JOURNAL OF CLINICAL MICROBIOLOGY, June 1998, p. 1737–1740 Vol. 36, No. 6
0095-1137/98/$04.00+0

Identification of *Pneumocystis carinii* f. sp. *hominis* Gene Sequences in Filtered Air in Hospital Environments

MATS OLSSON,[1,2]* CHRISTER LIDMAN,[3] SOPHIE LATOUCHE,[4,5] ANDERS BJÖRKMAN,[6] PATRICIA ROUX,[4] EWERT LINDER,[1] AND MATS WAHLGREN[1]

Swedish Institute for Infectious Disease Control[1] and Microbiology and Tumor Biology Center, Karolinska Institutet,[2] Stockholm, Department of Infectious Diseases, Huddinge University Hospital, Huddinge,[3] and Department of Infectious Diseases, Danderyd University Hospital, Danderyd,[6] Sweden, and Laboratoire de Parasitologie-Mycologie, Centre Hospitalier Universitaire Saint-Antoine,[4] and Laboratoire Commun de Biologie Moleculaire, Hôpital Saint-Antoine,[5] Paris, France

Received 11 December 1997/Returned for modification 31 January 1998/Accepted 19 March 1998

To evaluate the risk of a nosocomial spread of *Pneumocystis carinii* f. sp. *hominis* (*P. carinii hominis*), air filter samples from rooms of *P. carinii* pneumonia (PCP) patients, adjacent corridors, and other hospital environments have been investigated for the presence of *P. carinii hominis*. Amplified DNA from air filters and sputum or bronchoalveolar lavage samples from the PCP patients have been genotyped with the *P. carinii hominis* genes of the mitochondrial large-subunit (mtLSU) rRNA and the internal transcribed spacers (ITS1 and ITS2) of the rRNA. Genotypes of the two loci were identified by direct sequencing, and for site 85 of the mtLSU locus, three allele-specific PCR assays were used. *P. carinii hominis* DNA was identified in the air of five of seven PCP patient rooms and in the air of two of four air filtrations from the ward corridors. The *P. carinii hominis* genotypes were the same in four of the five room air samples as those in the corresponding patients, suggesting a risk of person-to-person transmission of *P. carinii hominis* from PCP patients. Three of 16 air samples collected in infectious disease wards without the presence of PCP patients and one sample from a cardiology unit in a separate hospital building were also positive, which further strengthens the possibility of acquisition of *P. carinii hominis* from the environment.

The reservoir and the transmission route of *Pneumocystis carinii* f. sp. *hominis* (*P. carinii hominis*, human-derived *P. carinii*) remain unclear, despite the recognized existence of the opportunistic pathogen, causing *P. carinii* pneumonia (PCP), in immunocompromised patients since the 1960s. The hypothesis of a reactivated latent infection, based on serological studies (23), has been challenged by experimental data from animal studies. These indicate that PCP is acquired as a new infection (7, 27), probably by airborne transmission of the organism (25, 26, 28). This is supported by the reclassification of *P. carinii* as a primitive fungus (4) and the recent findings of *P. carinii* DNA in indoor and outdoor environments (1, 21, 29). Reported outbreaks of PCP among immunodeficient patients not infected with human immunodeficiency virus (5) further suggest an airborne person-to-person transmission of infection.

To evaluate the risk of nosocomial transmission of *P. carinii hominis* in hospitals, we have applied air filtration equipment, used in animal experiments (19), for identification of *P. carinii hominis* DNA in the air surrounding PCP patients and in other hospital environments. Direct sequence analysis was performed by nested DNA amplification of two *P. carinii hominis* loci: mitochondrial large-subunit rRNA (mtLSU rRNA) (31) and the two internal transcribed spacer (ITS) regions of rRNA (ITS1 and ITS2) (18). These markers have previously been used to study strain variations in *P. carinii hominis* isolates from AIDS patients (16–18) and from different geographical locations (13, 30) as well as from patients with recurrent pneumocystosis (9, 15). To detect amplified copies representing mtLSU RNA types not seen by direct sequencing, allele-specific PCR (AS-PCR) assays have been developed (10). In the present study, we applied these assays to patient and air isolates to study the occurrence of minority populations of *P. carinii hominis*, as a complement to direct sequencing.

