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Detection of *Pneumocystis carinii* Sequences by Polymerase Chain Reaction: Animal Models and Clinical Application to Noninvasive Specimens

KAZUHIRO KITADA,[1] SHINICHI OKA,[2] SATOSHI KIMURA,[2] KAORU SHIMADA,[2] TADAO SERIKAWA,[3] JUNZO YAMADA,[3] HAJIME TSUNOO,[4] KOHJI EGAWA,[1] AND YOSHIKAZU NAKAMURA[1]*

Departments of Tumor Biology[1] and Infectious Diseases,[2] The Institute of Medical Science, The University of Tokyo, P.O. Takanawa, Tokyo 108, Institute of Laboratory Animals, Faculty of Medicine, Kyoto University, Kyoto 606,[3] and Meiji Institute of Health Science, Odawara 250,[4] Japan

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***Pneumocystis carinii* is a eukaryotic microbe which causes fatal pneumonia in patients with AIDS. Oligonucleotide primers were used to amplify the 5S rDNA sequence of *P. carinii* by the polymerase chain reaction (PCR) in various clinical and animal samples. Of 35 independent lung specimens tested, PCR detected the *P. carinii* sequence in all 23 cases which were known to be *P. carinii* infected, i.e., 15 from mice, 1 from rat, 3 from human autopsy, and 4 from biopsy of AIDS patients by needle aspiration. The results were consistent with clinical and microscopic diagnosis. The detection was highly sensitive and specific. Direct sequencing of these amplified DNAs revealed homogeneity of 5S rDNA sequences of independent isolates from mice, rats, and humans. Preliminary trials manifested efficacy of the PCR method to detect *P. carinii* sequences in induced sputum or blood from AIDS patients, the latter case suggesting that *P. carinii* might enter peripheral blood via phagocytosis or direct intrusion. Development of less-invasive or noninvasive PCR diagnostic techniques to detect *P. carinii* infection would greatly facilitate therapeutic and prophylactic management of *P. carinii* pneumonia.**

Pneumocystis carinii is an opportunistic pathogen which often causes fatal pneumonia in patients under immunosuppressed or immune deficient conditions due to AIDS, cancer chemotherapy, or immunosuppressive therapy for organ transplantation. More than 60% of AIDS patients suffer from *P. carinii* pneumonia at some time in the course of the disease (10). It is estimated that ~52,000 AIDS patients in the United States have developed *P. carinii* pneumonia during 1981 to 1988 (14). The current statistics predict occurrence of ~150,000 cases of *P. carinii* pneumonia among AIDS patients over the next 3 years in the United States.

P. carinii is a eukaryotic microorganism that can infect many mammalian hosts. It has two forms in the life cycle: one is a vegetative form, i.e., a small unicellular trophozoite, and the other is a resting form, i.e., a cyst containing eight intracystic bodies called sporozoites (22). Continuous in vitro culture of *P. carinii* has not been established, and diagnostic identification of *P. carinii* is based on microscopic detection of the organism in pulmonary materials after Grocott, toluidine blue O, and Giemsa staining or staining with monoclonal antibodies raised against *P. carinii* (17). However, these methods are not always successful in detecting the organism because of the inherent lack of specificity in cytochemical stains and the uncertainty of the sensitivity of immunochemical stains of clinical specimens and the antigenicity of any given isolate. In fact, previous studies have shown that *P. carinii* isolates from rats and humans have species-specific, as well as shared, antigenic determinants (3, 18). Therefore, potential variability in the antigenic structure cannot be excluded in clinical isolates of *P. carinii*, which may limit the use of monoclonal antibodies for diagnosis. Hence, it is urgent to establish a highly sensitive, highly specific, and widely applicable method to detect *P. carinii* infection to cope with increasing cases of *P. carinii* pneumonia.

