Pneumocystis colonization in asthmatic patients not receiving oral corticosteroid therapy

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ABSTRACT

Pneumocystis jirovecii can colonize patients with chronic obstructive pulmonary disease. To determine if colonization occurs in asthma patients, sputum samples from 10 patients with mild asthma, who were not receiving oral corticosteroids, were evaluated by a sensitive real-time PCR assay that targets a multicopy gene of *P. jirovecii*. 2 patients (20%) had *Pneumocystis* DNA detected; 1 patient had 3 positive samples over an 11-day period. Thus, *Pneumocystis* colonization occurs in asthma patients, and further studies are warranted to evaluate its role in airways disease.

Trial registration number NCT01113034.

INTRODUCTION

Pneumocystis jirovecii is a fungal pathogen that causes clinically significant *Pneumocystis* pneumonia (PCP) exclusively in immunosuppressed patients.[1] However, a number of recent studies have demonstrated colonization or subclinical infection in individuals not typically considered immunosuppressed, including those with underlying pulmonary diseases such as cystic fibrosis[2] and chronic obstructive pulmonary disease (COPD).[3] Asthma, like COPD, is an obstructive pulmonary disease, with an estimated prevalence of ~8–9% in the USA (http://www.cdc.gov/asthma/asthmadata.htm). Recent studies with animal models suggest an association between *Pneumocystis* infection and both COPD and asthma.[4 5]

While oral corticosteroids are frequently used to treat asthma and are considered an independent risk factor for developing PCP, PCP is highly unusual in patients with asthma with no other recognized risk factors.[6] It is currently unknown if and to what extent persons with asthma may be colonized with *Pneumocystis*. We thus undertook a study to prospectively examine the frequency of colonization in a small group of well-controlled asthmatics participating in a safety study of DAS181, a drug being developed for treatment of respiratory virus infections; DAS181 is a recombinant protein with sialidase activity that selectively cleaves sialic acid residues from host cells, thus preventing binding by viruses such as influenza that require sialic acid as receptors.[7]

MATERIALS AND METHODS

Ten patients who were screened or who enrolled in a placebo-controlled cross-over trial of DAS181 were included in this substudy. Detailed information on the primary study, including the methods and enrollment criteria, have been recently reported.[7] Patients eligible for enrollment included those with well-controlled asthma who were in good health otherwise, were not currently receiving oral corticosteroids, and had received no oral corticosteroids for at least the prior 3 months. Inhaled and topical corticosteroids were permitted. Daily 10 mg doses of DAS181 or placebo were given via Cyclohaler, a dry powder inhaler, for 3 days. After 18 days, the patient received the alternative (DAS181 or placebo) in a similar manner. The protocol and amendment were approved by the institutional review board of the National Institute of Allergy and Infectious Diseases, and all patients provided signed informed consent.

Induced sputum sample collections were scheduled for multiple times during the study for bacterial culture as a safety parameter. After initiation of the protocol, the study was amended to allow *Pneumocystis* PCR to be performed; thus, PCR was not performed on all study samples. Induced sputa were processed and *P. jirovecii* DNA was detected utilizing a real-time PCR assay that targets the multicopy major surface glycoprotein (*msg*) gene family, as previously described.[8] This assay is routinely performed in the microbiology department at NIH for the diagnosis of *Pneumocystis* infection, and utilizes appropriate quality control measures to prevent contamination as previously described.[8] The result is either positive or negative for *Pneumocystis* but is not quantitative.

RESULTS

The median age of the 10 patients who provided sputum samples for analysis was 22.9 years (range, 20–62) (table 1). Six (60%) participants were women, six (60%) were white, two (20%) were black or African-American, one (10%) was Asian, and one (10%) was multiracial. No patient had a signficiant comorbidity, specifically no one had diabetes mellitus or an underlying immunological defect. All patients had mild asthma; asthma-related study medications at study entry are

Table 1 Baseline clinical characteristics for the 10 patients

Characteristic	Number (%) or median (range)
Gender	
Male, n (%)	4 (40%)
Female, n (%)	6 (60%)
Age in years, median (range)	22.9 (20–62)
Race	
White, n (%)	6 (60%)
Black, n (%)	2 (20%)
Asian, n (%)	1 (10%)
Multiracial, n (%)	1 (10%)
Medications	
Albuterol, prn inhaled, n (%)	9 (90%)
Fluticasone, inhaled, n (%)	2 (20%)
Fluticasone/salmetrol, inhaled, n (%)	1 (10%)
Montelukast, oral, n (%)	2 (20%)
Cetirizine, oral, n (%)	1 (10%)
Loratidine, oral, n (%)	1 (10%)
Diphenhydramine, oral, n (%)	1 (10%)
Topical hydrocortisone	1 (10%)
FEV1, % predicted, median (range)	88.5 (73–108)
Frequency of rescue inhaler use in prior 3 months, n, median (range)	0 (0–12)

summarized in table 1. Three patients were receiving inhaled fluticasone, and one patient was receiving topical hydrocortisone.