* Corresponding author. Mailing address: Swedish Institute for Infectious Disease Control, S-105 21 Stockholm, Sweden. Phone: 46 (8)-735 1300. Fax: 46 (8)-735 1162. E-mail: Mats.Olsson@smi.ki.se.

MATERIALS AND METHODS

Patients. Seven patients, admitted to the infectious disease (ID) departments of Huddinge University Hospital, Huddinge, Sweden, Danderyd University Hospital, Danderyd, Sweden, or Hôpital Tenon, Paris, France, were included in the study. They all had clinical signs of PCP, such as cough and/or dyspnea, and the diagnosis was confirmed microscopically with sputum or bronchoalveolar lavage (BAL) samples after cyto- or immunocytochemical staining. Treatment was provided immediately upon diagnosis. Clinical data for the patients are summarized in Table 1. Five patients had single-bed rooms, whereas patient 1 shared a room with another PCP patient, during the second filtration period (night). This patient was not included in the study because no information about the study was given to the patient. Patient 7 shared a room with a patient without PCP. The patients were free to leave their rooms and visit the corridors and leisure rooms, which were located inside the ID units. No specific considerations were taken in selecting rooms for the PCP patients. The study was approved by the ethical committee of Huddinge University Hospital, and all patients gave their consent after they had been informed about the study.

Filtrations. Air samples were collected from different hospital environments: seven PCP patient rooms (six in the Swedish hospitals and one in the Parisian hospital) and four ward corridors adjacent to the PCP patient rooms (Table 1). The filters in the cassettes and the air pumps were used as described previously (12, 21). For practical reasons, the air filtrations in PCP patient rooms and the corresponding corridors started 12 to 24 h after initiation of antipneumocystis treatment. The filter cassettes in the patient rooms were placed about 1.5 m from the floor, 2 to 3 m from the patient's face; in the corridors they were at the same height, approximately 25 m from the PCP patient rooms. During one filtration period (approximately 18 h), the amount of filtered air was 2.5 m^3 in the Swedish hospitals, whereas about 1 m^3 of air was filtered in the Parisian hospital. Air was also filtered in 16 other hospital environments, including ID wards. No PCP patients were hospitalized in the ID wards during these control filtrations, and the cardiology, hematology, and orthopedic units are located in separate buildings (Table 2). The filters were set out in the corridors, as mentioned previously, and run for longer periods (Table 2).

DNA amplification. DNA from the sputum, BAL, and filter samples was prepared as previously described (13, 20, 21). Nested amplification of the mtLSU rRNA gene portion (29), using the primer pair pAZ102-H and pAZ102-E or

TABLE 1. Clinical data for PCP patients hospitalized in ward rooms where the air was filtrated for identification of *P. carinii hominis* genotypes

Patient	Hospital location	Date of filtration experiment	Sex[a]/age (yr)	Previous PCP episodes	$CD4^+$-cell count/μl	Level of *P. carinii* organisms in clinical samples
1	Huddinge	July 1995	M/38	1994	260	Intermediate
2	Huddinge	August 1995	M/31		110	Many
3	Danderyd	April 1996	M/45		174	Few
4	Huddinge	April 1996	F/49	1991 and 1996	40	Few
5	Huddinge	June 1996	F/41		90	Intermediate
6	Huddinge	September 1996	M/39		50	Intermediate
7	Paris	December 1996	M/43		66	Intermediate

[a] M, male; F, female.

pAZ102-X/R1 and pAZ102-Y/R1, was the main amplification method used. In order to save the limited amounts of DNA extracts from filters, 1 μl of DNA extract was used in the first phase of experiments. All negative air samples were then amplified with 6 μl of DNA extract. Two modifications of the protocol were introduced: a hot-start procedure, by adding the MgCl solution at 80°C before starting the program, and addition of 1 μl (2%) of the template volume from the first PCR to the nested-PCR mixture. The generated 267-bp PCR amplimer was then used for direct sequencing. The ITS PCR was carried out on patient samples 2, 3, 4, 6, and 7.