The polymerase chain reaction (PCR) technique is a method for amplifying nucleic acids by repeated cycles of temperature changes to promote template denaturation, oligonucleotide primer hybridization, and polymerase-mediated extension (8). Because of $>10^5$-fold amplification over the input quantity of nucleic acid, the PCR is well suited for identifying low-copy-number genetic sequences, particularly infectious viruses, or microbes at the early phase of infection. We have isolated the cytoplasmic 5S rRNA of *P. carinii* from rats and determined the RNA sequence (19). Up to now, the 5S rRNA sequences of ~700 organisms have been investigated (12, 20). This has enabled extensive evolutionary study of the organisms on the basis of the 5S rRNA sequence; thus, the phylogenic association of *P. carinii* with the protista fungi group has been suggested (19). A similar conclusion has also been obtained by the sequence analysis of the 16S rRNA gene from *P. carinii* (1). The large collection of sequence data of 5S rRNAs is also useful for choosing oligonucleotide primers of high specificity for the PCR assay. Thus, the 5′- and 3′-terminal sequences of 5S rRNA were chosen as a set of PCR primers in this study because of the inherent heterogeneity in these sequences between *P. carinii* and other related organisms.

The clinical samples for diagnosis of *P. carinii* infection by microscopic analysis are mostly from samples of open lung biopsy, bronchoscopic alveolar lavage, and in some cases patients' sputa. However, lung biopsy and bronchoscopy are not easily handled, especially in AIDS cases, and induced sputa are not always reliable for visual detection by staining. In this article, we utilized PCR to diagnose *P. carinii* infection from noninvasive clinical specimens, including

* Corresponding author.

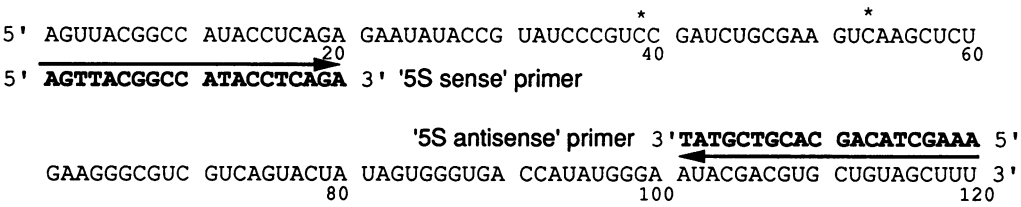

FIG. 1. 5S rRNA sequence of *P. carinii* and oligonucleotide primers used in the PCR. Asterisks at positions 39 and 52 denote revised nucleotides.

blood. All of the AIDS patients available in The Institute of Medical Science Hospital were examined. Model animal experiments ensured the accuracy of the PCR detection of the *P. carinii* sequence.

MATERIALS AND METHODS

Animals and parasites. The *P. carinii* derived from mice used in this study was isolated in the Animal Facility of Kyoto University (11). Specific-pathogen-free (SPF) nude mice (BALB/c *nu/nu*) were purchased from Japan SLC Co., Ltd. (Shizuoka, Japan). *P. carinii*-infected nude mice were kept in isolators to exclude infection with other pathogens. SPF nude mice were added to these isolators every month to maintain a *P. carinii*-infected mouse colony by airborne transmission. To obtain independent isolates of *P. carinii*, SPF mice were distributed to 43 animal facilities in Japan, maintained for 6 months in these facilities, and examined for *P. carinii* infection by the morphological test (11). Of 43 groups tested, 12 were infected with *P. carinii*; the infection in these mice was established by microscopic examinations after Grocott or toluidine blue O staining. Mice from these 12 groups were used independently in the present experiments. The *P. carinii* from rats used in this study has been described previously (13). The SPF athymic (*rnu/rnu*) nude rats (Rowett hooded; purchased from Japan CREA Co., Ltd., Kawasaki, Japan) were inoculated intranasally with a suspension of passaged lung homogenate of the *P. carinii*-infected rat and were treated with cortisone acetate and tetracycline until the onset of pneumonia (13).

Human autopsy specimens. Lung specimens were obtained from three patients who died from *P. carinii* pneumonia and stored at −80°C until used. *P. carinii* cysts were detected in these lung specimens by staining with toluidine blue O. Three normal control lung specimens contained no detectable *P. carinii* by microscopic examinations.

