BRIEF REPORT

Distribution of *Pneumocystis carinii f. sp. hominis* Types in the Lung of a Child Dying of *Pneumocystis* Pneumonia

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***Pneumocystis f. sp. hominis* causes pneumonia in immunocompromised persons. In order to determine the types and distribution of *P. carinii* organisms within a single human lung, multiple samples were obtained from the lung of a child who died of *P. carinii* pneumonia. *P. carinii* DNA was detected in all of the samples and 2 different genotypes of *P. carinii* were identified, with uneven distribution in the lung, demonstrating that infection of the human lung is not necessarily clonal, and that different *P. carinii* genotypes may predominate in different areas of the lung.**

Pneumocystis carinii f. sp. hominis is a significant cause of morbidity in immunocompromised adults and children. Diagnosis of *P. carinii* pneumonia relies on the identification of *P. carinii* organisms in respiratory tract samples, usually bronchoalveolar lavage (BAL) samples. Previous studies using BAL samples suggest that there is an uneven distribution of *P. carinii* organisms in the lung, with an increased *P. carinii* load in the upper lobes when compared to the middle and lower lobes [1]. Radiographically, some patients appear to have *P. carinii* infection restricted exclusively to the upper lobes [1, 2]. The predominance of *P. carinii* in the apices was originally associated with use of aerosolized pentamidine as prophylaxis for *P. carinii* [2], but was subsequently shown to occur irrespective of prior administration of this drug [1, 3] . In addition, analysis of BAL samples suggests that mixed infections with >1 type of *P. carinii* may occur [4–6]. In this study the distribution of *P. carinii* organisms in different sites of the lung was examined, using human lung tissue from a whole lung infected with *P. carinii* rather than respiratory tract samples. *P. carinii f. sp. hominis* organisms were genotyped at the internal transcribed spacer (ITS) regions of the ribosomal RNA operon [4, 5]. Multiple samples were examined in order to determine the pattern of *P. carinii* organisms throughout the lung, whether >1 type of *P. carinii* was present, and the distribution of different types at different sites within the lung.

Methods. A girl, aged 2 years and 4 months, with medulloblastoma of the brain stem diagnosed in April 1997, received vincristine and cisplatinum on 11 March 1998. Since diagnosis, she had developed multiple metastases to the spinal cord. On 25 May 1998, she was admitted to hospital with pneumonia. She was empirically started on iv ceftriaxone and amikacin, together with high-dose trimethroprim-sulfamethoxazole. Increasing dyspnea and hypoxemia determined connection to mechanical ventilation on 27 May when bronchoscopy was performed; abundant *P. carinii* cysts were visualized by methenamine silver staining of BAL fluid. Hydrocortisone was added to the treatment. The patient progressively deteriorated and by 1 June had developed total radiological opacity of both lungs. The patient died on 15 June.

An autopsy was performed, limited to the lung, and the complete right lung was removed intact within 4 h of death. Each lobe was dissected separately and all necessary precautions were taken to eliminate the possibility of cross-contamination between samples. Each lobe was divided into pieces (2–3 cm^3), which were stored at −70°C until analysis.

DNA extraction and amplification. Samples of tissue (~1 cm^3) were mechanically disrupted using sterile scalpels and digested with 1 mg/mL Proteinase K, in 10 m*M* EDTA pH8.0, and 0.5% sodium dodecyl sulphate at 60°C overnight, after which a further 1 mg/mL Proteinase K was added and the sample was incubated for a further 24 h. DNA was purified by phenol-chloroform extraction, followed by a DNA binding

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Informed written consent was obtained from the child's mother to donate the lungs for research, and the guidelines of the Research Ethics Committee of the University of Chile School of Medicine were followed in the conduct of this research.

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resin (Wizard DNA Clean-Up System, Promega) [7] . All precautions were taken throughout these procedures to eliminate the possibility of cross-contamination of samples. All handling of samples took place in a laminar flow cabinet and negative controls were included in the extraction procedure to monitor for contamination.

DNA amplification was carried out as described elsewhere [4], using primer pair N18SF and N26SRX in the first round PCR, and primer pair ITSF3 and ITS2R3 in the second round PCR. Cloning of the amplification products was carried out [4] . Four clones from each sample were sequenced using the BigDye Terminator Cycle sequencing kit (Applied Biosystems), and the ABI Prism 377 DNA Sequencer, running the data collection software, version 2.1 (Applied Biosystems). Sequence data analysis was performed using Chromas 1.44 software (C. McCarthy, Griffith University). Sequence alignments were performed using the University of Wisconsin GCG sequence analysis software, version 10.1 (Genetics Computer Group).

Results. The diagnosis of *P. carinii* pneumonia was made in the patient on admission, on the basis of typical clinical features and positive methenamine silver staining of a BAL sample. Diagnosis was confirmed by PCR at the mitochondrial large subunit rRNA (mt LSU rRNA) locus on the BAL sample [8].

