



## Aspergillus colonisation and antifungal immunity in cystic fibrosis patients.

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Abstract:	<p>Cystic fibrosis (CF), caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, is the most common inherited life-limiting disease in North European people affecting 90,000 people worldwide. Progressive lung damage caused by recurrent infection and chronic airway inflammation is the major determinant of survival with a median age at death of 29 years. Approximately 60% of CF patients are infected with <i>Aspergillus fumigatus</i>, a ubiquitous environmental fungus, and its presence has been associated with accelerated lung function decline. Half of the patients infected with <i>Aspergillus</i> are &lt;18 years of age. Yet, time of acquisition of this fungus and determinants of CF-related <i>Aspergillus</i> disease severity and progression are not known. CFTR expression has been demonstrated in cells of the innate and adaptive immune system and has shown to be critical for normal function. Research delineating the role of CFTR-deficient phagocytes in <i>Aspergillus</i> persistence and infection in the CF lung, has only recently received attention. In this concise review we aim to present the current understanding with respect to when people with CF acquire infection with <i>A. fumigatus</i> and antifungal immune responses by CF immune cells.</p>

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3 *Aspergillus* colonisation and antifungal immunity in cystic fibrosis  
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32 Running title: *Aspergillus* in Cystic Fibrosis  
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34 Keywords: Aspergillosis, *Aspergillus fumigatus*, cystic fibrosis, colonisation, innate immunity  
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## Abstract

Cystic fibrosis (CF), caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, is the most common inherited life-limiting disease in North European people affecting 90,000 people worldwide. Progressive lung damage caused by recurrent infection and chronic airway inflammation is the major determinant of survival with a median age at death of 29 years. Approximately 60% of CF patients are infected with *Aspergillus fumigatus*, a ubiquitous environmental fungus, and its presence has been associated with accelerated lung function decline. Half of the patients infected with *Aspergillus* are <18 years of age. Yet, time of acquisition of this fungus and determinants of CF-related *Aspergillus* disease severity and progression are not known.

CFTR expression has been demonstrated in cells of the innate and adaptive immune system and has shown to be critical for normal function. Research delineating the role of CFTR-deficient phagocytes in *Aspergillus* persistence and infection in the CF lung, has only recently received attention. In this concise review we aim to present the current understanding with respect to when people with CF acquire infection with *A. fumigatus* and antifungal immune responses by CF immune cells.

## Introduction

In cystic fibrosis (CF), 90% of morbidity and mortality is a consequence of chronic suppurative lung disease.<sup>1</sup> Aggressive targeted and chronic treatment of airway infection is the mainstay of clinical management, reducing lung function decline and improving survival.<sup>2</sup> The introduction of CFTR modulators into clinical practice reduces (but does not abolish) pulmonary exacerbations and rate of lung function decline, and will not reverse existing lung damage (bronchiectasis). Even with new treatment modalities targeting CFTR dysfunction, pulmonary infections will continue to remain the major prognostic problem in CF. Research and treatment has conventionally focussed on the role of bacterial pathogens with little attention being paid to the role of fungal species.

*Aspergillus fumigatus* is a ubiquitous fungus: inhalation of *Aspergillus* spores and their airway deposition is fact of everyday life. Susceptibility to *Aspergillus*-related lung disease in CF is reflected by clinical phenotypes ranging from persistent *Aspergillus* infection and bronchitis to allergic and airway invasive aspergillosis.<sup>4</sup> About 60% of CF patients are infected with *A. fumigatus* and this has been associated with accelerated lung function decline.<sup>5-8</sup> Little attention has been paid to the role of *A. fumigatus* in the pathogenesis of non-ABPA (allergic bronchopulmonary aspergillosis) *Aspergillus* lung disease in CF, despite its' frequent recovery in respiratory samples.

In vitro studies have demonstrated that CF phagocytes display reduced killing activity against typical CF bacterial pathogens such as *Pseudomonas aeruginosa* and *Burkholderia cepacia*.<sup>9-11</sup> Others have reported defects in a number of host immune mechanisms including the release of antimicrobial peptides, intracellular alkalization, diminished production of hypochlorous acid, and increased release of pro-inflammatory cytokines.<sup>12-14</sup>

Only a few studies can be found in the literature addressing innate antifungal immune

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3 responses by CFTR-defective phagocytes and epithelial cells against *A. fumigatus*.<sup>15-17</sup> In  
4  
5 addition, 2 studies have addressed the persistent inflammation evoked by *A. fumigatus*  
6  
7 (non-allergic) infection in an experimental CF murine model.<sup>18,19</sup>  
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10 This concise review summarizes our current understanding addressing the highly relevant  
11  
12 questions of when people with CF acquire infection with *A. fumigatus* and why the absence  
13  
14 of a functional CFTR protein in immune cells renders them susceptible to develop  
15  
16 *Aspergillus* airway disease.  
17