Human biopsy specimens. Fine-needle aspiration biopsy of the lung was performed in 10 AIDS patients with pneumonia, as described previously (4). Coagulation factors were administered to hemophiliac patients prior to the biopsy. These aspirates were suspended in physiological saline and kept frozen at −80°C until used. Parts of the aspirates were subjected to microscopic detection for *P. carinii* after staining with periodic acid methenamine silver; four specimens were positive and six were negative. The same samples were also used in the PCR assay in a blind experiment.

Patients' blood samples. Three blood samples were obtained from two AIDS patients (patients 1 and 2); two samples were obtained from patient 1 before (19 March 1990) and after (2 April 1990) the onset of pneumonia as determined by a clinical diagnosis of *P. carinii* pneumonia. Patient 2 was diagnosed as having *P. carinii* pneumonia by microscopic examination and PCR by using lung aspirates on 5 March 1990, and then pentamidine was administered. The blood sample of patient 2 was taken on 19 March 1990 after marked recovery from the pneumonia. The blood was heparinized and frozen at −80°C until used. The clinical data of these two patients were kept in confidence from the PCR experiment.

DNA preparation. Lung homogenates or aspirates were incubated at 60°C for 6 h in 0.5% sodium dodecyl sulfate and 200 mg of proteinase K per ml. DNAs were extracted three times with phenol-chloroform, precipitated with ethanol, and dissolved in TE buffer (10 mM Tris-HCl [pH 7.5] and 1 mM EDTA). Heparinized blood (1 ml) was centrifuged, and the precipitates were washed three times with lysis buffer containing 2% Triton X-100 to lyse cells. The precipitate was incubated at 60°C for 6 h in a solution (50 μl) containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.5 mg of gelatin, 0.25% Nonidet P-40, 0.25% Tween-20, and 10 mg of proteinase K. The samples were boiled for 10 min and used directly as PCR templates without phenol extraction or ethanol precipitation. Induced sputum (~2 ml) was mixed with 2 ml of sterile water containing 66 mM dithiothreitol by vigorous shaking and incubated at 37°C for 10 min for mucolysis as described previously (15). The suspension was vortexed again vigorously, washed three times with phosphate-buffered saline, and treated with Triton X-100, Nonidet P-40, Tween-20 and proteinase K as described above.

DNA amplification and gel electrophoresis. The 5S rDNA sequence of *P. carinii* was amplified by the PCR method (8) in a reaction mixture (100 μl) containing 10 mM Tris-HCl (pH 8.3); 50 mM KCl; 1.5 mM $MgCl_2$; 1 mg of gelatin; 200 mM (each) dATP, dTTP, dCTP and dGTP; 50 pmol of oligonucleotide primers; 2.5 U of Amplitaq DNA polymerase (Perkin Elmer Cetus, Norwalk, Conn.); and 10 μl of DNA solution (containing ~1 μg of DNA in the case of lung autopsy samples and 0.1 to 0.01 μg of DNA in the other cases). The primers were 5S sense (5'-AGTTACGGCCAT ACCTCAGA-3') and 5S antisense (5'-AAAGCTACAGC ACGTCGTAT-3'), generating a 120-bp product by PCR (Fig. 1). Denaturation was at 94°C for 1.5 min with annealing at 55°C for 2.5 min and extension at 72°C for 1.9 min; amplification was done by 25 or 40 cycles. As a precaution to prevent template contamination, preparation of DNA samples, preparation of reaction solutions, and PCR amplification were performed in the different rooms using the safety cabinet or a clean bench and Microman pipetters (Gilson Medical Electronics, Villiers-le-Bel, France). The amplified products were subjected to electrophoresis in 12% polyacrylamide or 2% agarose gels and stained with ethidium bromide.

DNA sequencing. DNAs amplified by PCR were isolated from agarose gels after electrophoresis, extracted with phenol-chloroform, and precipitated with ethanol. The resulting double-stranded DNAs were directly sequenced by the dideoxy chain-termination method (9) with [α-^{32}P]dCTP and the 5S sense and 5S antisense primers as used in the PCR.

