The Effect of Aspergillus fumigatus Infection on Vitamin D Receptor Expression in Cystic Fibrosis

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Rationale: Aspergillus fumigatus (A. fumigatus) in cystic fibrosis (CF) is increasingly recognized. Although allergic bronchopulmonary aspergillosis (ABPA) leads to deterioration of pulmonary function, the effect of A. fumigatus colonization in the absence of ABPA remains unclear.

Objectives: To address this, we examined individuals with CF with A. fumigatus who were ABPA negative to identify the effects of itraconazole therapy on Aspergillus-induced lung inflammation.

Methods: The effect of A. fumigatus on nuclear vitamin D receptor (VDR) expression was investigated using qRT-PCR and Western blotting. IL-5 and IL-13 levels were quantified by ELISA. The effect of itraconazole was assessed by a combination of high-resolution computed tomography, lung function test, and microbiological analysis.

Results: Measurements and Main Results: We demonstrate that A. fumigatus down-regulates VDR in macrophages and airway epithelial cells and that the fungal metabolite gliotoxin (Gt) is the main causative agent. Gt overcame the positive effect of 1,25-OH vitamin D3 on VDR expression in vitro, resulting in increased IL-5 and IL-13 production. In vivo, A. fumigatus positivity was associated with increased Gt in CF bronchoalveolar lavage fluid and increased bronchoalveolar lavage fluid levels of IL-5 and IL-13. After airway eradication of A. fumigatus with itraconazole, we observed decreased Gt, IL-5 and IL-13, improved respiratory symptoms, and diminished high-resolution computed tomography mosaic pattern consistent with sustained pulmonary function.

Conclusions: This study provides a rationale for the therapeutic effect of itraconazole and implied that the therapeutic potential of vitamin D supplementation in preventing ABPA is only feasible with concurrent elimination of A. fumigatus to permit VDR expression and its positive functional consequences.

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AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject
In individuals with cystic fibrosis, the effect of azole treatment on patients with Aspergillus fumigatus colonization in the absence of allergic bronchopulmonary aspergillosis is unknown.

What This Study Adds to the Field
Aspergillus colonization increases T-helper 2 cytokine production by structural and innate immune cells of the airways. The mechanism of action involves down-regulation of vitamin D receptor expression. The overall effect of itraconazole treatment was elimination of A. fumigatus bioburden, thereby permitting vitamin D receptor expression, resulting in decreased T-helper 2 cytokine production and significant clinical improvement.

Keywords: cystic fibrosis; vitamin D receptor; Aspergillus fumigatus; itraconazole; inflammation

Cystic fibrosis (CF) is a systemic heritable disorder. Pathogenesis of lung disease induced by CF is a direct result of decreased chloride secretion and hyperabsorption of sodium, resulting in the retention of dehydrated mucus within the airways. Pathogenic colonization and chronic infection occurs, giving rise to recurrent exacerbations, respiratory failure, and death.

Our understanding of the airway microbiome within the CF milieu has evolved with fungi emerging as recognized colonizers and potential pathogens (1–4). Although yeasts and filamentous fungi are identified in CF, the most commonly isolated member of the latter group is Aspergillus fumigatus. In the immune-competent host, exposure to A. fumigatus spores leads to an immune response stimulating prompt fungal clearance. Occasionally, however, in immunocompromised individuals, this ubiquitous fungus can cause life-threatening invasive disease. In most cases, although it is acknowledged as a potential problem, the impact of A. fumigatus on airway disease and inflammation in CF remains unclear (4).