A median of 4.5 sputum samples were provided per patient (table 2).Two of the 10 patients (20%; 95% CI 3.6% to 51%) tested positive for *Pneumocystis* DNA at one and three time-points, respectively (table 2). Neither of them had been using inhaled or topical corticosteroids. All four samples were obtained prior to any administration of DAS181. The first patient had a positive sputum on the first sample tested, with four subsequent samples being negative. The second patient had a positive sputum on the first two samples tested, then had two negative sputum tests before the third positive test, which was obtained 9 days after the first positive test. A subsequent sample taken 11 days later was again negative. The remaining patients were negative at all time-points tested.

Table 2 Proportion of induced sputum samples positive for *Pneumocystis* by PCR

Patient no.	Proportion PCR-positive	Time span of sample collection (days)	Day(s) of positive sample
1	0/1	1	
2	0/7	78	
3	0/8	42	
4	1/5	44	1
5	3/6	22	1, 2, 11
6	0/3	7	
7	0/4	40	
8	0/1	1	
9	0/1	1	
10	0/7	78	

All three positive samples from the second patient had a high cycle threshold (C_t), with C_t ranging from 31.5 to 35.9, compared with 20.8 to 21.6 for the positive control of 10,000 gene copies/assay. This indicates a very low copy number of the targeted gene in these three samples. The C_t for the single sample for the first patient was not recorded.

DISCUSSION

This study, the first to examine *Pneumocystis* colonization in asthmatics using a sensitive detection method, detected *P. jirovecii* DNA in induced sputum samples from 20% of asymptomatic patients with a history of asthma. While the number of patients examined is small, this nonetheless suggests that patients with asthma may be at increased risk for colonization or subclinical infection by *Pneumocystis*. Although chronic use of corticosteroids, especially in the setting of an underling inflammatory disease or malignancy, is a risk factor for PCP as well as for colonization,[9] none of the patients in the current study had received oral corticosteroids. While three patients were receiving inhaled corticosteroids, none had a positive PCR. Inhaled corticosteroids have only very rarely been associated with *Pneumocystis* infection, and were not the only potential risk factor in two reported cases.[10–12]

One person had a single sample that was positive, while a second had three positive samples during an 11-day period, with two negative samples in between. This suggests that colonization is intermittent and of short duration or, alternatively, that the level of colonization may be low, near the threshold of sensitivity of the assay. The latter possibility is supported by the high Ct values for all three positive samples from the second patient. The efficiency of sputum induction in recovering organisms may also be variable.

The clinical significance of colonization in asthmatic patients is uncertain. Clinically significant PCP has only rarely been reported in asthmatics with no other predisposing factors.[6 13] *Pneumocystis* colonization has been associated with increased airway obstruction, as measured by pulmonary function tests, in HIV-infected patients, even after correcting for smoking.[14] COPD, another obstructive pulmonary disease, has also been associated with colonization with *Pneumocystis*, and animal models as well as human studies have suggested it may be associated with more severe disease, though a causal association needs validation in rigorously controlled studies.[3 4 15] Systemic inflammation, as defined by higher serum levels of IL-8, TNF-α, and IL-6, has been associated with higher rates of *Pneumocystis* colonization in patients with COPD, suggesting that an inflammatory state may predispose to such colonization, though alternatively the colonization may be causing such inflammation.[16] Whether a similar relationship is true in asthmatic patients is currently unknown. Thus, while conjectural at this time, it is possible that, at least in a subset of asthmatic patients, *Pneumocystis* colonization may contribute to the frequency or severity of disease exacerbations.

Colonization with *Pneumocystis* has been identified by PCR in a number of other populations, including patients with rheumatological diseases, cystic fibrosis, and other

chronic diseases.[2] Studies of patients without known underlying diseases have yielded variable results, ranging from no detectable colonization to rates of ~20%.[17] Differences in methodology may account at least partially for the different results. Many studies have utilized a nested PCR approach, which is sensitive but has a higher risk of false positives due to contamination. We utilized a highly sensitive single tube real-time PCR methodology, performed using the standard clinical assay run by our microbiology department that includes rigorous quality control measures to minimize contamination.

In summary, 20% of patients with even well-controlled asthma in this small cohort study were colonized with *Pneumocystis* at one or more times. Given that oral corticosteroid use is an independent risk factor for PCP, it is possible that asthmatic patients receiving oral corticosteroids for a disease flare have an even higher risk of colonization. Although these patients do not appear to be at high risk for developing clinically significant pneumonia, nonetheless it remains possible that colonization (or even subclinical infection) may contribute causally to the airways reactivity experienced by some patients during these flares. Further studies are needed to verify these data in a larger cohort, ideally including well-controlled asthmatics as well as patients receiving chronic oral corticosteroids for unstable disease, in order to better understand the potential role that the presence of *Pneumocystis* in the airways may play in exacerbation of asthma.

Contributors ELD, REC, RTD, KNO, and JAK are responsible for study conception and design; REC, CF, GF, and KNO performed study procedures and collected data; ELD, GF, KNO, and JAK analyzed and interpreted results; ELD, RTD, KNO, and JAK are responsible for drafting the manuscript; all authors are responsible for review of the manuscript.

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Competing interests None declared.

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