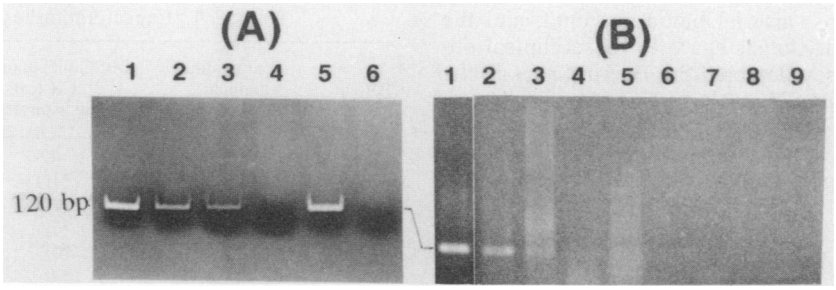

FIG. 2. PCR amplification of the 5S rDNA sequence of *P. carinii* in animal specimens. Experimental procedures and conditions of DNA preparation and PCR are as described in Materials and Methods. The amplified product was subjected to electrophoresis in a 12% polyacrylamide gel (A) or 2% agarose gel (B) and detected by ethidium bromide staining. (A) Lung specimens. Lane 1, *P. carinii*-infected mouse autopsy; lane 2, paraffin section of *P. carinii*-infected mouse lung; lane 3, thymic mouse autopsy; lane 4, SPF mouse autopsy; lane 5, *P. carinii*-infected rat autopsy; lane 6, SPF rat autopsy. (B) Blood samples. Blood (~0.5 ml) bled from supraorbital vein of *P. carinii*-infected mice was divided into two portions. One was used to prepare total blood lysates (lanes 1 through 5) and the other half was used to prepare serum fractions (lanes 6 through 9). Lanes 2 and 6, 3 and 7, 4 and 8, and 5 and 9 were from the same mice, respectively.

RESULTS

Model animal experiments. The DNA from lung homogenates of *P. carinii*-infected rats yielded a 120-bp product by PCR, whereas that of uninfected rats generated no positive signal (Fig. 2A, lanes 5 and 6, respectively). The 120-bp product encoded the entire 5S rRNA sequence as deduced below.

The mouse *P. carinii* is not identical to the rat *P. carinii* with respect to immunodeterminant structures, and cross-infection is restricted to some extent (2, 5). However, the above-described primers amplified effectively the 120-bp product by PCR in lung homogenates from *P. carinii*-infected mice (Fig. 2A, lane 1). PCR efficiently amplified the 5S rDNA sequence in lung specimens from mice which were independently infected with *P. carinii* in 12 different animal facilities (data not shown). On the other hand, the samples from control lungs of SPF mice failed to produce an amplified product (Fig. 2A, lane 4).

To develop methods that were more widely applicable for diagnosis of *P. carinii* infection by using the animal model, blood samples from mice severely infected with *P. carinii* were examined by PCR. When whole-blood DNAs were tested, three of five blood samples were positive and two were negative (Fig. 2B, lanes 1 to 5). Positive signals were not obtained when sera from the same five blood samples were used in PCR (Fig. 2B, lanes 6 to 10). These results were interpreted as indicating that we detected the *P. carinii* organism or, more specifically, its DNA after it had entered the peripheral blood by phagocytosis or by direct intrusion.

PCR assay of clinical specimens from humans. Six human lung autopsy samples were tested for PCR amplification. Three were from patients diagnosed with *P. carinii* pneumonia and the others were from patients not having *P. carinii* pneumonia. The PCR amplified the 120-bp product in the former three samples, whereas it did not in the latter three samples (Fig. 3A). The amplified 120-bp product encoded the 5S rRNA sequence as described below.