Postmortem, 10 samples were analyzed from different anatomical regions of the right lung, 4 from the upper lobe, 3 from the middle lobe and 3 from the lower lobe. *P. carinii* DNA was detected in all the samples, using PCR at the ITS regions of the rRNA operon (table 1). Amplification products were cloned and the DNA sequence determined. An ITS sequence type was assigned to each clone (4 clones from each sample), as described elsewhere [4]. Two different ITS sequence types were found. The ITS sequence type Ca_3 was found in 3 samples, type B_1b_2 in 3 samples and a mixture of both types in 4 samples (Table 1).

Discussion. In this study the distribution of *P. carinii* organisms in different sites of the lung was examined, using human lung tissue from a whole *P. carinii* infected lung. Previously, this type of analysis has only been possible using multiple respiratory tract samples, rather than samples of lung tissue. *P. carinii* organisms were found in all parts of the lung, which was consistent with the severity of the radiological abnormalities and of the disease. Isolates of *P. carinii f. sp. hominis* were typed at the ITS regions of the rRNA operon, as this is the most informative locus to date [4, 5] and is present in 1 copy per genome [9]. Two different types of *P. carinii f. sp. hominis* were found, Ca_3 and B_1b_2 (corresponding to Fg and Ec, as described by Lee et al. [5]). This gives additional support to the view that infection with *P. carinii* is not necessarily clonal, and that infection with >1 type of organism can occur [4, 5]. Type Ca_3 was the predominant type in the lower lobe, type B_1b_2 in the middle lobe and a mixture of both types in the upper lobe.

Table 1. Distribution of *Pneumocystis carinii f. sp. hominis* genotypes in the patient's right lung.

Site in lung	Presence of *P. carinii*	*P. carinii* ITS genotype
Upper lobe		
Apical segment	+	Ca_3, B_1b_2
Anterior segment, upper	+	Ca_3, B_1b_2
Anterior segment, lower	+	Ca_3
Posterior segment	+	B_1b_2
Middle lobe		
Medial segment, upper	+	B_1b_2
Medial segment, lower	+	Ca_3, B_1b_2
Lateral segment	+	B_1b_2
Lower lobe		
Anterior basal segment	+	Ca_3
Medial basal segment	+	Ca_3
Posterior basal segment	+	Ca_3, B_1b_2

NOTE. ITS , internal transcribed spacer; +, *P. carinii* DNA.

These data are most consistent with a model in which infection was initiated by >1 organism, with each organism forming a focus of infection. As the disease progressed with an expansion in the numbers of organisms, some regions of the lung remained infected by a single type, whereas in other regions, a mixture of both types was established. This was most obvious in the upper lobe, and is consistent with the finding of a heavier burden of *P. carinii* organisms in this region as described by other investigators [1, 3].

The results of this study are in contrast to those from a study in which 2 BAL samples, one from the right upper and the other from the right middle lobe were taken from 10 HIV-infected patients with *P. carinii* pneumonia [6]. Using genotyping at the mt LSU rRNA, the same genotype was found in both samples from every patient. However, the mt LSU rRNA is less informative at distinguishing between types of *P. carinii f. sp. hominis* than the ITS regions, and mixed infection with >1 mt LSU rRNA genotype was only found in 1 of the 10 patients [6].

It is not yet possible to conduct analyses of this nature in animal models of *P. carinii* infection such as the rat model, since the markers which are necessary to distinguish between different types of *P. carinii* do not exist and have only been developed for *P. carinii f. sp. hominis*. Indeed, the level of diversity found in *P. carinii f. sp. hominis* is not present in *P. carinii f. sp. carinii* (rat-derived *P. carinii*) [7]. Mixed infections of *P. carinii*, however, have been observed in rats but consisted of a mixture of different *formae specialis* of rat-derived *P. carinii*, which were much more divergent than the different ITS genotypes observed in this study. A total of 5 different *formae*

speciales have been found in lung samples from wild rats. Mixed infections were detected in 50% of these samples [7]. In laboratory rats, mixed infection with *P. carinii f. sp. carinii* and *P. carinii f. sp. ratti* has been found, and it has been suggested that there may be a dynamic interaction between the 2 types, and that *P. carinii f. sp. carinii* may be dominant over *P. carinii f. sp. ratti.* This form of interaction of types may also occur in *P. carinii f. sp. hominis* infection. It has been reported that ITS type B_2a_1 is more common in second and subsequent infections and is associated with mild disease [10].

The samples analyzed in this study originated in Chile, and unlike the majority of samples in other studies [4, 5, 6, 7, 10], were from a young child, immunosuppressed by cytotoxic drugs rather than by HIV infection. The 2 ITS types identified in this study have previously been observed in samples from the Northern Hemisphere. Of note, the ITS sequence type Ca_3, is uncommon in *P. carinii f. sp. hominis* isolates from the United Kingdom (4%) [10], and from samples from Denmark and the United States (1%) [5].

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