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Case Report

Improvement in *Exophiala dermatitidis* airway persistence and respiratory decline in response to interferon-gamma therapy in a patient with cystic fibrosis

Christopher P. Eades [a,b,*], Darius P.H. Armstrong-James [c,d], Jimstan Periselneris [c,d], Andrew Jones [d], Nicholas Simmonds [d], Peter Kelleher [b,d,e], Anand Shah [c,d]

[a] *Department of Clinical Infection, Chelsea and Westminster NHS Foundation Trust, London, UK*
[b] *Department of Infection and Immunity, North West London Pathology NHS Trusts London, UK*
[c] *National Heart and Lung Institute, Imperial College London, UK*
[d] *Department of Respiratory Medicine, Royal Brompton and Harefield NHS Foundation Trust, London, UK*
[e] *Centre for Immunology & Vaccinology, Department of Medicine, Imperial College London, UK*

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Airflow obstruction infection and inflammation are key components of respiratory morbidity in patients with cystic fibrosis (CF). Chronic respiratory fungal infections, most commonly with moulds of the genera *Aspergillus and Exophiala,* are common in this patient cohort. An increased susceptibility to fungal infections may be observed in individuals with defects in IL-17RA signalling pathways, a situation characterised by excessive mucus production, increased Th2 cytokine, IL-17F, IL-33 and reduced IFN-γ secretion in patients without CF [1]. Apart from its well-recognised role in control of surface airway volume and ciliary function, CFTR regulates a number of intracellular immunological signalling pathways within respiratory epithelial cells. Loss or reduction of CFTR is associated with increased secretion of TNF-α, IL-6 and IL-8, reduction in NO secretion and inhibition of STAT signalling pathways which lead to reduced respiratory mucosal and systemic IFN-γ secretion [2]. Interferon-γ therapy has been used as treatment and prophylaxis for *Aspergillus* lung disease in patients with chronic granulomatous disease (CGD), a condition recognised increasingly as having immunological parity to the CF situation [3]. Indeed, IFN-γ may enhance anti-microbial activity of monocytes, neutrophils and respiratory epithelial cells and restrain excessive IL-1β associated inflammasome activation. In this report we outline the successful use of adjunctive IFN-γ therapy in a CF patient who had progressive respiratory morbidity secondary to *Exophiala dermatitidis* infection, despite azole, echinocandins and amphotericin B treatment.

A 24-year-old male with homozygous delta 508 CF, and asthma, was referred by his local CF centre with a history of increased production of thick dark colour sputum plugs, and worsening exertional dyspnoea. Baseline pulmonary function tests (PFTs) showed a forced expiratory volume in one second (FEV_1) of 2.44 litres (L) (51% predicted) and a forced vital capacity (FVC) of 3.73 L (67% predicted) (Fig. 1). Sputum cultures liberated a heavy growth of the melanised mould, *Exophiala dermatitidis* (sensitive to amphotericin B, itraconazole, posaconazole and voriconazole), for which he was commenced on itraconazole 200 mg twice daily. *Pseudomonas aeruginosa* was also isolated and he was treated successfully with periodic courses of oral ciprofloxacin, in combination with long-term nebulised colismethisate sodium (colistin) prophylaxis. High resolution computed tomography (HRCT) imaging of the thorax revealed florid 'tree-in-bud' change and mucous-plugging.

Abbreviations: IL-12, interleukin-12; LPS, lipopolysaccharide; PHA, phytohaemagglutinin; aCD3, activated CD3; PMA-IONO, phorbol myristate acetate-ionomycin; TNF-α, tumour-necrosis factor-alpha.

* Corresponding author at: Department of Clinical Infection, Chelsea and Westminster NHS Foundation Trust, London, UK.
E-mail address: Christopher.eades@nhs.net (C.P. Eades).