Next, lung biopsy specimens obtained by fine-needle aspiration from four AIDS patients were examined by DNA amplification in a blinded fashion. As shown in Fig. 3B, two were positive in PCR detection (lanes 5 and 6), whereas the other two were negative (lanes 3 and 4). These PCR results were consistent with the clinical findings, i.e., patients in lanes 3 and 4 suffered from severe pneumonia but the *P. carinii* organism was not found in lung aspirates by Giemsa stain, whereas patients in lanes 5 and 6 had pneumonia, and *P. carinii* organisms were detected in these biopsy samples by microscopic examinations. The former two *P. carinii*-negative patients did not have clinical signs of *P. carinii* pneumonia during an 8-month follow up; one of them was diagnosed with miliary tuberculosis and the other was diagnosed with cytomegalovirus and *Aspergillus* infection.

Detection of *P. carinii* 5S rDNA sequences in blood from patients with *P. carinii* pneumonia was then attempted. Three blood samples were obtained from two AIDS patients, one from patient 2 after administration of pentamidine and two from patient 1 before (19 March 1990) and after (2 April 1990) the onset of *P. carinii* pneumonia, which was inter-

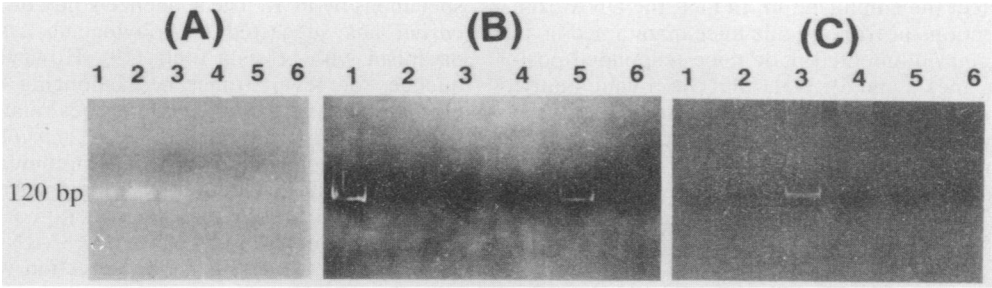

FIG. 3. PCR tests of clinical specimens. Experimental procedures and conditions are as described in Materials and Methods. (A) Lung autopsy. Lanes 1 through 3, patients diagnosed with *P. carinii* pneumonia; lanes 4 through 6, patients unrelated to *P. carinii* pneumonia. (B) Lung biopsy. Lane 1, positive control (*P. carinii*-infected mouse lung); lane 2, negative control (water); lanes 3 through 6, needle aspirates from individual AIDS patients. (C) Blood samples. Blood (0.5 ml) from individuals was used in the reaction. Lane 1, patient 2; lane 2, patient 1 on 19 March 1990; lane 3, the patient 1 on 2 April 1990; lanes 4 through 6, three individual healthy human controls.

preted by the symptoms, arterial blood gas data, and the chest X-ray photograph. Consistent with these clinical observations, the PCR assay detected the *P. carinii* 5S rDNA sequence in the blood sample of 2 April but not of 19 March (Fig. 3C, lanes 2 and 3). Pentamidine administration, 4 mg/kg of body weight per day, was started on 5 April, and the symptoms markedly improved in a week. The other samples, including three control blood samples, were negative (Fig. 3C, lanes 1 and 4 to 6).

PCR assay of induced sputa. Sputa obtained from three AIDS patients (patients 3, 4, and 5) one immunosuppressed organ transplant patient (patient 6) and one control human (patient 7) were subjected to the PCR assay (Table 1). Patient 3 was diagnosed with *P. carinii* pneumonia by microscopic examination of the lung aspirates, and pentamidine administration was started. Sputum, after 3 days of administration of pentamidine, amplified the 5S rDNA sequence by PCR; however, sputa obtained later than 8 days after administration of pentamidine were negative. The sputum from patient 6 also yielded a positive signal which later became negative upon pentamidine administration. The Diff-Quik staining method detected *P. carinii* only in one of six sputum samples from patients 3 and 6 which were all positive in the PCR detection. Sputa from the other two patients and the control sputa were negative in the PCR amplification. Patient 4 suffered from pneumonia, but the biopsy lung sample was negative in the Diff-Quik stain and in the PCR assay, whereas patient 5 did not present any clinical sign of pneumonia even though located in the same room as patient 3. These observations revealed consistency of the PCR results from sputum with other clinical information on the patients.

