Letters to the Editor

Potential Coinfections Complicate Typing of *Pneumocystis carinii* f. sp. *hominis*

Lee et al. reported on the nucleotide sequence variation of the two internal transcribed spacers (ITSs), ITS1 and ITS2, of the rRNA operon of *Pneumocystis carinii* f. sp. *hominis* (3). Thanks to their work, the number of polymorphic nucleotide positions recognized has increased and the number of recognized alleles has passed from 2 to 15 for ITS1 and from 3 to 14 for ITS2. Also, the number of different combinations of the alleles of the two loci, each defining a *P. carinii* f. sp. *hominis* type, has increased from 4 to 59. This great diversity of types will undoubtedly be useful for epidemiological studies. Accordingly, Lee et al. concluded that nucleotide sequence determination of PCR products from these regions is the method of choice for typing *P. carinii* f. sp. *hominis* isolates.

However, typing of *P. carinii* f. sp. *hominis* is complicated by the fact that coinfections of single patients are likely to occur (for a review, see reference 1). To approach this problem, Lee et al. used as a screening step five oligonucleotides hybridizing specifically to only two alleles of ITS1 and three alleles of ITS2. A coinfection was assumed if, for one locus, the PCR product from a specimen hybridized to more than one oligonucleotide. However, (i) this method does not allow the differentiation of polymorphisms located outside the annealing site of the oligonucleotide. Also, (ii) an oligonucleotide may fail to hybridize to alleles with unrecognized polymorphisms at the annealing site. According to the data of Lee et al., the first possibility probably occurred for both ITSs and the second possibility occurred for only ITS2. Thus, the presence of more than one allele, revealing possible coinfection events, may be missed by their screening step.

Given these considerations, we feel that only efficient detection of potential coinfections, either by direct subcloning of the PCR product from the ITSs followed by sequencing of numerous clones or by a multiple-target approach using single-strand conformation polymorphism (2), will help the progress of *P. carinii* f. sp. *hominis* epidemiology.

REFERENCES

1. **Hauser, P. M., D. S. Blanc, J. Bille, and P. Francioli.** Typing methods to approach *Pneumocystis carinii* genetic heterogeneity. FEMS Immunol. Med. Microbiol., in press.
2. **Hauser, P. M., P. Francioli, J. Bille, A. Telenti, and D. S. Blanc.** 1997. Typing of *Pneumocystis carinii* f. .sp. *hominis* by single-straing conformation polymorphism of four genomic regions. J. Clin. Microbiol. **35:**3086–3091.
3. **Lee, C.-H., J. Helweg-Larsen, X. Tang, S. Jin, B. Li, M. S. Bartlett, J.-J. Lu, B. Lundgren, J. D. Lundgren, M. Olsson, S. B. Lucas, P. Roux, A. Cargnel, C. Atzori, O. Matos, and J. W. Smith.** 1998. Update on *Pneumocystis carinii* f. sp. *hominis* typing based on nucleotide sequence variations in internal transcribed spacer regions of rRNA genes. J. Clin. Microbiol. **36:**734–741.

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Author's Reply

We agree with the comments of Hauser et al. stating that additional methods are required to detect all coinfections with *Pneumocystis carinii* f. sp. *hominis* in the same patient. Although the use of the five type-specific oligonucleotide probes that we have developed previously may not be sufficient to detect all coinfections, it has enabled us to detect multiple types of *P. carinii* f. sp. *hominis* in many specimens. Using this approach, we have identified approximately 60 types of *P. carinii* f. sp. *hominis.* This new sequence information will enable the development of additional type-specific probes and other typing methods for *P. carinii* f. sp. hominis.

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