Colonization rates vary between centers. In one decade-long study, A. fumigatus was isolated in over one third of lung cultures from people with CF (5), a proportion similar to that detected by our group (6). Additionally, we and others have demonstrated that the frequency of A. fumigatus isolation from CF sputum does not correlate with rates of allergic bronchopulmonary aspergillosis (ABPA), gender, pancreatic status, or cirrhosis but is associated with higher recovery rates of Pseudomonas aeruginosa infection.
Aspergillus A. fumigatus pulmonary radiological abnormalities on high-resolution computed tomography (HRCT) are more pronounced in an A. fumigatus–colonized cohort compared with a matched non–A. fumigatus–colonized CF group, a finding not reflected by pulmonary function testing alone (12). A. fumigatus colonization has additionally been shown to be an independent risk factor for hospital admission in CF (1, 13), yet studies assessing the effect of eradication of A. fumigatus from the CF airway unaffected by ABPA are limited. Although work on azole-based agents (e.g., itraconazole or voriconazole) in CF-ABPA is ongoing, the anti-inflammatory effects of vitamin D and other VDR agonists in the setting of A. fumigatus colonization without ABPA remain unaddressed. This study clarifies the effect of A. fumigatus on VDR expression in the CF airway and for the first time evaluates treatment effectiveness of itraconazole in A. fumigatus colonization without ABPA. Some of the results of these studies have been previously reported in the form of an abstract (14–16).

METHODS

Patient Selection and Recruitment

All patients with CF attending Beaumont Hospital, Dublin, Ireland, between July 2008 and June 2010 (2-year period) were screened for study eligibility (n = 117) (see Figure E1 in the online supplement). Inclusion criteria required a confirmed diagnosis of CF by sweat chloride concentration greater than 60 mmol/l on at least two separate occasions and subsequent confirmatory genotyping. Patients included had to be colonized with A. fumigatus. Colonization was defined as detection of the fungus in sputum and/or bronchoalveolar lavage fluid (BALF) on at least two separate and consecutive occasions at least 12 weeks apart in the year before study commencement. Before recruitment, patients had to be exacerbation free over the preceding 6-week period with no therapy in any form. All participants had serum IgE levels less than 450 units/ml and negative cutaneous reactivity to P. aeruginosa and Staphylococcus aureus.

RESULTS

The Effect of A. fumigatus Culture Filtrates on VDR Gene and Protein Expression in CF Tracheal Epithelial and CF Bronchial Epithelial Cells

We and others have previously shown that A. fumigatus colonization affects approximately one third of people with CF (5, 6), an observation further confirmed in this study (Figure E1). The percentages of dominant immune cells in A. fumigatus–colonized CF BALF has been previously reported to include neutrophils (61%) and macrophages (31%) and, to a much lower extent, lymphocytes (4%) (21). By Western blotting, however, VDR protein expression was found abundant in macrophages but not in neutrophils and at a very low level in T cells (Figure E2). CF airway epithelial cells were also found to express VDR protein, and consequently this study examined the effect of A. fumigatus on VDR expression by structural (bronchial and tracheal epithelial cells) and immune cells (macrophages) of the airways.

VDR gene expression was significantly down-regulated by approximately 80% in CF tracheal epithelial (CFTE) cells (P = 0.001) (Figure 1A) and was completely inhibited in CF bronchial epithelial (CFBE) cells (P = 0.04) (Figure 1B) in response to treatment with 3-day culture filtrates for 16 hours. VDR gene expression was decreased by approximately 33% 2 hours after addition of Aspergillus culture filtrate in CFTE
The effect of A. fumigatus culture filtrates (1–4 d) on VDR protein expression was evaluated by Western blotting. Significant down-regulation of VDR protein expression was observed in CFTE and CFBE cells after treatment with 4-day culture filtrates for 5 hours (P = 0.01 and P = 0.009, respectively) (Figures 1F and 1G). To confirm the effect of Aspergillus colonization on VDR gene expression in vivo, bronchial brushings were performed on 6-day cultures (Figure 1H). Collectively these results indicate in vitro and in vivo down-regulation of VDR gene and protein expression by A. fumigatus.