Due to the limited amounts of air filter DNA extracts, genotyping of the ITS1 and ITS2 gene portions of *P. carinii hominis* was performed only on seven air samples which previously were positive by the mtLSU PCR. The ITS PCR was run according to the method of Lu et al. (18): 5 μl of DNA extract was used in the first PCR with primers 1724F and 3454R, followed by use of the primers ITS1F and ITS2R1 in the second step. Positive controls (*P. carinii hominis* DNA) and negative controls (without DNA) were included in all amplifications. The amplification results were controlled by ethidium bromide staining after agarose gel electrophoresis.

Sequencing. The amplified products of the mtLSU fragment were purified with the QIAquick PCR purification kit (Qiagen, Kebo Lab, Stockholm, Sweden) and the ITS gene fragments were purified with the Wizard PCR Preps DNA purification system kit (Promega, Coger, France). Sequence analysis, from both ends, was performed directly (11) by using the DNA sequencing kit with Dye Terminator AmpliTaq FS (22) (Applied Biosystems, Division of Perkin-Elmer, Warrington, United Kingdom), and sequences were separated on an automated sequence analyzer (model 373A; Applied Biosystems, Division of Perkin-Elmer).

AS-PCR. By using direct sequencing, the dominating fraction of the amplified DNA fragments is detected and sequenced. To detect minor fractions of amplified fragments, representing mtLSU rRNA types 1 to 3 (corresponding to T, A, or C in position 85) (16), not seen by direct sequencing, we used three sensitive AS-PCR assays. Two have been used previously, for detecting types 2 and 3 (AS/A-PCR and AS/C-PCR, respectively) (10), but AS-PCR for type 1 was designed by us. All three specific primers were designed with an additional A at the penultimate 3′ position (3, 10), and the assays were run as three different seminested PCR amplifications, with pAZ102-E as a downstream primer. As a template, we used 1 μl of the PCR product amplified with the outer mtLSU rRNA primers, pAZ102-H and pAZ102-E. To avoid competition effects with these primers, the product was purified with the QIAquick purification kit. The seminested amplifications were run as previously described (10), with one modification: the annealing temperature for AS/A-PCR and AS/T-PCR was 50°C.

Lowest detection level for AS-PCR. The lowest detection levels of the AS-PCR assays were estimated by using single mtLSU type 1, 2, and 3 isolates, amplified with the outer mtLSU primers and purified with the Qiagen kit. After DNA concentration measurement, the dilutions were reamplified as templates with the outer primers. The PCR products were again purified with the Qiagen kit and amplified with the seminested AS/T-PCR, AS/A-PCR, and AS/C-PCR assays.

TABLE 2. *P. carinii hominis* mtLSU genotypes detected in filtrated air from hospital environments without the presence of PCP patients

Hospital location	Department[a]	Filtration period	mtLSU genotype(s) determined by:	
			Sequencing	AS-PCR
Huddinge	ID	72 h		
Huddinge	ID[b]	72 h		
Huddinge	ID[b]	50 h		
Huddinge	ID	48 h	3	
Huddinge	ID	1 wk	1	1
Huddinge	ID	1 wk		
Huddinge	ID	1 wk		
Huddinge	ID	24 h		
Huddinge	ID	24 h		
Danderyd	Orthopedics	24 h		
Danderyd	Orthopedics	24 h		
Danderyd	Cardiology	24 h	3	1, 3
Danderyd	Cardiology	24 h		
Danderyd	Hematology	24 h		
Danderyd	Hematology	24 h		
Huddinge	ID	48 h	2	1, 2

[a] Ward corridor unless otherwise indicated.
[b] Room specifically used for prophylactic inhalations of pentamidine.

RESULTS

Specific *P. carinii hominis* mtLSU DNA was observed in seven air filtrations, which were collected in five of the seven ward rooms harboring PCP patients (Table 3). A positive mtLSU amplification was also observed for six air samples collected in two ward corridors, about 25 m from the patient room (Table 3). mtLSU types 1 to 3, but not type 4 (C-to-T base change at position 248) (16), were detected by direct sequencing in the positive amplifications. No typing was possible with position 288, because this site was not spanned by the primers. Two sequence types, simultaneously present, were seen in patient 3 and on two air filters from one of the rooms.