### 18 19 20 21 ***Aspergillus* colonisation** 22

23  
24 The isolation of *Aspergillus* species, in particular *A. fumigatus*, in respiratory secretions of  
25  
26 patients with CF is a common occurrence.<sup>20,21</sup> *Aspergillus fumigatus* is a ubiquitous fungus  
27  
28 and its conidia are dispersed in the air we breathe. In patients with CF, the impaired  
29  
30 mucociliary clearance and airways being filled with thick mucus, the inhaled *Aspergillus*  
31  
32 conidia are easily trapped. Numerous studies have reported a high variety in prevalence  
33  
34 rates (ranging from 3.2% to 56.7%) and this most likely reflects the differences in culture  
35  
36 techniques, the frequency of sampling, the interpretation of the culture results and if fungal  
37  
38 cultures are performed as routine practice in clinical management. Authors have aimed to  
39  
40 differentiate between transient colonisation ( $\leq 1/\text{yr}$ ) and persistent or chronic ( $\geq 2/0.5\text{-}1\text{yr}$ )  
41  
42 colonisation, although no consensus exists for those definitions, and some studies have  
43  
44 used even stricter definitions. Table 1 provides an overview of studies in which either results  
45  
46 of fungal cultures were reported as a secondary outcome or in which fungal colonisation  
47  
48 was the primary focus of the study.<sup>6,22-57</sup> Colonisation with *Aspergillus* species may be an  
49  
50 apparently isolated finding, but may also be seen in the context of fungal sensitization,  
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3 ABPA or *Aspergillus* bronchitis. The majority of the studies summarized in table 1 lack  
4  
5 information on this important aspect, and make it difficult to assess *Aspergillus* colonisation  
6  
7 on its own as being harmful in terms of CF lung disease progression. To address this issue, a  
8  
9 novel diagnostic classification system of *Aspergillus* infection and disease has been proposed  
10  
11 and was consequently used to provide estimates on the prevalence of the different  
12  
13 phenotypes of aspergillosis in CF using national and international CF registry data.<sup>7,58</sup> As this  
14  
15 novel classification system was tested in a cohort of adult patients, it remains to be seen if  
16  
17 these frequencies can be used on the total CF population as almost 50% of CF patients are ≤  
18  
19 18 yrs of age. The estimated prevalences reported did not include patients colonized with  
20  
21 *Aspergillus* in the absence of fungal sensitisation, ABPA or *Aspergillus* bronchitis.  
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24  
25 Galactomannan and *Aspergillus* PCR on sputum samples have been proposed to improve  
26  
27 the detection of *A. fumigatus* and *Aspergillus* disease in CF.<sup>58</sup> Unfortunately, no definition  
28  
29 was proposed how those 2 markers should be included to define persistent colonisation.  
30  
31