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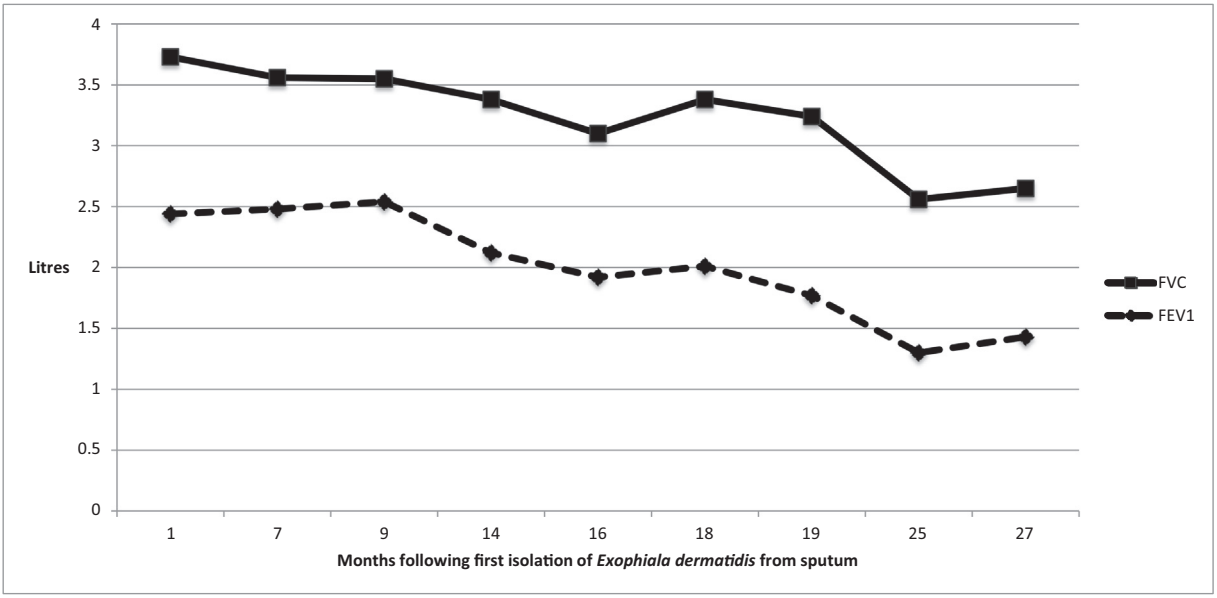

Fig. 1. Pulmonary function tests (PFTs) over period of prolonged sputum colonisation with *Exophiala dermatitidis*. Over the 27-month period of observation, the patient experienced a marked deterioration in lung function with a conspicuous worsening in airways obstruction. We attribute this directly to the observed *Exophiala* colonisation. IFN-γ-1b therapy was instituted at month 25 (*), which halted this trend. A modest improvement in PFTs was observed at month 27, four weeks after stopping therapy.

Investigations to exclude occult (culture-negative) *Aspergillus*-associated disease (aspergillus-specific immunoglobulins (Ig) G and IgE, serum galactomannan and β-D-glucan, BDG) were negative. No obvious domestic or occupational exposure to mould or damp conditions was elicited on enquiry.

Despite a 50% dose-reduction, the patient failed to tolerate itraconazole due to severe diarrhoea despite a sub-therapeutic level (total itraconazole level = 0.2 mg/mL; local RR = 1–4 mg/mL). The patient experienced intolerable neuropsychiatric and visual disturbances with voriconazole, again with sub-therapeutic plasma levels (0.5 mg/L; RR = 1.3–5.7 mg/L). Dual therapy with oral posaconazole 300 mg twice daily and IV caspofungin was instituted, but after initial improvement was unsuccessful in improving symptomatology, lung function and decreasing exacerbation frequency. *Exophiala dermatitidis* was grown persistently in sputum cultures throughout this time. An in-hospital trial of IV liposomal amphotericin B (Ambisome®) had to be abandoned due to progressive acute kidney injury. A trial of nebulised voriconazole and amphotericin B deoxycholate - recognised as experimental, unlicensed salvage therapy - was halted due to poor tolerability (bronchospasm) and a lack of discernible efficacy. Further combination triple therapy (IV caspofungin, nebulised voriconazole and oral terbinafine) was similarly ineffective, and oral isavuconazole was not tolerated due to severe diarrhoea. By this juncture, and from the first isolation of *Exophiala* in the sputum, the patient had spent a total of 148 days as a hospital inpatient, during which he required consecutive courses of broad-spectrum IV anti-bacterial concomitant with the antifungals described.

By month 25, the patient had deteriorated further, and was unable to climb a flight of stairs without stopping due to severe dyspnoea, and was dependent on high-dose oral and inhaled corticosteroids for worsening asthma (prednisolone 40 mg per day and nebulised budesonide 500 micrograms twice-daily). Pulmonary function tests had deteriorated to a nadir: FEV_1 = 1.3 L (27% predicted), FVC = 1.3 L (23% predicted) (Fig. 1). A full immunological work-up was requested on the basis of chronic fungal colonisation. Baseline analysis of humoral immunology revealed normal serum IgG [6.4 g/L; reference range (RR) = 6.4–16.0], but with normal subclass levels, undetectable IgA (<0.1 g/L; RR = 0.7–3.2 g/L), normal IgM (1.5 g/L; RR = 0.8–2.5) and reduced IgE levels (<5 IU/L RR 5–120). Polyvalent (23-serotype) pneumococcal IgG was low (25.0 mcg/L; RR = >70), in keeping with an impaired response to a history of previous vaccination, although the anti-tetanus IgG was considered protective (0.23 IU/ml; protective RR = >0.15). Analysis of lymphocyte revealed normal CD4+ and CD8+ T cell, and CD19+ B cell counts. However, the proportion of class switched memory B cells were reduced at 4.7% (RR = 7.5–32.0). Further immunological data, demonstrating a deficiency in IFN-γ and IL-17 in response to appropriate stimuli, are outlined in Table 1.