All three bases were detected at position 85 by the AS-PCR assays, which showed a high sensitivity, enabling detection of a single copy of the diluted model templates. As expected, the AS-PCR assays confirmed the presence of the sequence mtLSU types, but they also detected a minority of the mtLSU types 1, 2, and 3 in patients and on air filters (Table 3). Two or three mtLSU AS genotypes were found in four patients, and two types were also found in 9 of the 13 mtLSU DNA-positive air filter isolates, both from patient rooms and corridors.

In four of the five ITS-amplified patient samples, four ITS1 and four ITS2 genotypes were detected: sample 2, A3 and c1, respectively; sample 4, B2 and a1, respectively; sample 6, B1 and a2, respectively; and sample 7, B6 and b1, respectively. Only two of the seven air samples were positive by ITS PCR and used for subsequent direct sequencing (Table 3). Patient sample 3 was negative by the ITS PCR, and no comparison with genotypes found in room air was possible (Table 3). Only single genotypes were detected by the ITS PCR and subsequent sequence analysis.

For four patients, 2, 3, 4, and 6, the *P. carinii hominis* mtLSU genotypes matched those of samples from the air filters, for both the dominating mtLSU identified by sequencing and the mtLSU minority population identified by AS-PCR (Table 3).

TABLE 3. *P. carinii hominis* mtLSU rRNA genotypes detected in BAL or sputum samples of hospitalized patients, in air filtrated in the patients' rooms, or in simultaneously filtrated air of the adjacent ward corridors

Patient and filtration period[a]	mtLSU rRNA genotype(s) determined by indicated method in:					
	Patient samples		Room air		Corridor air	
	Sequencing	AS-PCR	Sequencing	AS-PCR	Sequencing	AS-PCR
1	3	3				
I					ND[b]	ND
2	3	1, 3				
I			1, 3	1, 3	ND	ND
II			1, 3	1, 3	ND	ND
3	1, 2	1, 2				
I					2	1, 2
II[c]			2	1, 2	1	1
III					1	1
IV			ND	ND		
4	3	1, 2, 3				
I			3[d]	3[d]	3	3
II			3	1, 3	3	2, 3
III			ND	ND	3	1, 3
5	1	1, 3				
I						
6	2	2				
I						
II						
III			3	2, 3		
7[e]	2	2				
I			3	1, 3	ND	ND

[a] I is the first night of filtration, II is the second night, III is the third night, and IV is the fourth night.
[b] ND, not done.
[c] *P. carinii hominis* ITS genotypes in the room air, B6 and a2.
[d] Only 2 h of filtration was done because of technical failure.
[e] *P. carinii hominis* ITS genotypes in the patient were B6 and b1; in the room air, they were A1 and c1.

In addition, the types detected in the two corridor filtrations matched the types found in the respective patients. The nonmatching pattern of mtLSU types, as detected by sequencing and AS-PCR, in patient 7 and in the air of the room occupied by the patient was confirmed by the distinct ITS types in the patient samples and in the room air sample (Table 3).

mtLSU genotypes were detected by direct sequencing and AS-PCR in 4 of the 16 filtrations in hospital locations without the presence of PCP patients (Table 2).

DISCUSSION

By using *P. carinii hominis* mtLSU rRNA and the ITSs, ITS1 and ITS2, we have detected and identified *P. carinii hominis* genotypes in filtered air samples collected in hospital locations with or without the presence of PCP patients. The dominating mtLSU and ITS types were detected by direct sequencing, while the minority of mtLSU types, involving position 85, not found by sequencing analysis were detected by AS-PCR. A concordance in genotypes was found between four patient isolates and air from the patients' rooms and, in two cases, air from ward corridors. This suggests a spread of the infection to the surrounding environment. We cannot exclude, however, the possibility that the genotypes found in the corridor air represent environmental strains of *P. carinii hominis*, as we also observed the presence of *P. carinii hominis* in hospital locations without the known presence of PCP patients, e.g., the cardiology ward (Table 2). The nonmatching result for the air filtration experiment in the room of patient 7 (Table 3) could therefore represent an environmental spread of *P. carinii hominis*. Such an environmental spread of *P. carinii hominis* was suggested for PCP patients living together in pairs and who were infected by genetically distinct strains of *P. carinii hominis* (14).

