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# Letters to the Editor

## Coinfection with *Tropheryma whippelii* and a Whipple's Disease-Associated Bacterial Organism Detected in a Patient with Central Nervous System Whipple's Disease

The actinomycete bacterium *Tropheryma whippelii* has been identified as the presumed cause of morbus Whipple (4). A second Whipple's disease-associated bacterial organism (WABO) was found in a duodenal biopsy specimen in a single case (2). DNA sequence data for the 16S rRNA gene place WABO evolutionarily close to the nocardioform bacteria. It is well-known that Whipple's disease may also involve the central nervous system (CNS) (3). Cohen et al. (1) amplified *T. whippelii* DNA from the cerebrospinal fluid (CSF) of a morbus Whipple patient with CNS involvement. We report for the first time a coinfection with *T. whippelii* and WABO in a patient with CNS Whipple's disease.

The patient, a 59-year-old male, had arthralgia and gastrointestinal symptoms before the diagnosis of morbus Whipple was made in 1995 by duodenal biopsy. He had suffered from increasing loss of memory since 1994 and grand mal epileptic seizures since May 1995. Examination of his CSF in 1995 gave a cell count of 11 Mpt/liter and a protein concentration of 0.34 g/liter, with a number of sickle-form-particle-containing cells. After being treated with co-trimoxazole for more than 1 year, the patient showed remission and gained 3 kg. His neurological status improved, as well as his short-term memory. No seizures occurred for a period of 6 months. Analysis of specimens from two further lumbar punctures in June and November of 1996 still revealed a slight pleocytosis (13/15 Mpt/liter). Cells were mostly monocytes, with few lymphocytic cells. Periodic acid-Schiff stain-reactive cells were present in both samples.

DNA was extracted from the cellular content of the two 1996 CSF samples. A part of the 16S rRNA gene conserved in gram-positive bacteria was amplified in a first PCR (primers, p515FPL and P13B). THe 904-bp fragment served as a template in a second, nested PCR. A *T. whippelii*-specific 284-bp amplicon was obtained under hot-start conditions (primers, p3FE and p2RB). Negative controls were processed during DNA extraction and amplification. PCR products were directly sequenced (ABI PRISM-Syst. 373; PE Applied Biosystems). The DNA sequence obtained from the earlier 1996 CSF sample had a pattern intermediate between those of *T. whippelii* and WABO, showing numerous ambiguous base positions. Thus, the amplicon was apparently a mixed PCR product. Subsequently, the amplified DNA was cloned in a pGEM-T vector (Promega). Sequence analysis of recombinant clones proved the existence of two DNA segments of different origins. One sequence was almost identical (except for 1 of 225 bases) to the WABO sequence. The second sequence matched in all but one position with that of *T. whippelii*. The two DNA fragments were cloned in a ratio of about 3:1 in favor of WABO.

Direct DNA sequencing of PCR products obtained from the second 1996 lumbar puncture produced a clear *T. whippelii* sequence but revealed no WABO DNA sequence.

Our findings show that DNAs from both *T. whippelii* and WABO could be detected in the CSF of a patient with CNS Whipple's disease. It remains unclear whether WABO is a disease-causing agent which influences clinical manifestations (e.g., CNS involvement) or whether it represents an opportunistic infection. Apparently the ratio of the two bacterial organisms may change during the course of the disease.

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