Down-regulation of VDR Expression by Gliotoxin, an Immunomodulatory Mycotoxin from A. fumigatus

The VDR modulator present within culture filtrates of A. fumigatus (ATCC-26933) was characterized. The inhibitory activity was stable within culture filtrates stored at -70°C and subsequently used against CFBE cells at 37°C but was significantly abolished by heat inactivation (99°C) for 20 minutes (P = 0.003) (Figure 2A). Passage of culture filtrates through a 3 kD exclusion filter removed approximately 65% of the VDR inhibitory activity (P = 0.007), which suggested the presence of predominantly low-molecular-weight (<3 kD) VDR inhibitory factors.

Secreted by the hyphal form of A. fumigatus, gliotoxin (Gt) has previously been characterized as a small, heat-inactivated toxin of 326.4 daltons (22). Gt has been shown to provoke airway inflammation and tissue damage with consequential long-term airway remodeling (23). To confirm Gt as a fungal agent capable of VDR down-regulation, the effect of a low-Gt-producing A. fumigatus strain (CaF1) on VDR expression was explored. Strain CaF1 produced minimal levels of Gt compared with the high-Gt-producing strain ATCC-26933 after 96 hours of fungal growth (2 ± 0.05 and 252 ± 2.5 ng/mg hyphae, respectively) (Figure E3). Moreover, VDR expression in CFTE and CFBE cells remained relatively unchanged when treated with 1- to 4-day culture filtrates of CaF1, in comparison to the ATCC-26933 strain, which resulted in significant down-regulation of VDR (P = 0.01 and P = 0.009 for CFTE and CFBE cells compared with untreated cells, respectively) (Figures 2C and 2D). The clinical relevance of these findings was confirmed when BALF obtained from Aspergillus-positive patients illustrated high levels of Gt, as quantified by HPLC analysis of chloroform extracts (Figure 2E).

The effect of purified Gt on VDR expression by CF and non-CF cells was next explored. By HPLC analysis of cultures of A. fumigatus strain ATCC-26933, no significant difference was detected in Gt levels produced on Day 3 (280 ± 29 ng/mg hyphae) compared with Day 4 (252 ± 2.5 ng/mg hyphae) (P > 0.05) (Figure E3A). This concentration produced by the fungus equates to approximately 0.8 μM commercial Gt, which was therefore used in this study. Gt used at this concentration significantly down-regulated VDR protein expression in CFBE
cells ($P = 0.03$) to a similar level obtained after exposure to 4-day culture filtrates of *A. fumigatus* (ATCC-26933), indicating that Gt is most likely the main VDR inhibitory factor secreted by the fungus (Figure E3B).

In vitro studies further revealed that 0.8 μM Gt significantly down-regulated VDR gene expression in CFTE ($P = 0.001$), CFBE ($P = 0.02$), and human bronchial epithelial (HBE) ($P = 0.003$) cell lines (Figures 3A–3C, respectively). HBE cells were used in this latter experiment to provide evidence that down-regulation of VDR by Gt was independent of dysfunctional CFTR. In addition, CFBE cells cultured under submerged monolayer (Figure 3D) or air–liquid interface (Figure 3E) of Gt in *A. fumigatus* colonized cystic fibrosis BALF ($n = 3$, Asp+) compared with noncolonized samples ($n = 3$; Asp−). Gt was quantified by HPLC and standardized to total BALF protein (mg). All assays were performed in triplicate on three consecutive days. Data shown are mean ± SE.

**Figure 2.** Characterization of the *Aspergillus* virulence factor responsible for vitamin D receptor (VDR) down-regulation.

