

## Preliminary Results of *Pneumocystis carinii* Strain Differentiation by Using Molecular Biology

SOPHIE LATOUCHE,<sup>1,2\*</sup> PATRICIA ROUX,<sup>1</sup> J. L. POIROT,<sup>1</sup> ISABELLE LAVRARD,<sup>1,2</sup>  
BRIGITTE HERMELIN,<sup>2</sup> AND VÉRONIQUE BERTRAND<sup>2</sup>

Laboratoire de Parasitologie-Mycologie, Centre Hospitalier Universitaire Saint-Antoine, 75012 Paris,<sup>1</sup> and  
Laboratoire Commun de Biologie Moléculaire, Hôpital Saint-Antoine, 75571 Paris Cedex 12,<sup>2</sup> France

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**The mode of *Pneumocystis carinii* transmission is controversial. Recent studies point to exogenous inoculation rather than reactivation, and person-to-person transmission has also been suggested. Comparison of nucleotide sequences of the large-subunit mitochondrial rRNA gene of *P. carinii* from human immunodeficiency virus-seropositive patients showed strain differences.**

*Pneumocystis carinii* pneumonia (PCP) remains the most frequent opportunistic infection in AIDS. Despite major improvements in diagnosis and therapy, epidemiological data are fragmentary. Recent studies indicate that *P. carinii* is rarely detectable by sensitive techniques such as PCR in bronchoalveolar lavage (BAL) fluid or lung biopsy specimens from patients without PCP (1, 9, 11). This suggests that exogenous inoculation rather than reactivation may be involved in PCP. Contamination through inhalation of cysts does occur, but the mode of transmission, whether environmental and/or from infected individuals, is uncertain.

The occurrence of small epidemics in human immunodeficiency virus-seronegative patients hospitalized simultaneously with HIV-seropositive patients suggests that nosocomial infection can occur (2, 4, 5).

Antigenic studies (7, 16) and karyotyping techniques such as pulsed-field gel electrophoresis have revealed genetic variations in *P. carinii* strains infecting humans, ferrets, and rats (10, 13, 14). It has also been demonstrated that coinfection with different *P. carinii* strains in the same host is possible (3, 6). Recently it was shown that humans are infected by multiple strains of *P. carinii* (8). In the preliminary study presented here, we attempted to identify different strains of *P. carinii* by studying genomic variations in a fragment of the gene encoding the large subunit of mitochondrial rRNA of human *P. carinii* isolates.

This study was conducted in two Paris hospitals (Hôpital Saint-Antoine and Hôpital Tenon) between February 1992 and May 1994. We examined *P. carinii* DNA in 37 BAL specimens from 28 HIV-seropositive patients with PCP proven by direct examination with standard stains (Giemsa and toluidine blue O) and indirect immunofluorescence. Two or three BAL procedures were performed for 8 of the 28 patients who showed no improvement after receiving specific therapy.

BAL fluid was centrifuged, and DNA in the pellet was prepared by proteinase K digestion followed by phenol-chloroform extraction. *P. carinii* DNA was amplified by PCR with the primer pairs and cycle conditions described by Wakefield et al. (15). The PCR products were electrophoresed in a 2% agarose gel with 1× tris-borate-EDTA and ethidium bromide and purified by using the Wizard PCR Preps DNA Purification

System (Promega). The purified products were then sequenced directly on an automated sequencer (model 373 A; Applied Biosystems) and by using the Prism Ready Reaction Dye Deoxy Terminator Cycle Sequencing kit (Applied Biosystems).

