine and chemokines such as leukotriene B4 and platelet-activating factor, which induce smooth muscle contraction, vascular permeability and attract eosinophils. RANTES (regulated on activation, normal T-cell expressed and secreted), eotaxin and monocyte chemotactic protein (MCP)-3 are other important chemoattractants for eosinophils. The receptor for eotaxin, chemokine receptor 3 (CCR3) is selectively expressed by Th2, eosinophils and basophils, and is upregulated by IL-4. Th2-derived IL-5 is essential for increased eosinophil production from the bone marrow and their activation but appears dispensible for *A. fumigatus*-induced hyperreactivity in mice [81]. Nevertheless, these cells are a prominent feature of ABPA and are highly present in bronchial alveolar lavages suggesting their products to inflict tissue damage under chronic conditions [19]. Chemokines are implicated in various allergic conditions; however, their exact role in ABPA requires further refinement as the blockade of these by therapeutics may control the inflammatory cellular composition and local tissue destruction.
It has long been recognised that mucosal-associated immunity, especially in the gut, appears to be regulated by T-lymphocytes expressing IL-10 and or TGF-β [82]. Recently, CF patients colonised by *A. fumigatus* were shown to have increased levels of FoxP3-positive T-reg cells that expressed higher levels of surface TGF-β upon *A. fumigatus* stimulation, and confer tolerance to oral antigens in mice [61, 71]. The role of IL-10 producing T-reg cells (sometimes termed Tr1 cells) in ABPA is not clear; however, IL-10 promoter polymorphisms have been associated with fungal diseases and ABPA [73]. Adoptive transfer of T-reg cells is effective in lowering inflammatory conditions in multiple animal models suggesting that modulation of the number and activation of these cells in humans may control excessive inflammation in ABPA.

In conclusion, inflammatory mediators of Th2 cells including IL-4, IL5 and IL-13 play a dominant role in the induction and maintenance of the hypersensitivity response in ABPA. These promote IgE and IgA isotype switching and attract typical innate effector cells associated with hypersensitivity responses such as eosinophils, basophils and mast cells. Genetic variation in these pathways predispose for ABPA; however, ranking these for their role in ABPA disease development will prove difficult considering the impact of environmental variables and limited patient numbers. Based on homology with other hypersensitivity disorders, the mechanisms that underlie Th differentiation in ABPA can begin to be understood; however, the characterisation of Th subsets and their role in ABPA development has only just started.

**CFTR-related immunological disease mechanisms in ABPA**

In general, the immune mechanisms in CF are normal; however, there is evidence to support that ABPA, specifically, may also result from the abnormal function of CFTR in immune cells next to the epithelial cells. The association between CF patients and allergic disease was reported in 1949 [83]. CF patients have mutations in CFTR that encodes an adenosine triphosphate (ATP)- and cyclic adenosine monophosphate (cAMP)-regulated anion channel that regulates the composition of excretions [84]. CFTR in the lung epithelium regulates the air–surface liquid layer that underlies the mucus layer, which impacts the mucociliary clearance and functions of humoral components [85].

CFTR is expressed in multiple other tissues including the immune system, suggesting that the hyperinflammatory status of CF patients that was previously believed to be secondary to infection may result from a dysregulated immune response caused by a CFTR mutation [86–89]. Genetic studies in mice support a role for CFTR in macrophages, DCs and lymphocytes [90, 91]. In human innate cells the impaired bacterial clearance by phagocytes has been observed; however, the capacity of these cells to present antigens to T-cells has not been thoroughly assessed [92, 93]. MULLER et al. [91] reported that CFTR deficiency in mice provokes a stronger hypersensitivity response to *A. fumigatus*, and a shift from a predominant cytokine profile of IL-5 to IL-4. Recently, CD3 lymphocytes were implicated in the hypersensitivity response towards *A. fumigatus* by adoptive transfer experiments [94]. Conditional knockouts that lack CFTR in lymphocytes have enhanced basal and *A. fumigatus*-induced IgE levels, further supporting that CFTR is functional in murine CD4+ lymphocytes by limiting Th2-skewing.