(A) Down-regulation of VDR protein expression was reversed by heat inactivation (HI) of *A. fumigatus* (ATCC-26933) culture filtrates (AFCF). Cystic fibrosis bronchial epithelial (CFBE) ($1 \times 10^5$) were untreated (Con) or exposed to 4 days of AFCF or HI-AFCF for 5 hours. The Western blots illustrated are from one representative experiment out of three and converted to densitometry units in respective graphs. (B) AFCF down-regulated by a small (< 3 kD) secreted molecule. AFCF (4 d) was passed through a 3 kD membrane, and the resulting flow through (FT < 3 kD) or top supernatant (AFCF SUP > 3 kD) was added to CFBE cells ($1 \times 10^5$) for 5 hours. (C and D) The effect of CaF1 (white bars) and ATCC-26933 (black bars) AFCF grown for 1 to 4 days on VDR protein expression in cystic fibrosis tracheal epithelial (CFTE) (C) and CFBE cells (D). (E) Levels of Gt in *A. fumigatus* colonized cystic fibrosis BALF ($n = 3$, Asp+) compared with noncolonized samples ($n = 3$; Asp−). Gt was quantified by HPLC and standardized to total BALF protein (mg). All assays were performed in triplicate on three consecutive days. Data shown are mean ± SE.

**Figure 3.** Effect of purified gliotoxin on vitamin D receptor (VDR) expression. (A–C) Purified gliotoxin (Gt) down-regulates VDR in a dose-dependent manner. VDR gene expression of cystic fibrosis tracheal epithelial (CFTE) (A), cystic fibrosis bronchial epithelial (CFBE) (B), and human bronchial epithelial (HBE) (C) cells ($1 \times 10^5$) treated for 5 hours with 0.16 or 0.8 μM Gt. RNA was analyzed by qRT-PCR, normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH), and compared with untreated cells (Con). (D–F) Gt down-regulates vitamin D–induced VDR protein expression in vitro. CFBE cells cultured under submerged monolayer ($1 \times 10^5$) (D) or air–liquid interface ($5 \times 10^5$) (E) or U937 differentiated macrophages ($1 \times 10^5$) (F) were untreated (Con) or exposed to 1,25D$_3$ (10$^{-9}$ M) in the presence or absence of Gt (0.8 μM) for 24 hours, and VDR protein expression was analyzed by Western blotting using mouse monoclonal VDR and rabbit polyclonal GAPDH antibodies. All assays were standardized to GAPDH and were performed in triplicate on three separate days. Data shown are mean ± SE.
pared with cells treated with 1,25D3 only (P = 0.04 and P = 0.005, respectively). This effect was also observed by treating macrophages with Gt (P = 0.03) (Figure 3F). Control experiments indicated that Gt did not affect cell viability or induce cell apoptosis as determined by trypan blue exclusion assays or a lack of cleaved (17/19 kD) caspase-3, respectively (result not shown). Collectively these experiments suggest an overwhelming inhibitory effect of Gt on VDR expression.

Gliotoxin Enhances the Production of Th2 Cytokines, Which Are Reduced after Itraconazole Treatment

It has been shown that VDR knockout mice have elevated levels of IL-5 and IL-13 production (10), and vitamin D has also been observed to modulate Aspergillus-induced Th2 cytokines IL-5 and IL-13 expression in CD4+ T cells (9). In line with this, we investigated the effect of Gt on IL-5 and IL-13 expression in vitro. CFBE cells or macrophages were treated with 1,25D3 (10−9 M) in the presence or absence of 0.8 μM Gt. Gt significantly increased IL-5 and IL-13 gene (Figures 4A and 4B) and protein (Figures 4C–4F) expression in CFBE submerged monolayers (P = 0.05 and P = 0.05, respectively) (Figures 4A and 4B), air-liquid interface CFBE cultures (P = 0.05 and P = 0.01, respectively) (Figures 4C and 4D), and macrophages (P = 0.05 and P = 0.05, respectively) (Figures 4E and 4F). In support of this result, high levels of IL-5 and IL-13 were observed in CF BALF samples before itraconazole treatment (Figures 4G and 4H). After itraconazole treatment, a significant decrease in IL-5 (P = 0.01) and IL-13 (P = 0.002) protein expression was detected in vivo, suggesting a role for itraconazole in reducing the Th2 inflammatory response in these patients.