32 Most colonisation studies have focussed on *A. fumigatus*<sup>23-25,27,28,33-36,38,39,42,43,45-49,51-55,57</sup>,  
33  
34 being the most prevalent *Aspergillus* species encounters in human colonisation and disease,  
35  
36 while others have reported *Aspergillus* colonisation without defining the actual  
37  
38 species<sup>6,22,26,29,30,32,37,40,41,50,56,59</sup>. Studies reporting on the relative number of *A. fumigatus*  
39  
40 versus non-*fumigatus* *Aspergillus* species have shown that between 36% and 58% of  
41  
42 *Aspergillus* colonisation in patients with CF is caused by *A. fumigatus*.<sup>38,40-42,46</sup> Jubin et al  
43  
44 showed that mixed *Aspergillus* species colonisation may occur in up to 33% of the  
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46 patients.<sup>41</sup>  
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49  
50 Although *Aspergillus* infection and disease in CF is considered to become a problem during  
51  
52 adolescence and adulthood, epidemiological studies about when patients with CF acquire  
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54 *Aspergillus* colonisation are scarce. Epidemiological studies conducted in France, Australia  
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3 and in Greece, reported a mean age of the patients at date of first isolation of *A. fumigatus*  
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5 to be 12.9 yrs, 9 yrs and 13 yrs, respectively.<sup>33,36,49</sup> Two more recent studies, reported an  
6  
7 age of acquisition being 16.4 yrs (median, range 12.2-22.0 yrs) in the US<sup>56</sup>, 13.5 yrs (mean,  
8  
9 SD +/- 4yrs) in France<sup>57</sup>, and 9.0 yrs (median, range 3.1-16.2 yrs) in the UK<sup>51</sup>. Two other  
10  
11 studies have broken down the prevalence of *A. fumigatus* colonisation into different age  
12  
13 categories showing colonisation rates of 16.4% up to 28% in children < 12 yrs of age.<sup>39,43</sup> By  
14  
15 differentiating transient ( $\leq 1/\text{yr}$ ) from persistent ( $\geq 2/\text{yr}$ ) colonisation, Saunders et al showed  
16  
17 that children < 12 yrs of age were only transiently colonized in a minority of the cases (21%),  
18  
19 with the majority of children  $\geq 12$  yrs (59%) being persistently colonised with *Aspergillus*.<sup>51</sup>  
20  
21 Cultures from BAL-fluid showed a significantly higher yield of *A. fumigatus* compared to  
22  
23 sputum samples taken in the same period.<sup>51</sup> Valuable data were obtained from an  
24  
25 Australian study in which infants, diagnosed with CF after newborn screening, underwent  
26  
27 bronchoalveolar lavage (BAL) at the age of 3 mo, 1 yr and 2 yrs.<sup>6</sup> *Aspergillus* was cultured  
28  
29 from BAL-fluid of 7 out of 56 infants (12.5%).<sup>6</sup> The difficulties in obtaining sputum samples  
30  
31 from infants and young children (< 8 yrs) most likely leads to an underestimation of the  
32  
33 prevalence of *Aspergillus* colonisation and infection in this age group, and precludes any  
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35 conclusion about when patients with CF acquire *Aspergillus* infection.  
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### 44 ***Aspergillus* colonisation and lung disease**

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47 Several studies have investigated the role of *Aspergillus* colonisation on lung function in CF  
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49 with conflicting results. Several studies have reported either more pulmonary exacerbations  
50  
51 requiring hospitalizations and/or significant lower lung function parameters and/or a  
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53 steeper lung function decline in *Aspergillus* colonized patients.<sup>5,42,45,55</sup> However, other  
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3 studies do not support an independent effect of *Aspergillus* colonisation on lung  
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5 function.<sup>29,32,37,41,43,54,56</sup>

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7 McMahon showed that *Aspergillus* colonisation, defined as at least two sputum cultures  
8  
9 positive for *Aspergillus* spp. at least four weeks apart in the year prior to study inclusion,  
10  
11 resulted in more severe and significant bronchiectasis scored on HRCT-chest.<sup>60</sup>

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14 Noni et al showed that paediatric patients (n=121, mean age 14 yrs) with chronic *Aspergillus*  
15  
16 colonisation ( $\geq 2$  positive sputum cultures/yr) had a lower FEV1 at baseline and a faster  
17  
18 deterioration of their lung function in the 7 yrs after baseline.<sup>49</sup> In a large retrospective  
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20 study using registry data from over 16,000 CF patients, lower FEV1 percent predicted was  
21  
22 not a predictor of development of persistent *Aspergillus* colonisation.<sup>56</sup>

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25  
26 In a systematic review by Harun et al<sup>61</sup>, which included studies describing lung function  
27  
28 progression over age in CF patients, only 2 out of 39 publications (between 1990-2015)  
29  
30 recognized *Aspergillus* colonisation or disease as a possible risk factor influencing lung  
31  
32 function decline.<sup>5,62</sup> This reflects that *Aspergillus* infection and disease has not been  
33  
34 recognized as playing a major role in the lung function decline in patients with CF, resulting  
35  
36 in the absence of systematic approaches to monitor *Aspergillus* infections and disease  
37  
38 longitudinally in CF patients from an early age onwards. A recent survey among paediatric  
39  
40 and adult CF consultants in the UK showed that one third of the respondents considered  
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42 *Aspergillus* colonisation to be potentially harmful and would therefore treat this condition.<sup>63</sup>

