BRIEF REPORT

Prophylaxis Failure Is Associated with a Specific *Pneumocystis carinii* Genotype

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To investigate the possible association between *Pneumocystis carinii* types and various clinical and demographic parameters, we used molecular typing to analyze 93 bronchoalveolar lavage specimens from patients with *P. carinii* pneumonia (PCP). Multivariate regression analysis revealed an association between being infected with a specific *P. carinii* genotype and receiving anti-PCP prophylaxis (odds ratio, 4.4; 95% confidence interval, 1.0–18.6; $P = .05$), although no association with a specific drug was detected.

Pneumocystis carinii pneumonia (PCP) is an important opportunistic infection among immunocompromised patients. In the absence of a reliable method for in vitro culture of the infecting organism, many aspects of PCP are poorly understood. Cotrimoxazole is the first-line drug for prophylaxis and treatment. Recently, nonsynonymous mutations in the *P. carinii* gene encoding the target of this drug, dihydropteroate synthase, were shown to be associated with failure of cotrimoxazole treatment [1] and an increased mortality rate [2]. It has been suggested that mutations altering the active site of cytochrome b, which is the target of atovaquone, a second-line drug, also confer resistance [3]. The isolation of mouse-derived strains of *P. carinii* that do not respond to cotrimoxazole and that lack alterations at the active site of dihydropteroate synthase suggests that other resistance mechanisms, such as altered drug uptake or detoxification, may also exist [4].

Received 4 December 2000; revised 20 February 2001; electronically published 22 August 2001.

Financial support: Swiss National Program on AIDS Research (grant 97-7299) and the Swiss National Fund (grant 32-56715.99).

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Clinical Infectious Diseases 2001;33:1080–2

1058-4838/2001/3307-0024$03.00

We recently developed a molecular typing method for strains of *P. carinii* that infect humans; this method consists of amplification of 4 variable regions of the genome, followed by detection of polymorphisms by the single-strand conformation polymorphism technique (SSCP) [5, 6]. This method was used to investigate the possible association between *P. carinii* genotypes and various clinical and demographic parameters of infected patients. The results show that failure of primary or secondary anti-PCP prophylaxis is associated with a specific *P. carinii* genotype; this suggests that drug resistance may be detected by molecular typing with the use of markers that are unrelated to resistance.

Ninety-three bronchoalveolar lavage (BAL) specimens were obtained from 91 patients with PCP who were admitted to 11 hospitals in European countries, as follows: Belgium (Brussels, 1 patient), Denmark (Copenhagen, 8), France (Lyon, 10; Paris, 9), Germany (Münster, 3), and Switzerland (Geneva, 4; La Chaux-de-Fonds, 1; Lausanne, 21; Lugano, 5; St.-Gallen, 1; Zurich, 28). The specimens were selected for study because data were available on the following variables: the patient's sex, age, HIV status, number of PCP episodes, CD4 count, anti-PCP prophylaxis regimen, and geographical origin, and the infecting *P. carinii* genotype. *P. carinii* organisms were typed using the PCR-SSCP method, as described elsewhere [5, 6]. The possible association between *P. carinii* genotypes and various clinical and demographic parameters was analyzed with SPSS 8.0 statistical software (SPSS). An association was considered to be statistically significant if $P \leq .05$ (χ^2 test for proportion).

The 91 patients ranged in age from 23 to 82 years (median, 35 years), and most (75%) were men. The majority of the 93 specimens (85%) were from AIDS patients. Eighty-one of the specimens were from patients with their first episode of PCP, 11 were from patients with their second episode, and 1 was from a patient with a third episode. Sixty-three episodes of PCP occurred in patients who had not received PCP prophylaxis, and 30 episodes occurred in patients receiving prophylaxis with the following drugs: aerosolized pentamidine (14 patients), cotrimoxazole (8), dapsone (4), atovaquone (1), and an undetermined drug (3). Of the 30 patients receiving prophylaxis, 4 did not comply with their drug regimen during the month before PCP was diagnosed.

Twenty-eight different *P. carinii* types were identified by use of the PCR-SSCP method. Forty-three specimens contained a single *P. carinii* type, 49 specimens contained 2 types, and 1 specimen contained 3 types. The same types were identified in

specimens obtained at different hospitals several years apart. The 2 patients with 2 episodes of PCP were infected with different types at each episode.