Specificity and sensitivity of PCR detection. The PCR primers used in this study did not amplify 5S rDNA sequences from other rat or mouse pulmonary pathogens, e.g., *Corynebacterium kutshceri*, *Pasteurella pneumotropica*, *Pasteurella multocida*, and *Bordetella bronchiseptica*, nor from other human fungal pathogens involved in pulmonary diseases, *Candida albicans*, *Candida glabrata*, *Aspergillus fumigatus*, *Cryptococcus neoformans*, and *Mycobacterium tuberculosis* (data not shown).

The sensitivity was tested quantitatively by diluting the infected mouse lung homogenates containing ~10^6 *P. carinii* including cysts and trophozoites. The DNA sample diluted up to a few organisms per PCR solution efficiently amplified the 5S rDNA sequence, demonstrating a high sensitivity of PCR detection of *P. carinii* under these conditions (data not shown). We assumed this high sensitivity is due to multiple copies of the 5S rRNA gene in the *P. carinii* genome, as described below. Pretreatment of specimens with ribonuclease A did not affect the amplification. In fact, the DNA from small paraffin sections or from thymic mice maintained in an isolator with *P. carinii*-infected nude mice responded positively (Fig. 2A, lanes 2 and 3). Note that the thymic mouse tested had no clinical sign of *P. carinii* pneumonia though the serum antibody titer against *P. carinii* increased to a 100-fold level in the immunofluorescence assay. Thus, the PCR method was sensitive enough to detect the latent infection. Furthermore, PCR was able to detect *P. carinii* (in sputum samples) which were not detectable by the Diff-Quik staining method (Table 1).

Sequence of the amplified 5S rDNA. The amplified 120-bp DNAs from mice, rats, and clinical specimens were sequenced directly by using the same oligonucleotide primers used in PCR. Twenty nucleotides at both 3′ and 5′ termini of these PCR products were directed from the primer sequence itself, and thereby the internal 80-nucleotide sequence was synthesized de novo, depending on *P. carinii* organisms contained in each specimen. The deduced sequences were all identical, suggesting that the *P. carinii* 5S rDNA sequence was conserved beyond the difference in the host range specificity (Fig. 1) (data not shown). Two sequence errors in the published 5S rRNA sequence were found and revised: C for U at positions 39 and 53 (Fig. 1).

TABLE 1. Expectoration tests of *P. carinii*[a]

Patient	Pentamidine administration[b] (days)	Diff-Quik staining[c] and PCR tests of lung aspirates	Mucolysed sputa	
			Diff-Quik staining[c]	PCR tests
3	− (0)	+	−	ND
	+ (3)	ND	−	+
	+ (8)	ND	−	−
	+ (14)	ND	−	−
	+ (20)	ND	−	−
6	− (−3)	+	+	+
	− (0)	ND	−	+
	+ (3)	ND	−	+
	+ (5)	ND	−	+
	+ (8)	ND	−	+
	+ (11)	ND	−	−
	+ (13)	ND	−	−
	+ (17)	ND	−	−
4	−	−	−	−
5	−	ND	−	−
7	−	ND	−	−

[a] Induced sputa were collected from three AIDS patients (patients 3, 4, and 5), one immunosuppressed organ transplant patient (patient 6), and one normal human (patient 7) according to the procedure described by Valerie et al. (15). Patients 3, 4, and 6 suffered from pneumonia. Patients 3 and 5 were in the same room. These sputa were treated with mucolysis, and template DNAs were prepared as described in Materials and Methods. Experimental procedures and conditions of PCR amplification (40 cycles) of 5S rDNA sequence are as described in Materials and Methods. ND, not done.