Our results confirm previous animal experiments showing *P. carinii* DNA in the air surrounding infected rats (21, 29) and by the matching in genotypes between rodents and the air (12). In addition, our results also support a recent hospital study suggesting a risk of nosocomial transmission of *P. carinii hominis* in a hospital environment (2). The authors of that study did not, however, investigate air samples from the corridors adjacent to the PCP patient rooms. An air filtration system similar to that used in the present study was applied to document the aerosolization of varicella-zoster virus (VZV) in hospital environments. VZV DNA was successfully detected in air samples obtained from hospital rooms housing patients with active VZV infection and from hospital corridors (24).

By using the AS-PCR assays, we were able to detect more than one mtLSU genotype in most of the patient and air samples. Assuming that *P. carinii hominis* has a haploid genome (6), this indicates the simultaneous presence of distinct strains of *P. carinii hominis*. This has also previously been described for about 30% of patient samples in another study (19), but to our knowledge, the presence of multiple strains of *P. carinii hominis* in indoor air has not been reported, although mtLSU polymorphisms in three species-specific forms of *P. carinii* were detected in ambient air (29).

Although the discriminatory potential of the *P. carinii hominis* mtLSU locus is limited, with only four types identified (13, 17, 30), it has been used to demonstrate genotype switching in recurrent PCP (8, 15). The genotypes of the mtLSU locus were also demonstrated in samples from distant geographical regions and in samples obtained over a 4-year period (30). They did not appear randomly, nor were they related to technique or to whether the patients received prophylaxis or not (13). Moreover, the mtLSU amplification assay was more sensitive for detection of *P. carinii hominis* DNA on filter samples than the ITS PCR. Even so, the negative ITS amplification result for the sputum sample from patient 3 is puzzling, since the sputum contained, although in a low number, cysts and trophozoites after immunofluorescence staining.

In conclusion, by using DNA sequence analysis and AS-PCR assays, concordant *P. carinii hominis* genotypes have been found in PCP patients and in the air of their rooms and adjacent corridors, which supports the hypothesis of airborne transmission of *P. carinii hominis*. A risk of a nosocomial spread of *P. carinii hominis* can therefore not yet be excluded, which implies that prophylaxis for susceptible patients with a high risk of PCP is necessary, and until conclusive evidence of the transmission of *P. carinii hominis* is obtained, contacts between PCP patients and immunosuppressed patients at risk for PCP should be avoided. However, many questions remain unanswered regarding the occurrence and transmission of *P. carinii hominis*; the infectivity of air-trapped *P. carinii hominis* DNA is unknown, and it appears to be very important to establish the viability of the detected *P. carinii hominis* air forms and to demonstrate that they are infectious. Extensive molecular epidemiological studies are also needed to elucidate the natural reservoir of *P. carinii* organisms.

ACKNOWLEDGMENTS

This work was supported in part by Martin Rinds Fund, Stockholm, Sweden, and done in collaboration within the European Concerted Action (ECA) BioMed-1 (*Pneumocystis* and pneumocystosis).

We thank Sven Löfdahl and Philippe Hauser for valuable advice and Elsa Tynell and Britt-Marie Johansson for assistance in air filtration.