Itraconazole Treatment Effectively Reduces A. fumigatus Bioburden in CF Airways

Itraconazole therapy administered orally at 400 mg once daily for a 6-week period significantly reduced A. fumigatus bioburden within the CF airway over time as determined by CFU and conidia counts per gram of sputum and/or BALF (Figures 5A and 5B). Itraconazole therapy was well tolerated in the study cohort, with no reports of adverse effects or liver dysfunction during treatment and follow-up. Although CFU counts remained low at the 12-month follow-up (Figure 5A), A. fumigatus conidia burden was increased when compared with the 6-month follow-up sample (Figure 5B), suggesting that qPCR is a more reliable method of Aspergillus detection in CF sputum. In addition, corresponding to a reduction in Aspergillus bioburden at the 12-month follow-up, levels of Gt were significantly reduced in vivo from approximately 250 ± 80 to 75 ± 50 ng/mg BALF protein (P = 0.04) (Figure 5C). Moreover, after 6 weeks of itraconazole (400 mg daily) treatment for Aspergillus infection, VDR gene expression was significantly increased by greater than 2-fold (P = 0.04) (Figure 5D). This set of experiments demonstrates that itraconazole therapy significantly reduced A. fumigatus colonization within the CF airways and by corollary diminished Gt levels initiating up-regulation of VDR expression.

A. fumigatus Is Associated with a Mosaic Pattern on HRCT That Significantly Attenuates after Itraconazole Therapy

It has been shown that A. fumigatus colonization in CF has adverse effects upon the lung parenchyma (on HRCT) that do not correlate with pulmonary function testing (12). To establish the presence of radiological changes after eradication of A. fumigatus from the CF airway, HRCT scans were performed...
in people with CF colonized by *A. fumigatus* with no prior history of ABPA or corticosteroid treatment for suspected ABPA (n = 13). Three HRCT scans were performed at baseline (pretreatment), at 10 weeks after completion of itraconazole (posttreatment), and at 12-month follow-up.

No significant differences in the overall or component parts of the modified Bhalla score was identified after treatment or during follow-up. However, 92.3% (n = 12) of baseline (pretreatment) scans illustrated a mosaic pattern, which was significantly less evident after itraconazole eradication of *A. fumigatus*. Fewer scans after treatment (76.9%; n = 10) or during follow-up (53.8%; n = 7) illustrated evidence of the mosaic pattern observed at baseline (Figures 6A and 6B). In patients whom attenuation rather than elimination of the pattern occurred after itraconazole treatment, we further quantified the mosaic pattern by its conspicuity (obvious, subtle, or present on expiratory images only) and by percentage of lung parenchyma involved. A significantly diminished conspicuity and a lower percentage of lower lobe lung parenchymal involvement after treatment and during follow-up was observed (Figure 6C).

Pulmonary function (FEV₁ and forced expiratory flow, mid-expiratory phase [FEF₂₅₋₇₅]) remained stable without significant deterioration over the study course (Figure 6D). However, eradication of *A. fumigatus* from the CF airway resulted in a significant reduction in the number of infective exacerbations (Figure 6E) and requirement for intravenous antibiotic therapy (Figure E4), findings that were sustained over the study follow-up period. Respiratory symptoms (assessed by CFQ-R) illustrated significant improvement during follow-up that was maintained to study conclusion (Figure 6F). Additionally, mean serum levels of IgE decreased after treatment (178.2 ± 88.4 to 136.9 ± 60.7 IU/ml) and during follow-up (90.5 ± 32.0 IU/ml), albeit not significantly (P > 0.05). No patient developed ABPA over the course of the study. Radioallergosorbent test *Aspergillus* sampling validated this. In summary, these results indicate that eradication of *A. fumigatus* from the CF airway resulted in improved HRCT appearances, less infective exacerbations, fewer requirements for intravenous antibiotics, and improved CFQ-R respiratory symptom scores.