[b] Pentamidine was administered daily to the patients 3 and 6 at a dose of 4 mg/kg per day. Patient 6 was pretreated with sulfamethoxazole and trimethoprim for 4 days (days −3 to 0) and then shifted to pentamidine administration.

[c] *P. carinii* organisms in lung aspirates or mycolysed sputa were stained with a rapid Giemsa-like stain (Diff-Quik; American Scientific Products, Chicago, Ill.) and detected microscopically.

DISCUSSION

Oligonucleotide primers encoding the 3′ (antisense)- and 5′ (sense)-terminal sequences of 5S rRNA of *P. carinii* amplified the 5S rDNA sequence in animal and clinical specimens by PCR. The sequence study of 5S rRNA from *P. carinii* has suggested the phylogenic association of this organism with protista fungi (19). However, 5S rRNA sequences of several microbes belonging to or related to protista fungi or pathogenic microbes related to pneumonia differ significantly from that of *P. carinii* in the 5′- and 3′-terminal region (Fig. 4). This fact confers inherent specificity to the PCR detection by using primers encoding these terminal sequences. The large collection of sequence data of 5S rRNAs compared with that of 16S rRNAs proved useful for choosing primer sites. The detection was specific to *P. carinii*, and DNAs from uninfected hosts and several other pathogens tested did not interact with these primers. On the other hand, no sequence diversity of 5S rDNA was found in different *P. carinii* isolates from mice, rats, and humans as revealed in this study.

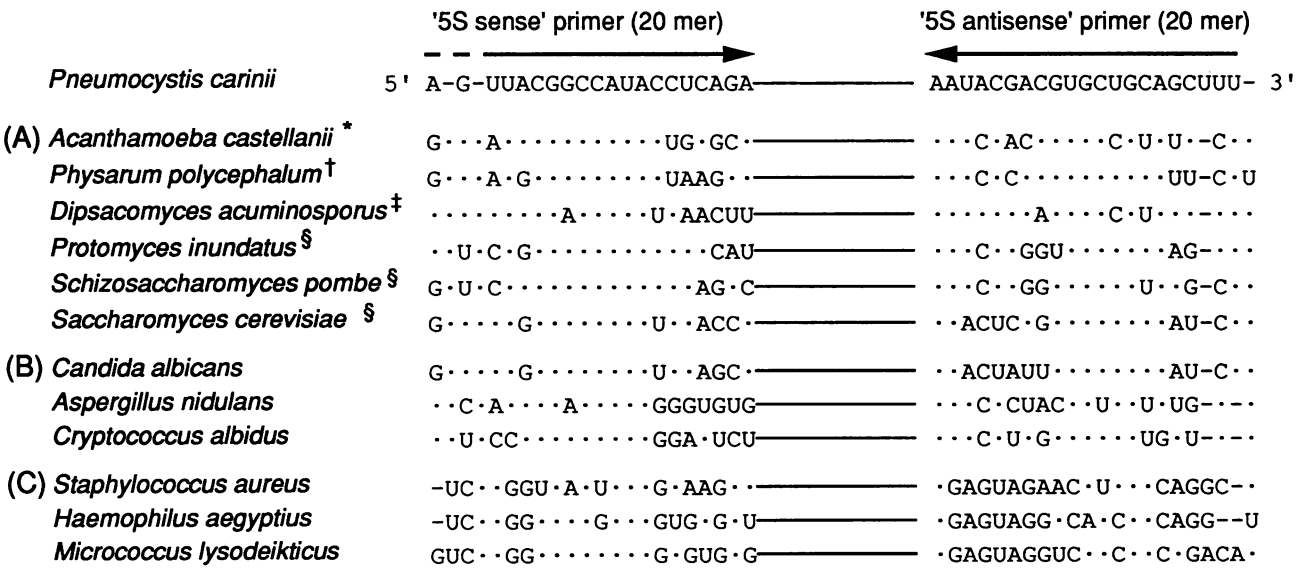

FIG. 4. Comparison of the 5'- and 3'-terminal sequences of 5S rRNA between *P. carinii* and other organisms phylogenically or pathologically related to *P. carinii*. Sequence alignment was as described previously (12, 20), and nucleotides different from the *P. carinii* sequence are indicated. (A) Organisms phylogenically related to *P. carinii*. *, amoeba; †, myxomycota; ‡, zygomycota; §, basidiomycota. (B) Pathogenic fungi or their related species involved in pulmonary diseases. (C) Normal flora and related microbes in the upper airway of human.