## 43 44 45 46 47 48 49 **Innate fungal immunity**

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52 CFTR-defective epithelial cells  
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3 Direct experimental evidence of the interaction between CF epithelial cells and *Aspergillus*  
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5 conidia is limited. However, there is evidence for the contribution of healthy epithelial cells  
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7 to the innate immune response to *A. fumigatus* and for impairments in epithelial cell  
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9 function in CF, from which insights can be gained. CFTR protein is highly expressed in lung  
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11 epithelial cells and its loss leads to reduced airway surface hydration and impaired  
12  
13 mucociliary transport.<sup>64</sup> This is significant as *Aspergillus* conidia are regularly inhaled into  
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15 the airways and the mucociliary escalator is a key mechanism for preventing colonisation.  
16  
17 Pentraxin 3 is a soluble pattern recognition receptor that is secreted by epithelial cells in  
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19 response to *A. fumigatus*<sup>65</sup> and is important for *Aspergillus* clearance.<sup>66</sup> Pentraxin 3 levels  
20  
21 have been found to be reduced in sputum from CF patients.<sup>67</sup>  
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23  
24 Although not the primary phagocytes of the airway, epithelial cells have been shown in-vitro  
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26 to phagocytose conidia.<sup>68</sup> Adhesion and internalisation of conidia has been shown to be  
27  
28 impaired in CF bronchial epithelial cells.<sup>15</sup> In the same paper authors also demonstrated  
29  
30 increased baseline and *Aspergillus*-induced apoptosis, reduced chemokine secretion and  
31  
32 impaired killing by CF epithelial cells in-vitro. In an in-vivo murine model, they found  
33  
34 impaired clearance of *Aspergillus* conidia and evidence of epithelial necrosis and fibrin  
35  
36 deposition in CFTR-deficient but not wild-type mouse airways. Ceramide has been shown to  
37  
38 accumulate in CF bronchial and alveolar epithelial cells in mice and humans.<sup>69</sup> In *A.*  
39  
40 *fumigatus* infection, ceramide mediates inflammation and interferes with epithelial killing of  
41  
42 the fungus. Inhibition of de-novo ceramide synthesis reduced inflammatory cytokine  
43  
44 release, granulocyte infiltration and fungal colonisation in a murine model of *A. fumigatus*  
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46 infection.<sup>16</sup>  
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## 56 CFTR-defective neutrophils

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3 Human neutrophils express the CFTR protein in phagosomes and in the membranes of  
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5 secretory vesicles and has shown to play a critical role in regulating antimicrobial neutrophil  
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7 activities.<sup>70-72</sup> Upregulating of Toll-like receptor (TLR) 5 on human CF airway neutrophils  
8  
9 have been described and its signalling results in excessive cytokine production after  
10  
11 stimulation with *P. aeruginosa*.<sup>73,74</sup> A small number of functional studies implicate that the  
12  
13 absence of a functional CFTR-protein in phagocytes leads to inadequate intraphagosomal  
14  
15 chloride transport and less production of hypochlorous acid (HOCl) resulting in a diminished  
16  
17 killing of *P. aeruginosa*.<sup>9</sup> A study by Pohl et al. showed that impaired CFTR function in  
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19 neutrophils leads to decreased release of secondary and tertiary granules due to altered ion  
20  
21 homeostasis that is corrected by CFTR potentiator therapy.<sup>75</sup>  
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23 We have recently shown that although human CF neutrophils are capable of efficiently  
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25 phagocytose and kill *A. fumigatus*, this is at a cost of excessive reactive oxygen species  
26  
27 (ROS).<sup>17</sup> Previous studies investigating neutrophil ROS production in vitro have shown  
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29 contrasting results, with ROS production being either increased, decreased or normal in CF  
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31 neutrophils.<sup>76-78</sup> A possible reason for this inconsistency is that this response is stimulus  
32  
33 specific as a range of microbial and non-microbial stimuli were used in these studies. The  
34  
35 excessive amount of ROS induced by *A. fumigatus* in CF neutrophils is significantly  
36  
37 correlated to disease severity in terms of clinical exacerbations and lung function [Brunel  
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39 2018].<sup>17</sup> Our data suggest that the hyper inflammatory response by CF neutrophils upon  
40  
41 exposure to *A. fumigatus* is likely to contribute to progressive lung disease.<sup>17</sup> Increased  
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43 NLRP3 expression in murine *cftr*<sup>-/-</sup> neutrophils was demonstrated, while pNLR4 expression  
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45 was inherently lower.<sup>19</sup> These combined defects were shown to result in increased  
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47 neutrophil recruitment and IL-1 $\beta$  release during *Aspergillus* infection in CF mice.<sup>19</sup>  
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## CFTR-defective macrophages