Given the patient's progressive symptomatology despite aggressive anti-infective therapy, and evidence of a secondary defect in IL-17 and IFN-γ cytokine secretion, he was offered a trial of subcutaneous IFN-γ-1b in accordance with a local therapeutic protocol established for similar patients with chronic granulomatous disease (50 micrograms/m^2 three times weekly). Administration of subcutaneous IFN-γ-1b was associated with a marked reduction in mucous plugs, reversal of sputum colour from black to the patient's baseline green, and clearance of *Exophiala* growth from sputum cultures. The patient elected to stop IFN-γ therapy after three months due to severe flu-like symptoms (myalgia, asthenia and fatigue).

Table 1
Functional, whole blood immunological stimulation assays taken prior to interferon-γ therapy.

STIMULUS	IFN-γ		IL-17		TNF-α		IL-6		IL-10		IL-12	
	Control (C)	Patient (P)	C	P	C	P	C	P	C	P	C	P
LPS	<5	<5	–	–	608	770	8171	11909	442	401	<5	11
LPS/IL-12	615	81*	–	–	2452	4704			308	72	49	174
PHA	1017	303	331	25*	1037	91*	12828	86*	697	108	–	–
aCD3	2621	776	163	<2*	1850	34*	586	<1*	986	4*	–	–
aCD3+IL-2	369	90	180	64	2513	1143	631	26*	1663	60*	–	–
PMA/IONO	18215	7826	214	82	20222	24326	9768	15,182	247	208	94	118

Results of the patient's whole blood, pro-inflammatory cytokine-release assay, in response to various stimuli, are detailed above. Results marked * are deemed significant by laboratory and expert clinical criteria (± 10% of control value). In comparison to the healthy, experimental control(C) IFN-γ production in response to stimulation with LPS/IL-12 and PHA was markedly reduced, although preserved following PMA-IONO stimulation. Moreover, IL-17 production was significantly attenuated following stimulation with both PHA and aCD3. Taken together, these findings suggest a secondary impairment in Th-1/Th-17 signalling at the time of sampling.

However, he continues to remain stable with regard to pulmonary function with cessation of the rapid decline, and his functional capacity (performing light physical activity) has returned to baseline. His IV antimicrobial therapy requirement has reduced dramatically, and he remains on long-term anti-pseudomonal prophylaxis with nebulised colistin. Moreover, the patient has been able to reduce his very significant daily requirement for oral corticosteroids following clearance of the *Exophiala* colonisation. Whole-blood functional immunoprofiling performed 12 months post IFN-γ therapy however, continues to show persistently low Th17 and a more pronounced reduction in Th1 cytokine responses.

This case highlights the potential use of immunotherapy to treat refractory fungal infections and the limitations of existing drug therapy. Fungicidal azole-derivatives with anti-mould activity, including itraconazole, voriconazole and posaconazole, pose a number of difficulties in clinical practice - such as unpredictable bioavailability, multiple drug interactions (including with CFTR modulators, such as lumacaftor/ivacaftor), and significant systemic toxicities. The emergence of azole-resistant moulds, particularly *Aspergillus*, is also a growing concern. Second line agents such as the echinocandins and amphotericin B require intravenous (IV) administration, have limited coverage for some non-*Aspergillus* moulds, significant toxicity and echinocandins are fungistatic (rather than fungicidal) for *Aspergillus* spp. at therapeutic doses. Furthermore, as an emerging pathogen, the evidence base for the treatment of clinical respiratory infection caused by *Exophiala* is very poor, particularly in the CF population – in contrast to Aspergillus-associated disease [4]. Indeed, the optimal management is particularly ill-defined where azole-based therapy is unsuitable. We believe this case highlights the importance of emerging fungal pathogens, together with the potential clinical benefit of detailed immunological testing, in patients with CF.

Further results included normal CD4+ and CD8+ T-cell, and CD19+ B-cell counts. Phorbol myristate acetate (PMA) simulation of neutrophils showed a normal oxidative burst, refuting a diagnosis of chronic granulomatous disease (CGD), and the PHA T-cell proliferation assay was similar to a healthy control.

Conflict of interest

None.

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