The remarkable advantage of the PCR system is its sensitivity; even a few *P. carinii* organisms, presumably trophozoites, in one PCR mixture efficiently amplified the product which was readily detectable by ethidium bromide staining. The latent infection of *P. carinii* in thymic mice was detectable (Fig. 2A, lane 3), and *P. carinii* organisms in patients' induced sputa which were not detected by Diff-Quik staining were detectable by PCR (Table 1). This unusually high sensitivity of detection may be due primarily to high copy numbers of the 5S rRNA genes in the *P. carinii* chromosomes. In most eukaryotes, the 5S rRNA genes are repetitive (6); thus, one can expect $>10^2$ copies of the 5S rRNA gene in the haploid *P. carinii* genome. In fact, it has been reported that ^{32}P-labeled 5S rRNA of *P. carinii* hybridized to several different chromosomes separated by pulse-field gel electrophoresis (21).

Accuracy of detection by the PCR assay was high both in animal and clinical examinations when lung specimens were used. Of 35 independent lung samples tested, i.e., 17 from mice, 2 from rats, 6 from human autopsy, and 10 from human aspiration biopsy, the PCR successfully detected 23 positives and 12 negatives in agreement with the clinical and microscopic diagnose. Based on the efficacy and specificity of the PCR method manifested in these trials, we started its practical use for diagnosis of *P. carinii* pneumonia by using lung specimens from the aspiration biopsy. Up to now, two more positive patients have been found among six AIDS patients suffering with pneumonia. These PCR data were consistent with the microscopic diagnosis and the clinical response to pentamidine (data not shown). However, from a clinical point of view, open lung biopsy, bronchoscopy, and even the aspiration biopsy are not trivial examinations because of the risk with patients as well as human immunodeficiency virus (HIV) exposure to the medical staff. Therefore, some noninvasive or less invasive methods are desired. In this regard, the blood test and expectoration method were attempted. Very recently, Wakefield et al. reported similar application of PCR to clinical alveolar lavage specimens for detection of *P. carinii* by using primers that amplify the mitochondrial large rDNA sequence (16). They have also discussed the preferred use of noninvasive systems.

It has been shown that *P. carinii* antigen(s) is present in peripheral blood specimens from patients with *P. carinii* pneumonia (7). Of two AIDS patients examined in this study, the PCR assay detected *P. carinii* 5S rDNA sequence in the blood sample of patient 1 upon the onset of *P. carinii* pneumonia (Fig. 3C, lane 3). The clinical and microscopic observations were consistent with the PCR result and ensured the accuracy of the PCR detection of *P. carinii* in blood samples from patient 1. The failure of detection of *P. carinii* in the blood sample (of 19 March 1990) from patient 2 may be at least in part due to a prior administration of pentamidine which started on 5 March 1990.

We assume that detection by the PCR-ethidium staining method will be less sensitive in blood samples than in lung biopsy specimens. Of five *P. carinii*-infected mice, we failed to detect the *P. carinii* sequence in blood samples from two mice. This reduced sensitivity might be due to the low level of *P. carinii* which have entered peripheral blood via phagocytosis or direct intrusion. Oligoblotting instead of ethidium staining will certainly increase the sensitivity of the method and facilitate its practical use in blood tests. We are currently testing the efficacy of the blood-based and sputum-based detection methods in more patients. Noninvasive diagnosis of *P. carinii* infection by using the PCR method will be of great benefit for AIDS or immunosuppressed patients and asymptomatic human immunodeficiency virus carriers. This would also facilitate basic progress in the pathology or epidemiology of *P. carinii* infection.

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