Alveolar macrophages play a key role in controlling pulmonary fungal infections both through direct killing of phagocytosed pathogens and by regulating the inflammatory response generated by inhaled fungal pathogens.<sup>79</sup> There is evidence from work with bacteria that macrophage antimicrobial functions are impaired in CF but there is little experimental data looking at macrophage interactions with fungi in CF. CFTR expression has been demonstrated in human monocyte-derived macrophages<sup>11</sup> and murine and human alveolar macrophages<sup>80,81</sup>. These authors have reported impaired macrophage killing of *P. aeruginosa* and have proposed impaired phagosomal acidification as the mechanism underlying this defect. However, others have challenged this hypothesis.<sup>82</sup> Impaired autophagy has also been reported in CF macrophages and may contribute to the killing defect towards *B. cenocepacia* as observed in one study.<sup>10</sup> A similar autophagy defect has been reported in lung macrophages isolated from *A. fumigatus* infected CFTR-deficient mice.<sup>19</sup> Recently, correction of an anti-*Pseudomonas* killing defect by treatment of CF macrophages with the CFTR corrector Lumacaftor (VX-809) was reported.<sup>83</sup> Alveolar macrophages are the primary phagocytes of the airway. Failure to kill phagocytosed conidia will result in germination and fungal escape out of the cell. The hyphal form of *Aspergillus* releases proteases and gliotoxins, causing further damage to the host airway.

As well as failing to kill pathogens effectively, CF macrophages have also been observed to generate hyper-inflammatory responses to infectious stimuli. *B. cenocepacia* induced greater inflammatory cytokine release and inflammatory cell death in monocyte-derived macrophages from CF patients versus healthy controls.<sup>10,84</sup> Inflammatory cytokine release by CF murine and human macrophages stimulated with LPS was increased versus wild type

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2  
3 and healthy controls.<sup>13</sup> In a murine CF model of *A. fumigatus* infection, Ianitti et al observed  
4  
5 increased activation of the NLRP3 inflammasome in CF lung macrophages versus wild type  
6  
7 controls.<sup>19</sup>  
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## 10 11 12 CFTR-defective T-cells

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14 The role of T cells in immunity to pulmonary aspergillosis is well recognised with Th1, Th2,  
15  
16 Th17, Th9 and cytotoxic T cells all playing a role.<sup>85-87</sup> Th1 CD4+ T cells enable inflammation  
17  
18 and fungal clearance, whilst Th17 cells are important for neutrophil recruitment, Th2 cells  
19  
20 for allergic inflammation and Th9 responses play a role in fibrosis. Furthermore, a role for T  
21  
22 regulatory (Treg) cells has been described in regulation of innate and adaptive responses.<sup>88</sup>  
23  
24 CF lymphocyte unresponsiveness to bacterial pathogens has been long established<sup>89</sup>, with a  
25  
26 tendency for a pro-allergic Th2 response and increased tendency to develop allergic  
27  
28 *Aspergillus* lung disease in patients with CF.<sup>23,90</sup> This is consistent with CF murine models  
29  
30 where *A. fumigatus* challenge leads to a shift to IgE, IL-4 and IL-13 production.<sup>91</sup>  
31  
32 Furthermore, T helper cells from CF patients have lower levels of interferon- $\gamma$  production  
33  
34 and increased IL-10 production.<sup>92</sup> It has been hypothesized that this is a consequence of  
35  
36 increased calcium flux across the T cell membrane.<sup>93</sup> Calcium flux has been shown to be  
37  
38 regulated by CFTR, and is crucial for T cell activation. Murine studies have specifically  
39  
40 demonstrated that T cell receptor stimulation led to increased calcium entry in CFTR-  
41  
42 deficient T cells, and increased translocation of the calcineurin-dependent transcription  
43  
44 factor NFAT.<sup>94</sup> Thus, excessive calcium flux as a direct consequence of CFTR dysfunction in T  
45  
46 cells may be a major contributor to dysregulated immune responses in CF.  
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49  
50 Recent studies showed that CFTR<sup>-/-</sup> mice have increased innate lymphoid cell IL-9 production  
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52 in response to *A. fumigatus* challenge, which is linked to expansion of Th9 T cells.<sup>87</sup> IL-9  
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3 neutralisation led to an improvement in lung immunopathology and fibrosis. Further studies  
4  
5 in the murine model of pulmonary aspergillosis showed that CFTR dysfunction led to  
6  
7 defective indoleamine 2,3-dioxygenase activity, leading to alterations in tryptophan  
8  
9 metabolism with a subsequent imbalance of the Th17/Treg axis and excessive lung  
10  
11 inflammation.<sup>18</sup> Therapeutic modulation of this pathway to enhance indoleamine 2,3-  
12  
13 dioxygenase enabled resolution of excessive inflammation.<sup>18</sup> Clinical studies have also  
14  
15 shown that the co-stimulatory molecule, OX40 ligand, is critical for driving CD4+ Th2  
16  
17 responses in CF patients with ABPA.<sup>95</sup> This correlated with low vitamin D levels in serum.  
18  
19 Notably ex vivo therapy with vitamin D led to reduced OX40 ligand expression and reduced  
20  
21 Th2 responses.<sup>95</sup> These studies indicate that a better understanding of the hyper-  
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23 inflammatory T cell responses in CF may open up new immunotherapeutic avenues for the  
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25 treatment of fungal-driven lung disease.  
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### 33 **Summary and Future Directions**