Asthmatic non-CF individuals with ABPA frequently carry a mutant CFTR allele [95–98]. A recent study, which involved the extensive CFTR sequencing of ABPA patients with normal sweat chloride levels and pancreatic function, found that the CFTR mutation frequency in patients with ABPA was approximately 48 higher compared with the general population [98]. Whether certain CFTR mutations specifically cluster with ABPA remains to be seen, and as this is difficult to study due to low patient numbers it remains undetermined. The strong correlation of ABPA with CFTR heterozygocity is remarkable, as it has been generally accepted that approximately 20% residual function is sufficient for epithelial functioning. This may point out that other tissues are more strongly affected by CFTR deficiency, but cannot rule out epithelial involvement. The hypothesis that CFTR mutant lymphocytes are intrinsically Th2-primed, as may be expected from mice studies, requires further thorough investigation and should carefully address confounding factors, such as genetic background, infectious status and therapeutic regimen.
To summarise, ABPA is mostly prevalent in CF patients compared with a small percentage of asthma patients, and is a result of complex interactions between the invasive pathogen *A. fumigatus* and the human immune system. Th2-skewing of Th cells followed by a strong humoral IgE response and activation of IgE-responding effector cells are clear hallmarks of ABPA. To date, genetic variation in CFTR itself appears to be the strongest genetic factor associated with ABPA, also in asthmatics. *A. fumigatus*-driven hypersensitivity mouse models reflecting ABPA strongly support a role for CFTR within the T-cell compartment [91, 94]. The strong relationship between ABPA and CF may, therefore, not only result from impaired epithelial functioning but may also result from lymphocyte defects that only become apparent upon strong Th2 stimuli.

![Diagram of ABPA immunopathogenesis](image-url)
associated with \textit{A. fumigatus}. Therefore, it appears that next to environmental factors such as nutritional status, co-infection and long-term immune suppression, genetic variations in the systems underlying \textit{A. fumigatus} recognition, clearance and Th2 skewing may also drive patient-specific ABPA susceptibility. The identification of ABPA-related disease mechanisms will be crucial for future development of therapeutics that control immune-related tissue destruction without impairment of fungal clearance. Figure 3 illustrates the immunopathogenecity of ABPA.

**Clinical features and diagnostic approach**

Patients with ABPA typically present with symptoms such as a low-grade fever, productive cough, bronchial hyperreactivity, chest pain, wheezing, haemoptysis and expectoration of brownish sputum plugs. Sometimes patients are asymptomatic and diagnosed during routine screening tests in patients with asthma or CF. Physical examination can reveal wheezing or coarse crackles on auscultation, clubbing of the fingers in 15\% of patients and complications such as pulmonary hypertension and/or respiratory failure [99, 100]. The diagnostic criteria for patients with asthma are summarised in table 1. Because the primary disease symptoms in patients with CF can closely resemble ABPA, adapted criteria for ABPA have been formulated within this patient category (table 2). In CF, ABPA is diagnosed in the presence of the following: 1) acute or subacute clinical deterioration not attributable to another aetiology; 2) total serum IgE concentration of >500 IU\cdot mL^{-1}; 3) immediate cutaneous reactivity to \textit{A. fumigatus} or \textit{in vitro} demonstration of IgE antibody to \textit{A. fumigatus}; and 4) either precipitins to \textit{A. fumigatus} or \textit{in vitro} demonstration of IgG antibody to \textit{A. fumigatus} or new or recent abnormalities on radiological tests (CT scan or chest radiograph).

