JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 1997, p. 2511–2513 Vol. 35, No. 10
0095-1137/97/$04.00+0

Detection of *Pneumocystis carinii* DNA in Air Samples: Likely Environmental Risk to Susceptible Persons

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Received 8 May 1997/Returned for modification 5 June 1997/Accepted 30 June 1997

The means by which humans acquire *Pneumocystis carinii* is not well understood. Whether it can be acquired from specific environmental sources or transmitted from person to person has not been determined. This study was designed to detect nucleic acids of *P. carinii* in air samples from various locations, including *P. carinii*-infected patients' homes and hospital rooms, non-*P. carinii*-infected patients' hospital rooms, empty hospital rooms, offices at Indiana University, and other homes in different locations. DNA was extracted from cellulose-ester filters through which air samples had been filtered, and the *P. carinii* DNA was amplified by PCR with primers specific for the internal transcribed spacer regions of rRNA. *P. carinii* DNA was found in 17 of 30 air samples (57%) from the rooms of *P. carinii*-infected patients. It was also found in 6 of the 21 other hospital rooms sampled (29%) but was not found in any of the offices, storage areas, or control homes. Environmental sampling suggests that the airborne presence of *P. carinii* genetic material and infectious organisms is plausible. The organism was also detected in locations where *P. carinii* patients were not immediately proximate, such as the hospital rooms of non-*P. carinii*-infected patients.

The lack of a clear definition of the mode(s) of transmission of *Pneumocystis carinii* has hampered efforts to minimize exposure. Individuals who are immunocompromised are at risk for *P. carinii* pneumonia, and outbreaks have occurred at hospitals both among patients receiving chemotherapy for malignancies (15, 17) and in transplantation units (4). With the advent of the human immunodeficiency virus, the number of *P. carinii* pneumonia cases has increased, so that during the first 7 years of the epidemic, *P. carinii* pneumonia accounted for more than 60% of AIDS-defining illnesses and occurred in 80% of individuals with AIDS (16).

Even though anti-*Pneumocystis* prophylaxis is recommended for patients with decreased $CD4^+$ cells, *P. carinii* pneumonia remains an important infection (14, 16). Trimethoprim plus sulfamethoxazole is very effective for both treatment and prophylaxis (6, 11), but many individuals do not tolerate it (3), and other antimicrobics are less effective. Therefore, preventing infections is of great importance.

The traditional view was that most humans were infected with the organism early in life and harbored it in a quiescent state. Pneumonia was thought to occur when normal immune mechanisms were suppressed and the quiescent microorganisms began to proliferate (12). However, several reports have pointed to other modes of transmission (10, 13). For example, it has been noted that cancer patients treated at hospitals admitting AIDS patients had a higher incidence of *P. carinii* infections (5) and that the increased numbers of *P. carinii* infections in renal transplant patients might have resulted from the spread of the disease from patients with AIDS (2). In addition, differences in the types of *P. carinii* detected in recurrent episodes of *P. carinii* infection in the same patient demonstrate that new infections, rather than the exacerbation of older latent ones (7), often cause the later episodes. These reports suggest the possibility of transmission of the disease among patients. Transmission among rats by the airborne route has been documented, and *P. carinii* nucleic acids have been detected in air samples (1).

The development of a typing system (8, 9) has allowed epidemiologic evaluations and comparisons among types detected in patient samples from various geographic locations. The method uses the variability of the internal transcribed spacer (ITS) regions of the rRNA of human *P. carinii* to differentiate types. The ITS primers are specific for and amplify only human *P. carinii*. This typing system has been used to determine where in the environment *P. carinii* nucleic acids can be found. Learning more about where the organisms exist in the environment and about their means of transmission could suggest ways to prevent the exposure of those with impaired immune systems.

MATERIALS AND METHODS

Sample selection. Lung biopsy or bronchoalveolar lavage (BAL) specimens that contained *P. carinii* as determined by microscopic examination were sent from the University of Alabama at Birmingham (UAB) to Indiana University (IU) for *P. carinii* molecular typing. When possible, air from the hospital room and the home of the same patient was sampled. At IU, hospital rooms of patients with proven *P. carinii* pneumonia, hospital rooms of non-*P. carinii* patients, and empty hospital rooms were sampled, as were two clinic rooms where human immunodeficiency virus-infected persons were treated. The homes of the Indiana and Alabama investigators and offices in Indiana were also sampled, as was an outdoor setting in Indiana. Whenever possible, the *P. carinii* molecular type of the patient-derived lung sample was compared to the *P. carinii* molecular type of the associated hospital or room air sample.

Collection of air samples. Calibrated pumps were used to collect air samples through mixed cellulose-ester filters (SKC, Fullerton, Calif.) with a 25-mm diameter and a 0.8-μm pore size. Air sample volumes ranged from 303 to 2,860 liters of air (mean, 1,027); the flow rate was approximately 1 liter per min. Pumps were calibrated before and after sampling, and the total volume collected was determined by time-flow rate (liters per minute) calculations. The volume of air collected at UAB was similar to that collected at IU. Air filters were sealed in clean envelopes or plastic bags for transportation to the molecular biology laboratory at IU for processing.