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35 *Aspergillus* colonisation is commonly observed in patients with CF, with *A. fumigatus* most  
36  
37 frequently encountered, and can manifest in clinically recognized CF-related *Aspergillus*  
38  
39 diseases as ABPA and fungal sensitization. Persistent *Aspergillus* colonisation or *Aspergillus*  
40  
41 bronchitis are not well defined and should receive more attention. Only incomplete data  
42  
43 exists with respect to *A. fumigatus* colonisation dynamics and time of acquisition. In  
44  
45 addition, the impact of persistent *Aspergillus* colonisation (infection) on lung function  
46  
47 decline in CF has not been studied sufficiently. A systematic approach in which standardized  
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49 fungal diagnostic measurements are applied in a longitudinal way to a large cohort of  
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51 patients with CF, is urgently needed to assess the impact of persistent *Aspergillus*  
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3 colonisation. The findings of such investigations will inform clinical decision making with  
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5 respect to the need of therapeutic interventions.  
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8 Treatment of persistent *Aspergillus* colonisation in the absence or presence of clinical  
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10 symptoms of pulmonary exacerbation and/or a decline in lung function with antifungals has  
11  
12 not been properly assessed. The only study which aimed to target persistent *Aspergillus*  
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14 infection in patients with CF was flawed by largely undetectable serum itraconazole  
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16 concentrations.<sup>96</sup> Well-known adverse effects of long term azole antifungal therapies,  
17  
18 including the rapid emerging triazole antifungal resistance, toxicity and interaction with  
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20 CFTR modulators, will challenge the use of azole antifungals for persistent *Aspergillus*  
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22 colonisation and infection.  
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26  
27 Enhanced mechanistic insights in the impairments in innate antifungal immune mechanisms  
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29 is warranted to identify new targets for non-allergic *Aspergillus* infections in CF patients.  
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31 Currently, the observed *Aspergillus*-induced inflammatory responses by innate immune cells  
32  
33 in both in vitro, ex vivo and experimental models of infection, seem to be a potential target  
34  
35 to limit the pathological consequences of CF-related *Aspergillus* infection. A range of anti-  
36  
37 inflammatories are being developed for the general treatment of CF-related airway  
38  
39 disease.<sup>97</sup> It will be crucial to understand the impact both on reducing hyper-inflammatory  
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41 responses as well as whether there is an effect on direct antifungal killing mechanisms. The  
42  
43 CFTR modulators are the first causative treatment options for CF patients and have achieved  
44  
45 significant improvement in lung function and quality of life.<sup>98</sup> One study showed a clear  
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47 reduction in fungal colonisation in the CF lung - an effect that may be associated with the  
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49 mitigation of the impaired innate immune mechanisms observed in CF immune cells.<sup>50</sup> In

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55 **in vitro work, which showed that a defective CFTR is associated with impaired degranulation of**  
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3 antimicrobial proteins in neutrophils, has demonstrated that ivacaftor is able to correct  
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5 degranulation and to increase bacterial killing by activation of Rab27a.<sup>75</sup> Another example of  
6  
7 a small molecule based therapy targeting both the primary defect in CF as well as the  
8  
9 aberrant inflammation and immune response, is thymosin  $\alpha$ 1. Thymosin  $\alpha$ 1 is a natural  
10  
11 occurring polypeptide, used as an immunomodulator in viral infections, malignancies and  
12  
13 immunodeficiencies, and has recently been shown to increase CFTR maturation and to  
14  
15 reduce inflammation in preclinical models of disease.<sup>99</sup> Its mode of action seems to be the  
16  
17 induction of IDO1 which in turns induces autophagy and favourable influencing the balance  
18  
19 of protein folding versus degradation of the CFTR.<sup>99</sup>  
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24 There is considerable interest in immunotherapeutic approaches to the treatment of fungal  
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26 disease.<sup>100</sup> Recombinant cytokines have been used extensively in transplant-related fungal  
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28 disease<sup>100</sup>, and recently successful use of interferon-gamma in CF-related fungal disease was  
29  
30 reported.<sup>101</sup> Targeting the increased inflammasome activation and IL-1 $\beta$  release with the IL-  
31  
32 1-receptor antagonist Anakinra, has shown promising results in pre-clinical models of  
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34 disease.<sup>19</sup> Improved understanding of *Aspergillus* disease pathogenesis in CF patients will  
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36 lead to new therapeutic targets and should eventually result in new management options.  
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Table 1. Overview of studies reporting *Aspergillus* colonisation rates in patients with cystic fibrosis.