REFERENCES

1. **Bartlett, M., C. Lee, J.-J. Lu, N. Bauer, J. Bettz, G. McLaughlin, et al.** 1994. *Pneumocystis carinii* detected in air. J. Eukaryot. Microbiol. **41:**75S.
2. **Bartlett, M. S., S. H. Vermund, R. Jacobs, P. J. Durant, M. M. Shaw, J. W. Smith, X. Tang, J.-J. Lu, B. Li, S. Jin, and C.-H. Lee.** 1997. Detection of *Pneumocystis carinii* DNA in air samples: likely environmental risk to susceptible persons. J. Clin. Microbiol. **35:**2511–2513.
3. **Bottema, C. D. K., and S. S. Sommer.** 1993. PCR amplification of specific alleles: rapid detection of known mutations and polymorphisms. Mutat. Res. **288:**93–102.
4. **Eriksson, O.** 1994. *Pneumocystis carinii*, a parasite in lungs of mammals, referred to a new family and order (*Pneumocystidaceae*, *Pneumocystidales*, *Ascomycota*). Syst. Ascomycetum **13:**165–180.
5. **Hennequin, C., B. Page, P. Roux, C. Legendre, and H. Kreis.** 1995. Outbreak of *Pneumocystis carinii* pneumonia in a renal transplant unit. Eur. J. Clin. Microbiol. Infect. Dis. **14:**122–126.
6. **Hong, S. T., P.-E. Steele, M. T. Cushion, and P. D. Walzer.** 1990. *Pneumocystis carinii* karyotypes. J. Clin. Microbiol. **28:**1785–1795.
7. **Hughes, W. T.** 1982. Natural mode of acquisition for *de novo* infection with *Pneumocystis carinii*. J. Infect. Dis. **145:**842–848.
8. **Keely, S. P., and J. R. Stringer.** 1997. Sequences of *Pneumocystis carinii* f. sp. *hominis* strains associated with recurrent pneumonia vary at multiple loci. J. Clin. Microbiol. **35:**2745–2747.
9. **Keely, S. P., J. R. Stringer, R. P. Baughman, M. J. Linke, P. D. Walzer, and A. G. Smulian.** 1995. Genetic variation among *Pneumocystis carinii hominis* isolates in recurrent pneumocystosis. J. Infect. Dis. **172:**595–598.
10. **Keely, S. C., R. P. Baughman, A. G. Smulian, M. N. Dihn, and J. R. Stringer.** 1996. Source of *Pneumocystis carinii* in recurrent episodes of pneumonia in AIDS patients. AIDS **10:**881–888.
11. **Kwok, P. Y., C. Carlson, T. D. Yager, W. Ankener, and D. A. Nickerson.** 1994. Comparative analysis of human DNA variations by fluorescence-based sequencing of PCR products. Genomics **23:**138–144.
12. **Latouche, S., M. Olsson, B. Polack, M. Brun-Pascaud, C. Bernard, and P. Roux.** 1997. Detection of *Pneumocystis carinii* f. sp. in air samples collected in animal rooms. J. Eukaryot. Microbiol. **44:**46S–47S.
13. **Latouche, S., E. Ortona, E. Mazars, P. Margutti, E. Tamburrini, A. Siracusano, K. Guyot, M. Nigou, and P. Roux.** 1997. Biodiversity of *Pneumocystis carinii hominis*: typing with different DNA regions. J. Clin. Microbiol. **35:**383–387.
14. **Latouche, S., J. L. Poirot, E. Maury, V. Bertrand, and P. Roux.** 1997. *Pneumocystis carinii hominis* sequencing to study hypothetical person-to-person transmission. AIDS **11:**549.
15. **Latouche, S., J.-L. Poirot, C. Bernard, and P. Roux.** 1997. Study of internal transcribed spacer and mitochondrial large-subunit genes of *Pneumocystis carinii hominis* isolated by repeated bronchoalveolar lavage from human immunodeficiency virus-infected patients during one or several episodes of pneumonia. J. Clin. Microbiol. **35:**1687–1690.
16. **Latouche, S., P. Roux, J. L. Poirot, I. Lavrard, B. Hermelin, and V. Bertrand.** 1994. Preliminary results of *Pneumocystis carinii* strain differentiation by using molecular biology. J. Clin. Microbiol. **32:**3052–3053.