**DISCUSSION**

In the current study, we illustrate that *A. fumigatus* colonization down-regulates VDR and identify Gt as one fungal agent responsible for mediating this effect. We also found that in vivo *A. fumigatus* colonization is associated with increased Gt in CF BALF, mosaic pattern on HRCT, and an enhanced production of the Th2 cytokines IL-5 and IL-13. Treatment with the anti-fungal agent itraconazole in vivo rescued VDR expression, significantly attenuated Gt levels, and decreased the production of the Th2 cytokines IL-5 and IL-13, which drive the allergic process leading to ABPA (24, 25). Such eradication of *A. fumigatus* from the CF airway resulted in improved respiratory symptoms and diminished HRCT mosaic pattern, consistent with sustained pulmonary function.

Vitamin D influences the inflammatory and immune state in CF and acts via cognate nuclear VDR (26, 27). Although insufficient vitamin D (< 30 ng/ml) is problematic for bone health in CF, it is also acknowledged as a risk factor for ABPA in those colonized with *A. fumigatus* (9, 28). Consequently, supplemental administration of vitamin D is advocated for its potential to prevent ABPA (9). As a result, the protein level of VDR is prioritized to prevent the Th2 cytokine production through Gt, a toxin released by the hyphal form of the fungi. Compromised VDR in the face of *A. fumigatus* disrupts the inflammatory state in CF, with vitamin D unable to exert its positive impact. One method by which
vitamin D confers benefit by attenuating Th2 responses to A. fumigatus by CD4+ T cells in CF-ABPA (9). Th2 cytokines IL-5 and IL-13 are not restricted to CD4+ cells alone, and, although this source remains of primary concern in the ABPA context, release of these cytokines by eosinophil and mast cells (29, 30), and by airway epithelial cells and macrophages as shown in the present study, is of importance in people with CF colonized by A. fumigatus but unaffected by ABPA.

The unique structural motif of Gt is essential for its damaging effects, including the inhibition of immune cell activation, phagocytosis, and ciliary beat frequency, together preventing fungal clearance (22, 31, 32). Gt concentrations have been reported in the setting of a murine model of invasive aspergillosis (33); however, to our knowledge this is the first study to report Gt levels in vivo in the context of CF and its relationship to VDR expression. However, Gt-mediated VDR down-regulation is independent of CFTR dysfunction; this phenomenon was observed during in vitro experiments using non-CF HBE cells. Gt is also able to overcome the positive effects conferred by vitamin D on VDR expression, a novel mode of action within the pulmonary milieu. Placing our findings in the context of those reported by Kriendler and colleagues (9), vitamin D may attenuate Th2 cytokines and potentially reduce or prevent CF-ABPA, although it requires the presence of VDR to carry out such functions. Hence, the elimination of Gt remains as crucial as the administration of vitamin D.

This observational study provided unique insights into the treatment effectiveness of itraconazole on microbiological, radiological, and clinical outcomes, the former two not addressed by prior work (34). Sputum culture alone has been previously reported as insufficient at detecting the presence of A. fumigatus in CF (6, 35). Despite this, CFU counts for A. fumigatus remain the most accessible and affordable method of assessment and therefore remain in routine clinical practice. Failure to detect A. fumigatus because of a negative sputum culture can have deleterious clinical consequences. In view of this, we chose
to assess for the presence of *A. fumigatus* from sputum using two separate approaches, CFU counts and qPCR for *A. fumigatus* conidia burden. Although itraconazole was effective at eliminating *A. fumigatus* after treatment, these effects were sustained over the 1-year follow-up by both methodologies. However, despite the reassuring CFU counts obtained at the 1-year time point, escalations of *A. fumigatus* conidia burden were detectable by qPCR at this time as compared with earlier time points, suggesting a slow return of *A. fumigatus* colonization and the potential need for retreatment. These data provide evidence to support use of qPCR as the optimal detection method for *A. fumigatus* from respiratory CF samples. However, the clinical parameters assessed in this study continue to show positive effects at 12 months after treatment, when a time qPCR counts for *A. fumigatus* conidia begin to slowly rise (35). Consequently, cost and clinical importance of such methods for *A. fumigatus* detection must be considered before recommending their routine use. Most benefit from such methodology is probably gained from its use in clinical scenarios of strongly suspected *A. fumigatus*-associated disease in the presence of negative sputum culture where a treatment decision is necessary and further microbiological evidence for such treatment is sought. This may be applicable to *A. fumigatus* beyond CF.