References	Focus	Country	Patient population	Duration and frequency of sampling*	Colonisation rates
Nelson, 1979 [22]	primary	USA	46 pts	once	56.7% Asp colonisation (no info if ABPA or sens)
Laufer, 1984 [23]	primary	USA	100 pts aged 2 – 34 yrs	once	9% Af colonisation overall 10% in those with ABPA
Bauernfeind, 1987 [24]	secondary	Germany	102 pts	22 mo period, multiple samples	5.9% Af colonisation (no info if ABPA or sens)
Mroueh, 1994 [25]	primary	USA	236 pts aged 1 – 41 yrs (mean age 14.5 yrs)	retrospective	25.4% overall Af colonisation 19% isolated Af colonisation 6.5% Af colonisation + ABPA based on single cultures
Flume, 1994 [26]	primary	USA	27 pts prior to lung transplantation	once	63% Asp colonisation (no info if ABPA or sens)
Cimon, J Med Mycol 1995 [in 27]	primary	France	210 pts	once	21.4% Af pos culture (only 1% ABPA in the study population)
Becker, 1996 [28]	primary	USA	49 adult pts	1 yr cross-sectional	16% Af colonisation (no data if ABPA or sens) based on single cultures
Milla, 1996 [29]	primary	USA	212 paediatric pts > 5 yrs mean age 17.2 +/- 9.2 yrs	multiple 1 yr study period	21.2% at least one Asp pos culture
Burns, 1998 [30]	secondary	USA	465 pts > 6 yrs	once	23.2% Asp colonisation (ABPA or sens not exclusion criteria for

					study)
Burns, 1999 [31]	secondary	USA	520 pts ≥ 6 yrs of age, FEV1 25%-75% predicted	twice (baseline and end of study)	25% Asp colonisation baseline 16.2% wk 20 (end of study) (18% vs 8%, tobra vs placebo)
Bargon, 1999 [32]	primary	Germany	104 adult pts	repeated cultures	41.3% Asp colonisation (no info if ABPA or sens)
Cimon, 2000 [33]	secondary	France	128 pts (98 adults and 30 paediatric pts)	longitudinal, 5 yrs, minimum 4 samples/pt	46.1% at least one Af pos culture (only 4% ABPA in the population studied)
Hodson, 2002 [34]	secondary	UK/Ireland	42 (tobramycin) + 37 (colistin) pts ≥ 6 yrs	once at end of study	5.7% Af pos in tobramycin group 3.2% Af pos in colistin group
Bakare, 2003 [35]	primary	Germany	94 pts, median age 28 yrs	multiple, 6 mo study period	45.7% Af colonisation 24.5% had ≥ 2 Af positive cultures (no info if ABPA or sens)
Skov, 2005 [36]	primary	Australia	270 paediatric and adult pts	2 samples/yr 4 yr study period	Increase from 7.4% to 18.8% (based on single Af pos cultures) ABPA 0.3 to 4%
Chotirmall, 2008 [37]	primary	Irish	50 pts	during exacerbations 5 yrs study period	30% at least one Asp positive culture 20% > 1 Asp pos culture 4% ≥ 10 Asp pos culture 12% ABPA
Valenza, 2008 [38]	primary	Germany	60 adult and paediatric pts [median 18 yrs, 6 -41 yrs]	multiple samples 1 yr study period	58.3% <i>A. fumigatus</i> pos 10% non- <i>fumigatus Aspergillus</i> pos (based on single positive cultures)
Paugam, 2010 [39]	primary	France	201 adult pts	multiple samples 2 yrs study period	56.7% in total study population (based on one Af pos culture) 28% age group 6-10 yrs