17. **Lee, C.-H., J.-J. Lu, M. S. Bartlett, M. M. Durkin, T.-H. Liu, J. Wang, B. Jiang, and J. W. Smith.** 1993. Nucleotide sequence variation in *Pneumocystis carinii* strains that infect humans. J. Clin. Microbiol. **31:**754–757.
18. **Lu, J.-J., M. S. Bartlett, M. M. Shaw, S. F. Queener, J. W. Smith, M. Ortiz-Rivera, M. J. Leibowitz, and C.-H. Lee.** 1994. Typing of *Pneumocystis carinii* strains that infect humans based on nucleotide sequence variations of internal transcribed spacers of rRNA genes. J. Clin. Microbiol. **32:**2904–2912.
19. **Lu, J.-J., M. S. Bartlett, J. W. Smith, and C.-H. Lee.** 1995. Typing of *Pneumocystis carinii* strains with type-specific oligonucleotide probes derived from nucleotide sequences of internal transcribed spacers of rRNA genes. J. Clin. Microbiol. **33:**2973–2977.
20. **Olsson, M., K. Elvin, C. Lidman, S. Löfdahl, and E. Linder.** 1996. A rapid and simple nested PCR assay for the detection of *Pneumocystis carinii* in sputum samples. Scand. J. Infect. Dis. **28:**597–600.
21. **Olsson, M., A. Sukura, L. A. Lindberg, and E. Linder.** 1996. Detection of *Pneumocystis carinii* DNA in filtration of air. Scand. J. Infect. Dis. **28:**279–282.
22. **Parker, L., H. Zakeri, Q. Deng, S. Spurgeon, P.-Y. Kwok, and D. A. Nickerson.** 1996. AmpliTaq™ DNA polymerase, FS Dye-Terminator sequencing: analysis of peak height patterns. BioTechniques **21:**694–699.
23. **Pifer, L. L., W. T. Hughes, S. Stagno, and D. Woods.** 1978. *Pneumocystis carinii* infection: evidence for high prevalence in normal and immunosuppressed children. Pediatrics **61:**35–41.
24. **Sawyer, M., C. Chamberlain, Y. Wu, N. Aintablian, and M. Wallace.** 1994. Detection of varicella-zoster virus DNA in air samples from hospital rooms. J. Infect. Dis. **169:**91–94.
25. **Sepkowitz, K., N. Schluger, T. Godwin, D. Armstrong, A. Cerami, and R. Bucala.** 1993. DNA amplification in experimental pneumocystosis: characterization of serum *Pneumocystis carinii* DNA and potential *P. carinii* carrier states. J. Infect. Dis. **168:**421–426.
26. **Sukura, A., L. Lindberg, T. Soveri, O. Guerrero, M. Chinchilla, K. Elvin, and E. Linder.** 1991. Establishment of *Pneumocystis carinii* infection in a rat population. Acta Vet. Scand. **32:**135–137.
27. **Vargas, S. L., W. T. Hughes, A. E. Wakefield, and H. E. Oz.** 1995. Limited persistence in and subsequent elimination of *Pneumocystis carinii* from the lungs after *P. carinii* pneumonia. J. Infect. Dis. **172:**506–510.
28. **Vogel, P., C. J. Miller, L. L. Lowenstine, and A. A. Lackner.** 1993. Evidence for horizontal transmission of *Pneumocystis carinii* pneumonia on simian immunodeficiency virus-infected rhesus macaques. J. Infect. Dis. **168:**836–843.
29. **Wakefield, A. E.** 1996. DNA sequences identical to *Pneumocystis carinii* f. sp. *carinii* and *Pneumocystis carinii* f. sp. *hominis* in samples of air spora. J. Clin. Microbiol. **34:**1754–1759.
30. **Wakefield, A. E., C. C. Fritscher, A. S. Malin, L. Gwanzura, W. T. Hughes, and R. F. Miller.** 1994. Genetic diversity in human-derived *Pneumocystis carinii* isolates from four geographical locations shown by analysis of mitochondrial rRNA gene sequences. J. Clin. Microbiol. **32:**2959–2961.
31. **Wakefield, A. E., F. Pixley, S. Banerji, K. Sinclair, R. F. Miller, E. R. Moxon, and J. M. Hopkin.** 1990. Amplification of mitochondrial ribosomal RNA sequences from *Pneumocystis carinii* DNA in rat and human origin. Mol. Biochem. Parasitol. **43:**69–76.