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Extraction of DNA from filters. Each filter was cut into small pieces with a pair of scissors and placed into a 1.5-ml Eppendorf centrifuge tube containing 300 μl of the cell lysis solution (Puregene DNA isolation kit D-5500; Gentra, Research Triangle Park, N.C.). After the filter was incubated at 37°C overnight, 300 μl of phenol-chloroform-isoamyl alcohol (25:24:1) was added. The mixture was vortexed for 1 min and then centrifuged for 10 min at 10,000 × *g* in an Eppendorf centrifuge. The supernatant was transferred to a new tube and extracted again with 300 μl of chloroform-isoamyl alcohol (24:1). The DNA in the aqueous phase of the extraction was precipitated with ethanol. The DNA pellet was washed with 70% ethanol, air dried, and then dissolved in 20 μl of water.

Amplification of DNA. The purified DNA was used as a template to amplify the region containing the ITSs by nested PCR with primers 1724F and 3454R for the first step, followed by primers ITS1F and ITS2R1 (9). Ten microliters of the DNA solution (containing approximately 200 ng of DNA) was used for PCR with the primer sets. The PCR mixture contained template DNA, PCR buffer a 0.2 μM concentration of each PCR primer, a 0.2 mM concentration of each deoxynucleoside triphosphate, and 2.5 U of *Taq* DNA polymerase in a total volume of 100 μl for amplification. For PCR with the primer set 1724F-3454R, the initial stage was a 10-min denaturation at 94°C; the second stage was 35 cycles of 1 min at 94°C, 1 min at 47°C, and 3 min at 72°C; and the final stage was a 10-min extension at 72°C. When the ITS1F-ITS2R1 primer set was used, the primer annealing was done at 55°C. BAL specimens from patients shown to have *P. carinii* pneumonia by histochemical stains were processed for PCR by incubation with proteinase K buffer (50 mM KCl; 15 mM Tris-HCl [pH 8.3], and 0.5% Nonidet P-40) containing 50 μg of proteinase K per ml for 45 min at 55°C; DNA was then extracted as described for the filters. The PCR conditions were also the same as those described for the filters.

Typing. Typing of *P. carinii* was performed as described by Lu et al. (9). PCR products were denatured with 0.4 N NaOH and then divided into five aliquots. Each aliquot was dotted onto a separate Nytran membrane with a dot blot apparatus. The membranes were dried, baked, prehybridized, and then hybridized separately with ^{32}P-labelled type-specific oligonucleotide probes 1-A, 1-B, 2-a, 2-b, and 2-c. After unhybridized probes were washed off, the membranes were exposed to X-ray film. The autoradiograms were then analyzed. As an example, a sample was considered to contain type Ac *P. carinii* if a PCR product hybridized with probes 1-A and 2-c.

RESULTS

Hospital rooms. A total of 30 air samples (29 from IU and 1 from UAB) were obtained from hospital rooms of patients with *P. carinii* pneumonia. (Diagnoses were made by demonstrating the presence of organisms in Giemsa-stained and methenamine-silver nitrate-stained BAL sediments.) Of the 29 air samples from hospital rooms of *P. carinii* pneumonia patients at IU, 16 were positive for the *P. carinii* ITS PCR and 13 were negative. The PCR results for the filters, the amounts of air sampled, and the types of *P. carinii* on filters and in BAL samples are shown in Table 1. The one filter from the hospital room of a *P. carinii* pneumonia patient at UAB was also positive in the *P. carinii* ITS PCR. The types of *P. carinii* found in patient BAL samples matched those found in room air samples in 10 instances. In two cases (no. 1 and 14) there were different *P. carinii* types in the BAL and air samples. In one case (no. 3) there were two different types (Ac and Bc) in the BAL sample but only one type (Ac) of *P. carinii* in the air sample. The air sample from one patient (no. 7) was not typed because of an insufficient quantity of PCR product. A BAL specimen was not available for PCR and typing from patients 13 and 17.

***P. carinii* patient homes.** Air samples from the homes of nine patients also were examined. Three of the nine patients were diagnosed with *P. carinii* pneumonia by morphological examination of Giemsa-stained BAL samples. The other six patients were suspected of having *P. carinii* pneumonia based on clinical parameters. One air sample from the home of one of the three patients with BAL-proven *P. carinii* pneumonia was positive in the *P. carinii* ITS PCR. Another air sample from the home of one of the six patients suspected of having *P. carinii* pneumonia was also positive in the *P. carinii* ITS PCR. Patient information, locations of air samples, and sample sizes are shown in Table 2. Unfortunately, the amounts of the amplified products from these two samples were not sufficient for typing.