**Skin testing**

In patients with bronchial asthma \textit{Aspergillus} skin testing is recommended for screening purposes. Intradermal injection is more sensitive in comparison to the skin-prick test [64, 102, 103]. A positive reaction to recombinant antigens of \textit{A. fumigatus} termed rAsp f 4 and/or 6 reached a sensitivity of 86.8\% (95\% CI 73.5–100\%) and a specificity of 92\% (95\% CI 83.9–100\%) in a study with 50 CF patients [102]. Of those 50 patients, 12 suffered from ABPA, 21 were sensitised for \textit{A. fumigatus} and 17 were control patients. However, less promising results were obtained by \textit{DE OLIVEIRA et al.} [104] who subjected 65 patients with asthma and a positive skin-prick test to

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<th>Table 1. Criteria for the diagnosis of allergic bronchopulmonary aspergillosis (ABPA) in patients with asthma</th>
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<tr>
<td><strong>Criteria</strong></td>
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<tr>
<td>For ABPA central bronchiectasis</td>
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<tr>
<td>Asthma</td>
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<tr>
<td>Central bronchiectasis, inner two thirds of chest CT field</td>
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<tr>
<td>Immediate cutaneous reactivity to \textit{Aspergillus} sp. or \textit{A. fumigatus}</td>
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<tr>
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<td>Chest roentgenographic infiltrates</td>
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<td>Serum precipitating antibodies to \textit{A. fumigatus}</td>
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<td>For ABPA seropositive</td>
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CT: computed tomography; \textit{A. fumigatus}. \textit{Aspergillus} \textit{fumigatus}: Ig: immunoglobulin. Reproduced from [101] with permission from the publisher.
recombinant antigen testing. 19 patients tested positive for at least one recombinant antigen; however, only six of them met the classical criteria for ABPA.

**Essential laboratory testing**

Total serum IgE is the most important laboratory test for ABPA and is essential for the diagnosis and monitoring of the disease. Normal levels of total serum IgE in patients that do not receive glucocorticoid therapy exclude ABPA as a diagnosis. In patients with asthma the total IgE levels should be >1,000 IU\cdot mL^{-1} (2400 ng\cdot mL^{-1}), whereas in CF patients IgE levels of >1,500 IU\cdot mL^{-1} can be detected. IgE levels are also used to monitor treatment. A reduction of 35–50% during treatment with systemic steroids is considered as a remission [105].

Increased levels of specific serum IgE antibodies to *A. fumigatus* distinguish ABPA from *A. fumigatus* hypersensitivity (AH), which is defined as a positive skin test, and other allergic conditions in asthmatics [106, 107]. The serum levels of *Aspergillus*-specific IgE are at least twice as high in ABPA compared with AH [108]. In patients with CF, specific serum IgE antibodies Asp f 3 and Asp f 4 are specific for ABPA and not for *Aspergillus* hypersensitivity [109].

**Supportive tests**

The presence of serum precipitins, *i.e.* precipitating IgG antibodies, are supportive to the diagnosis of ABPA [110, 111]. Peripheral eosinophilia is also regarded important in diagnosis; however, it may have relatively low specificity or sensitivity [112]. A total of >1,000 cells\cdot mL^{-1} has been set as a cut-off value. The differential diagnosis of peripheral eosinophilia includes a range of other disorders such as tuberculosis, sarcoidosis, drug-induced eosinophilia and Churg–Strauss syndrome that should all be carefully ruled out. Sputum cultures are rarely used for diagnosing ABPA as fungi can be prevalent in the lungs of many immunocompromised patients. Pulmonary function testing is not suitable as a diagnostic test and is only useful as a rough indicator for the

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**Table 2. Diagnostic criteria for allergic bronchopulmonary aspergillosis (ABPA) in cystic fibrosis (CF) patients as proposed during the 2003 CF Foundation Consensus Conference (Bethesda, MD, USA)**

**Classic case**

1. Acute or subacute clinical deterioration (cough, wheeze, exercise intolerance, exercise-induced asthma, decline in pulmonary function, increased sputum) not attributable to another etiology.
2. Serum total IgE concentration of >1000 IU\cdot mL^{-1} (2400 ng\cdot mL^{-1}), unless the patient is receiving systemic corticosteroids (if so, retest when steroid treatment is discontinued).
3. Immediate cutaneous reactivity to *Aspergillus* (prick skin test wheal of 3 mm in diameter with surrounding erythema while the patient is not being treated with systemic antihistamines) or *in vitro* presence of serum IgE antibody to *A. fumigatus*.
4. Precipitating antibodies to *A. fumigatus* or serum IgG antibody to *A. fumigatus* by an *in vitro* test.
5. New or recent abnormalities on chest radiograph (infiltrates or mucus plugging) or chest CT (bronchiectasis) that have not cleared with antibiotics and standard physiotherapy.