Further work performed by our group described the radiological evaluation of *A. fumigatus* colonization in CF (12) and reported a novel mosaic pattern associated with such colonization in the CF setting unrelated to ABPA. Quantification of this mosaic pattern over the study course and after itraconazole treatment illustrated elimination or significant attenuation, suggestive of an inflammatory benefit in the individuals with CF recruited to this study. Significant time-dependent effects, greatest at 12-month follow–up, were observed. Whether such findings confer prolonged benefit or represent a radiological lag in appearance compared with clinical benefit remains to be elucidated. Although images from an untreated placebo controlled group of *A. fumigatus* colonized patients with CF without ABPA would have provided stronger evidence for this radiological relationship, we feel it would have been unethical to recruit an untreated group in view of our previously published work illustrating worse radiological appearances in patients who are *A. fumigatus* colonized (12). Although our study findings are of interest, the small study population and the uncontrolled and open-label nature of the trial are notable weaknesses. However, itraconazole treatment produced positive outcomes across all the clinical parameters assessed, including decreasing exacerbations and stabilizing pulmonary function while improving CQ-R respiratory scores, findings contrasting prior work (34). Moreover, itraconazole is a potent inhibitor of CYP3A4 activity, and corticosteroids are partly metabolized through such enzymes; hence, it should be noted that a proportion of our patient cohort received inhaled corticosteroids at a low dose during the course of the study (Table E1). Systemic absorption of such steroid is accepted to be diminutive; hence, the effects on the CYP3A4 system conferred by itraconazole treatment during the study are probably minimal. However, this is a potential confounding factor to be considered when interpreting our results. No patient during the study developed ABPA by consensus criteria (17), and a reduced likelihood of ABPA occurring was demonstrated by decreasing levels of IgE, radioallergosorbent test to *Aspergillus*, and an attenuated Th2 cytokine response after treatment.

Despite the fact that IL-5 and IL-13 are linked to ABPA, none of the patients recruited to this study had ever had ABPA, although high levels of these cytokines were present in their lungs. These Th2 cytokines were significantly reduced after itraconazole therapy, which is suggestive of an important antiinflammatory role for the drug in addition to its antifungal properties. Minimal clinical data exist within the CF literature to assist the clinician in identifying individuals at risk of ABPA or those that can be predicted to develop ABPA in the setting of *A. fumigatus* colonization alone. Although our group has previously shown that sputum isolation of *A. fumigatus* does not correlate with occurrence of ABPA (6), the current study has uncovered high levels of Th2 cytokines IL-5 and IL-13 existing in vivo in the setting of *A. fumigatus* colonization without ABPA that may be attenuated by empirical itraconazole treatment. Whether such therapy can prevent ABPA in the long term in those asymptatically colonized by *A. fumigatus* remains to be determined.

In summary, although published work has suggested the therapeutic potential of vitamin D in preventing ABPA (9), our data imply that this approach is only feasible with concurrent elimination of *A. fumigatus* with itraconazole to permit VDR expression. This study raises awareness of the clinical importance of antifungal resistance (36, 37) and acknowledges the need for a more long-term study on the antifungal activity of triazole derivatives in the treatment of *A. fumigatus* in individuals with CF.

Author disclosures are available with the text of this article at www.atjsournals.org.

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