Univariate comparison revealed that there was a significant association between being infected with *P. carinii* type 6 and receiving anti-PCP prophylaxis ($P = .02$). None of the other genotypes was associated with this or other characteristics. Multivariate regression analysis, which included data on the year the specimen was collected, the patient's CD4 count, and the occurrence of coinfection, confirmed that there was an independent association between being infected with *P. carinii* type 6 and receiving anti-PCP prophylaxis (OR, 4.4; 95% CI, 1.0–18.6; $P = .05$; table 1). No association with a specific drug was detected. Infection with *P. carinii* type 6 occurred in patients receiving prophylaxis with pentamidine (5 patients), dapsone (3), and atovaquone (1). Seven of the 9 patients were compliant with prophylaxis during the month before PCP was diagnosed; compliance information was not available for 2 patients. The outcome of PCP episodes that involved *P. carinii* type 6 did not suggest drug resistance when therapeutic doses of anti-PCP drugs were used. The gene coding for dihydropteroate synthase of 3 isolates of *P. carinii* type 6 was amplified, sequenced, and compared with that of the wild-type allele (GenBank accession number U66283). No mutation was found (results not shown).

Among the 93 PCP episodes, we observed a broad diversity of infecting genotypes and a high frequency of coinfection. The stability of the genotypes that were found and their ubiquitousness were suggested by the observation of the same types in specimens obtained at different hospitals several years apart. These findings are in agreement with those of our previous studies [5–7].

The present study found an association between being infected with *P. carinii* type 6 and receiving anti-PCP prophylaxis. Being infected with type 6, rather than noncompliance with prophylaxis, seems to be associated with prophylaxis failure. Although the small numbers of patients limited the power of the study, the association between infection with type 6 and receiving prophylaxis was apparently independent of the drug received. Thus, a mechanism that confers multiple resistance may be present in *P. carinii* type 6. However, it is also possible that this genotype is more prone to accumulate mutations than are other types and thus is more likely to acquire drug resistance under selective pressure. Selection of resistant clones may have occurred in patients receiving prophylaxis; or it may have occurred in a limited number of patients, followed by dissemination of resistant clones. The fact that PCP episodes due to *P. carinii* type 6 responded favorably to treatment could be due to the higher doses of drugs given for treatment compared with the doses given for prophylaxis.

The findings of the present study are consistent with those of another study [10]. Indeed, the PCR-SSCP method assigned type 6 to *P. carinii* type B_2a_1 or type Ne, as defined by sequencing of the internal transcribed spacers of the nuclear rRNA operon [8, 9]. Type B_2a_1 has been reported to be associated with mild pneumonia and to occur most frequently in patients with recurrent episodes of PCP [10]. Both observations may be due to the drug resistance of this genotype. The mild severity could reflect reduced virulence that is due to the acquisition of resistance. The high frequency of type B_2a_1 in patients with recurrent episodes of PCP may have resulted from secondary prophylaxis.

Our results suggest that failure of primary or secondary anti-PCP prophylaxis is associated with a specific *P. carinii* genotype. Thus, typing with use of the rapid PCR-SSCP method may help predict resistance in a clinical situation and help improve treatment. Further study of *P. carinii* type 6 detected by PCR-SSCP may contribute to a better understanding of *P. carinii* drug resistance.

Table 1. Multivariate analysis of risk factors for infection with *Pneumocystis carinii* type 6 among 91 patients with *P. carinii* pneumonia.

Risk factor	No. (%) of patients, by infecting *P. carinii* genotype		Adjusted OR (95% CI)[a]	*P*
	Type 6	Other		
Received prophylaxis				.05
Yes	9 (69)	21 (26)	4.4 (1.0–18.6)	
No	4 (31)	59 (74)	Reference	
Period specimen was collected				.56
1989–1994	5 (39)	26 (32)	1.3 (0.3–6.5)	
1995–1996	7 (54)	35 (44)	Reference	
1997–1998	1 (7)	19 (24)	0.4 (0.0–3.9)	
CD4 count, cells/mm^3				.46
Unknown	3 (23)	31 (39)	1.0 (0.1–11.9)	
0–24	7 (54)	22 (27)	3.2 (0.3–34.8)	
25–49	2 (15)	11 (14)	2.7 (0.2–40.4)	
50–160	1 (8)	16 (20)	Reference	
Coinfection with >1 type of *P. carinii*				.26
Yes	3 (23)	47 (59)	0.4 (0.1–1.9)	
No	10 (77)	33 (41)	Reference	

NOTE. [a] OR for infection with *P. carinii* type 6 after adjustment for the 4 variables presented in the table.

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Acknowledgments

We thank A. Cruchon and C. Durussel, for excellent technical assistance, and Prof. A. E. Wakefield, for the gift of DNA from *P. carinii* type B_2a_1. For help with specimen collection, we also thank Prof. J. Lundgren (Copenhagen), Prof. S. Picot (Lyon), Prof. J.-C. Piffaretti (Lugano), and Prof. M. Struelens (Brussels).

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