A total of 37 340-bp fragments were compared with each other and with the prototype sequence reported by Sinclair et al. (12). No sequences were identical to the prototype: all had an A-to-C change at position 248 and a G-to-A change at position 288. On the basis of the nucleotide at position 85 (Fig. 1) we were able to divide the isolates into three groups: group 1 sequences had a T (6 patients), group 2 sequences had an A (7 patients), and group 3 sequences had a C (15 patients). We observed no sequence differences between initial and repeat BAL samples for any of the eight patients for whom BAL was repeated. This showed that during a given episode of PCP, a patient retained the same strain of *P. carinii*. Only one patient (Table 1) developed a second episode of PCP, which was due to failure of aerosol pentamidine prophylaxis. The two episodes occurred 7 months apart, and the *P. carinii* isolates were identical. This suggested that the recurrence was due either to reactivation of remnant organisms from the first episode or to de novo infection by the same strain.

Our comparative analysis indicated that nucleotide sequences of *P. carinii* from different patients may be different and suggested that there were three strains of *P. carinii* among the patients in this study. In addition, the sequences we observed were different from those described by Lee et al. (8), who did not find a C-to-A change at position 85 and an A-to-C change at position 248 in *P. carinii* isolates from American, Canadian, and Italian patients. This suggests that differences among *P. carinii* strains could reflect geographic origin. As shown in Table 1, there was no relationship between the date of BAL, the hospital, and the *P. carinii* strain. Group 3 appears

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TTGTGGTAAG TAGTGAAATA CAAATCGGAC TAGGATATAG CTGGTTTTCT 50
GCGAAATTG TTTTGGCAA TTTGTTAATC CTCTXAAAAA TAGTAGGTAT 100
AGCACTGAAT ATCTCGAGGG AGTATGAAAA TATTTATCTC AGATATTTAA 150
TCTCAAATA ACTATTTCTT AAAATAAATA ATCAGACTAT GTGCGATAAG 200
GTAGATAGTC GAAAGGGAAA CAGCCAGAA CAGTAATTTAA AGTCCCCCAA 250
TTAATATTTAA GTGAAATAAA AGTTGTTGGA TATCTAAAAC AGTTAAGAAG 300
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FIG. 1. Nucleotide sequence of a portion of the large-subunit mitochondrial rRNA gene of *P. carinii* from several patients. Underlined letters are mutations. X at position 85 is a T in group 1, an A in group 2, and a C in group 3.

\* Corresponding author. Mailing address: Laboratoire de Parasitologie-Mycologie, CHU Saint-Antoine, 27 rue de Chaligny 75012 Paris. Phone: 40 01 13 71 or 40 01 13 77. Fax: 40 01 14 99.

TABLE 1. Distribution of patients according to *P. carinii* nucleotide sequence group and hospital

Group	No. of patients			No. of BAL			Date(s) of BAL (day.mo.yr) <sup>a</sup>	
	Total	Hôpital Saint-Antoine	Hôpital Tenon	Total	Hôpital Saint-Antoine	Hôpital Tenon	Hôpital Saint-Antoine	Hôpital Tenon
1	6	5	1	8	6	2	9.3.1992, 14.9.1993, 2.9.1993 + 21.4.1994, <sup>b</sup> 20.9.1993, 1.4.1994	18.10.1993 + 25.10.1993
2	7	6	1	7	6	1	14.2.1992, 14.2.1992, 2.2.1994, 29.3.1994, 31.3.1994, 9.5.1994	7.4.1994
3	15	9	6	22	12	10	17.4.1992, 21.4.1992 + 29.4.1992, 30.9.1993, 4.10.1993, 12.10.1993, 21.1.1994, 2.2.1994, 18.2.1994 + 13.4.1994, 7.4.1994 + 5.5.1994	25.3.1992, 24.10.1992, 20.4.1993 + 10.5.1993, 15.9.1993 + 16.10.1993, 4.1.1994 + 14.1.1994 + 25.1.1994, 4.5.1994

<sup>a</sup> Dates joined by a plus sign show multiple BAL for one patient.

<sup>b</sup> This patient had two episodes of PCP.

to be overrepresented, but no statistical analysis was possible because of the small group sizes. The three mutations we detected on a 300-bp fragment point to a mutation rate of 1% and to the existence of conserved sequences.

To conclude this preliminary study, we are now sequencing a larger number of samples and others genes with sequence repeats.

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