**Minimal diagnostic criteria**

1. Acute or subacute clinical deterioration (cough, wheeze, exercise intolerance, exercise-induced asthma, change in pulmonary function, or increased sputum production) not attributable to another etiology.
2. Total serum IgE concentration of >500 IU\cdot mL^{-1} (1200 ng\cdot mL^{-1}). If ABPA is suspected and the total IgE level is 200–500 IU\cdot mL^{-1}, repeat testing in 1–3 months is recommended. If the patient is taking steroids, repeat when steroid treatment is discontinued.
3. Immediate cutaneous reactivity to *Aspergillus* (prick skin test wheal of 13 mm in diameter with surrounding erythema, while the patient is not being treated with systemic antihistamines) or *in vitro* demonstration of IgE antibody to *A. fumigatus*.
4. One of the following: precipitins to *A. fumigatus* or *in vitro* demonstration of IgG antibody to *A. fumigatus*; or new or recent abnormalities on a chest radiograph (infiltrates or mucus plugging) or chest CT (bronchiectasis) that have not cleared with antibiotics and standard physiotherapy.

Ig: immunoglobulin; *A. fumigatus*: Aspergillus fumigatus; CT: computed tomography. Reproduced from [19] with permission from the publisher.
severity of lung disease in general [113]. A promising serological test is for thymus and activation-regulated chemokine (TARC). Diagnostic accuracy was proven to be greater for TARC (93%) than for total IgE (74%), rAsp f 4 (75%) or rAsp f 6 (79%) in a small diagnostic study with 12 CF patients with ABPA and 36 control patients [114]. The definition of the diagnostic accuracy was the number of correctly positively categorised patients plus the correctly negatively categorised patients as a percentage of the total.

Radiology

Radiological imaging in most patients with ABPA shows centrally located, cylindrical bronchiectasis, while the presence of distal bronchiectasis is rare [115]. The radiological classification has predominantly prognostic implications as it cannot distinguish between bronchiectasis caused by ABPA or another factor [116]. HRCT scanning is regarded as the gold standard to identify bronchiectasis as a morphological diagnosis and correlates with the functional lung capacity of patients [117, 118]. Chest radiography lacks the sensitivity needed to rule out bronchiectasis and, therefore, HRCT is required if no abnormalities appear and ABPA is suspected. In ABPA, HRCT can be used to monitor disease progression and is directive for the therapeutic strategy.

Treatment

The treatment of ABPA depends upon two important factors: 1) glucocorticoids to dampen the immunological activity, and 2) antifungal agents to suppress the antigenic load.

Although glucocorticoids are the mainstay in ABPA treatment, no well-designed studies have been carried out. Neither the optimal dose regimen nor the optimal duration of therapy has ever been determined [119]. In asthmatics the optimal dose and treatment scheme as regarded by expert opinion is prednisone 0.5–1.0 mg·kg$^{-1}$·day$^{-1}$ for 2 weeks, followed by an alternate day regimen, which is tapered to zero during a 3–6-month period. In CF patients the prolonged use of glucocorticoids may induce severe side-effects such as glucose intolerance, growth suppression, cataracts and osteoporosis [120–122]. Therefore, the use of monthly pulses with methylprednisolone has been suggested as a treatment for ABPA in CF patients. Two small studies with 13 CF patients showed clinical and laboratory improvement after 0.3–1 mg·kg$^{-1}$·day$^{-1}$ and 10–15 mg·kg$^{-1}$·day$^{-1}$, respectively [123, 124]. Figure 4 illustrates the effect of systemic steroids in a CF patient with ABPA.