					59% age group 11-15 yrs 75% age group 16-20 yrs 61% age group 21-41 yrs
Nagano, 2010 [40]	secondary	Ireland	77 adult pts, median 28.5 yrs [18-59 yrs]	once	9.1% all Asp species 5.2% <i>A. fumigatus</i> (no info ABPA or sens)
Jubin, 2010 [41]	primary	France	85 paediatric pts mean age 8.5 +/- 9 yrs	multiple at least once/yr 6 yrs study period	21% Asp colonisation 14% isolated persistent Asp colonisation 4% Asp colonisation + ABPA
Sudfeld, 2010 [42]	primary	US	614 pts	10 yr study period, cohort	36.3% <i>A. fumigatus</i> (single pos culture) 26.1% non- <i>fumigatus Aspergillus</i>
De Vrankrijker, 2011 [43]	primary	Netherlands	259 pts non-ABPA 106 pts 0-12 yrs 99 pts 13-24 yrs 54 pts ≥25 yrs	multiple in one yr	23.6% (>50% of cultures Af pos) 16.4% age group 0-12 yrs 54.1% age group 13-24 yrs 29.5% age group ≥ 25 yrs
Wainwright, 2011 [44]	secondary	Australia	153 pts, BAL fluid from children < 5 yrs	during pulmonary exacerbations	9% Asp colonisation/infection
Fillaux, 2012 [45]	primary	France	206 pts median age 16.3 yrs [range 9.8-23.6]	routine samples, median follow-up 3.6 yrs [range 2.1-8.7]	18% persistent carriage = 3 Af pos cultures in 6 mo (non-ABPA or sens) 62.1% at least one Af pos culture 27.1% persistent carriage period at least once
Güngör, 2013 [46]	primary	Turkey	48 pts, mean age 11.6 yrs [range 2-38 yrs]	at least 3 sputum/deep throat swab samples	10.4% one <i>A. fumigatus</i> pos culture 8.3% non- <i>fumigatus Aspergillus</i> (no info ABPA or sens)

Fillaux 2014 [47]	primary	France	117 paediatric pts median age 9.9 yrs [IQR 6.0-13.2] at end of study	routine 8 yrs period	38.5% (= 3 Af pos cultures in 6 mo) without sensitization
Masoud-Landgraf, 2014 [48]	secondary	Austria	113 pts, median 20.3 yrs [range 2-57]	multiple samples 1 yr study period	78.8% Af pos cultures (no info ABPA or sens)
Noni, 2015 [49]	primary	Greece	121 paediatric pts	routine	32.2% one Af pos culture 11.6% chronic colonisation
Ramsey, 2014 [6]	primary	Australia	56 infants $\leq$ 2yrs of age	3 BAL samples 2 yrs study period	12.5% Asp colonisation
Heltshe, 2015 [50]	secondary	USA	151 pts: 38 pts 6-11 yrs 32 pts 12-17 yrs 81 pts $\geq$ 18 yrs	3 cultures as per study protocol	~15% and ~7.5% Asp pos cultures before and after ivacaftor (no info ABPA or sens)
Saunders, 2016 [51]	primary	UK	45 children < 18 yrs of age	multiple	29% Af pos BAL culture 14% Af pos sputum 42% one Af pos culture 22% persistent colonisation
Mirkovic, 2016 [52]	primary	Ireland	48 pts	8 sputum samples 2 year period	60.9% in Af sens pts 24% in non-sens pts (at least one pos)
Gernez, 2016 [53]	primary	US & Ireland	48 and 26 adult pts	At least 2 pos sputum cultures within previous 2 yrs	US 27.1% Af pos Ireland 30.8% Af pos
Reece, 2017 [54]	primary	Ireland	CF registry 749 pts median 18.1 yrs [range 4-	retrospective, 1 yr study period	11% overall Af colonisation (5% persistent and 6% intermittent) ABPA 5.9% <sup>#</sup>

			69]		$\leq 10$ yrs of age: 0 – 10% 11-20 yrs of age: 10-30% 21-30 yrs: 7-18% $\geq 31$ yrs: 0-18%
Brandt, 2018 [55]	primary	Germany	CF Registry 2599 pts (mean age 21 yrs +/- 12yrs)	1 yr study period	32.5% at least one Af pos culture/yr (no info if ABPA or sens)
Hong, 2018 [56]	primary	US	CF Foundation registry 16,095 pts (6 -45 yrs)	Retrospect 6 yrs study period at least 2 cultures/yr, Asp prevalent cases excluded	27.9% Asp pos overall 9.6% persistent Asp pos ( $\geq 2$ pos cultures during 12 mo; of which 5.4% ABPA) 18.4% transient of which 3.4% ABPA
Coron, 2018 [57]	primary	France	243 pts > 6 yrs (mean age 21.2 +/- 8.7 yrs)	prospective, samples annually or during exacerbation 3 yr study period	35.4% one Af pos culture adults 35.6% children 34.8%

\*based on sputum samples unless indicated otherwise; #patients intermittently or persistently colonised with *A. fumigatus* showed no increased prevalence of ABPA; Af, *Aspergillus fumigatus*; Asp, *Aspergillus* species; ABPA, allergic bronchopulmonary aspergillosis; sens, fungal sensitisation; pos, positive;