TABLE 1. Air samples from hospital rooms of patients with *P. carinii* pneumonia

Sample[a]	Air filter PCR result	Air vol (liters)	*P. carinii* type(s)	
			On filter	In BAL samples
1	+	899	Ac, Bb	Ba
2	+	1,062	Ba	Ba
3	+	1,182	Ac	Ac, Bc
4	+	1,680	Bb	Bb
5	+	—[b]	Ba	Ba
6	+	1,086	Ba, Bb	Ba, Bc
7	+	915	QNS[c]	Ac, Bc
8	+	489	Ba, Bc	Ba, Bc
9	+	1,003	Bb	Bb
10	+	333	Ba	Ba
11	+	2,860	Ac	Ac
12	+	999	Ba	Ba
13	+	833	Bc	ND[d]
14	+	1,231	Ba	Ac, Bc
15	+	1,481	Ba	Ba
16	+	1,095	Ba	Ba
17	+	1,012	QNS	ND
18	−	1,112		
19	−	1,176		
20	−	1,429		
21	−	1,265		
22	−	1,320		
23	−	1,468		
24	−	1,036		
25	−	929		
26	−	1,157		
27	−	952		
28	−	990		
29	−	1,250		
30	−	1,132		

[a] Sample 17 was from UAB; all other samples were from IU.
[b] —, unknown.
[c] QNS, quantity of PCR product was not sufficient for typing.
[d] ND, typing was not performed because of lack of specimens.

Empty and non-*P. carinii* patient hospital rooms and AIDS clinics. There were seven air samples obtained from the hospital rooms of patients with diseases other than *P. carinii* pneumonia at IU. Of these, two were positive in the *P. carinii* ITS PCR. Four of the 14 samples taken from empty hospital rooms also were positive in the *P. carinii* ITS PCR. One of the two air samples taken from AIDS clinics was positive in the *P. carinii* ITS PCR. These PCR products were not typed because of insufficient quantity.

TABLE 2. Air samples from patient homes[a]

Patient no.	BAL sample diagnosis	Air filter PCR results	Air vol (liters)	Location in home
0565915	+	+	1,474	Bedroom
1076313	ND	+	1,053	Over bed
1380056	ND	−	1,497	Living room
0549636	ND	−	1,513	Bedroom
1359037	ND	−	1,530	Bedroom
0632283	ND	−	998	Bedroom
1224279	ND	−	1,513	Living room
1201202	+	−	1,428	Bedroom
1415671	+	−	2,068	TV room

[a] All samples from UAB.
[b] ND, bronchoscopy was not done; *P. carinii* pneumonia was suspected based on clinical symptoms.

Homes of healthy individuals. There were four air samples from investigators' offices, one from a storage area, six from investigators' homes (four from IU and two from UAB), and one from out of doors. None of these 12 air samples were positive in the *P. carinii* ITS PCR.

DISCUSSION

The detection of *P. carinii* nucleic acid in air samples from the rooms of patients with documented *P. carinii* infections suggests the presence of the organisms in air and the possibility of aerosol spread. Not all infected patients had positive air samples recovered from their rooms; however, the room air circulation patterns relative to the sampling device, the distance from the patient to the air sampling device, the presence of a face mask or oxygen mask on the patient, and other unrecognized circumstances may have contributed to this variability. For example, one IU patient was moved to another room while the air sampler remained behind, so we did not know the volume of air sampled while the patient was in the room. Patients were sometimes taken to the X-ray facility or other locations for extended periods and we did not know how much of the air was sampled with the patient present. That we found positive samples in more than 50% of proven *P. carinii* pneumonia patients' rooms suggests that *P. carinii* organisms are in the air around *P. carinii*-infected patients. Whether these organisms are cysts or trophozoites and whether they are viable or infective cannot be determined by the methods used. That we found no *P. carinii* in any of the offices, storage areas, or investigators' homes also suggests that the organism is not a component of the normal airborne microflora. However, the presence of organisms in air from the rooms of some patients without *P. carinii* pneumonia suggests that it may be more common in hospitals. A previous study (1) has shown that air samples taken from a room where *P. carinii*-infected rats were housed were positive, while air samples from the Laboratory Animal Resource Center offices were negative.

Finding nucleic acids does not establish the presence of whole organisms which are potentially infectious. Therefore, it will be important to determine the viability of *P. carinii* in air samples to show that transmission may occur via the air. This is not yet technically feasible. It is likely that infection is acquired by the respiratory route, but the relative importance of patients and the environment as sources, the factors influencing survival in the environment, and whether there is a yet-undiscovered stage of the organism which does not require a human host are important questions for future investigation. The questions of whether infected patients should be isolated and whether nonhospitalized patients being treated for *P. carinii* pneumonia should avoid contact with susceptible persons need to be answered. The isolation of infected patients is expensive and therefore needs justification. The findings reported here nonetheless have important implications for the risk to susceptible persons from aerosolized *P. carinii* in hospitals, clinics, hospices, and homes.

More air samples are needed from a variety of locations to determine the potential risk to susceptible patients. If the spread of the organism can be associated with certain air samples, such as the air in an orchard as reported by Wakefield (18), it might be possible to decrease exposure by avoiding those areas. If specific sources can be identified in future studies, they could be avoided by those at risk. Preventing exposure could be an important step in diminishing infections. Now that a typing system is available to document specific *P. carinii* types in air samples and in patient BAL or biopsy specimens, it will be possible to perform extensive molecular epidemiologic studies to correlate types found in environmental air with types causing disease.

ACKNOWLEDGMENT

This work was supported in part by National Institutes of Health grant RO1-AI-34304.

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