Inhaled glucocorticoids

Recently it was reported that inhaled glucocorticoids are significantly linked with the prevalence of *Aspergillus* in lungs of CF patients [125], which might increase their risk of suffering from ABPA. The efficacy of inhaled steroids in patients with ABPA has never been documented and hence this treatment is not recommended in patients with CF. Some small case series in patients with asthma and ABPA indicate some beneficial effects of inhaled glucocorticoids [126, 127]. However, the single largest study with inhaled beclomethasone shows no beneficial effect at all [128]. Therefore, the use of inhaled glucocorticoids seems limited in CF patients and implicates limited value for patients with asthma.

![Figure 4. Chest radiograph of a 12-year-old cystic fibrosis patient with allergic bronchopulmonary aspergillosis a) before and b) after a 6-week course of systemic steroids.](image-url)
Antifungal agents

It has been suggested that itraconazole modifies the immunological activation associated with ABPA and can improve clinical outcome, at least over a 16-week period. The largest multicentre randomised controlled trial found significantly lower need for steroids decreased serum IgE concentrations and improved clinical findings in patients using itraconazole when compared with those who did not [129].

The most recent Cochrane review (updated in 2010) on the efficacy of itraconazole in the treatment of patients with CF concluded that evidence is limited and that further research is required [13]. Itraconazole might be used as an adjuvant to glucocorticoid treatment, presumably lowering the required dosage and thereby the side-effects of systemic steroids. The dosage of itraconazole is generally accepted to be 200 mg twice a day with a start dosage of 200 mg three times a day for 3 days. Liver function tests should be monitored monthly to prevent toxicity. A potential concern in patients using both inhaled corticosteroids and itraconazole is adrenal suppression due to an increase in steroid levels in serum [130].

Immunomodulatory therapy

With the progressing knowledge in the immunological mechanisms involved in patients with ABPA, the possibility of developing a more cause-related therapy becomes ever more apparent. In experimental settings some successes have been achieved. For example Asp f 1-derived peptide P1, prophylactically and therapeutically administrated to BALB/c mice is effective in regulating an allergic response to allergens/antigens of *A. fumigatus* [131]. The first results obtained by the administration of allergen-derived peptides to shift an *Aspergillus* specific Th2 response to a protective Th1 are promising.

An example of immunomodulative therapy in a clinical setting is the introduction of omalizumab in children with CF and ABPA. Omalizumab is a humanised monoclonal antibody against IgE. Currently, as documented in case reports, a total of seven children who were described as irresponsive to glucocorticoid treatment were found to have improved lung function after using 300–375 mg omalizumab subcutaneously every 2 weeks [132–135]. However, in order to introduce omalizumab in daily clinical routine, more clinical trials are warranted.

Conclusion

The aim of this chapter was to provide an overview of the clinical features of ABPA, the diagnostic criteria and the underlying pathophysiological immune mechanisms. ABPA consists of an *A. fumigatus*-driven hypersensitivity reaction in predominantly asthmatic and CF patients. Polymorphisms in genes that drive innate and adaptive immune mechanisms, as well as loss-of-function mutations in CFTR, are associated with the development of a strong Th2 response and ABPA. Continuous inhalation of *A. fumigatus* and resulting chronic infections, in combination with genetic predisposition, fuel a chronic inflammatory hypersensitivity response that eventually results in airway remodelling and functional impairment of the lung. The diagnostic process is characterised by a combination of tests evaluating lung function, serum hypersensitivity parameters (aspecific and specific for *A. fumigatus*), and radiological characteristics such as bronchiectasis. Treatment consists of dampening the immune response by the use of glucocorticoids and suppressing the fungal burden by antifungal agents.

Recent insights into the pathogenesis, diagnostic measures and treatment possibilities illustrate the ongoing effort aimed at preventing ABPA from causing invalidating lung disease. Promising examples are the establishment of CFTR mutations in ABPA pathogenesis, the superior test characteristics of TARC regarding the diagnosis of ABPA in CF patients, and the beneficial role of itraconazole to glucocorticoids in treatment.
Statement of interest

None declared.

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