Detection of *Tropheryma whippelii* DNA in a Patient with AIDS

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Received 9 January 1995/Returned for modification 25 January 1995/Accepted 9 February 1995

A case of an AIDS patient infected with the Whipple's disease bacterium, *Tropheryma whippelii*, is reported. A DNA fragment with sequence specificity for the 16S rRNA gene of the bacterium was detected by PCR in a duodenal biopsy specimen from a 55-year-old male patient with AIDS and diarrhea. The biopsy specimen contained periodic acid-Schiff stain-positive macrophages which did not, however, resemble the sickleform-particle-containing cells characteristic of Whipple's disease. This observation raises two possibilities: either the patient had a coincidence of AIDS and Whipple's disease or *Tropheryma whippelii* acted as an opportunist pathogen in this immunodeficient patient. The latter explanation is of interest in light of the ongoing discussion of immunologic abnormalities as predisposing factors for Whipple's disease.

Whipple's disease is a chronic multisystem disorder of bacterial origin (3). It is an uncommon disease, with only 617 cases reported worldwide between 1907 and 1986 (3). As the causative bacterium cannot be cultured, the conventional means for diagnosis are histology showing the presence of periodic acid-Schiff stain (PAS)-positive inclusions in histiocytes and electron microscopy showing rod-shaped bacteria with a typical trilaminar outer cell membrane (3, 15). Characterization of the bacterium was successful when PCR with primers for universally conserved regions of the bacterial 16S rRNA gene was applied (12, 17). On the basis of its 16S rRNA sequence, the bacterium was described as a new species and the name *Tropheryma whippelii* was proposed (12). The natural habitat of the bacterium and its route of infection are currently unknown. However, besides the presence of the causative bacterium, which usually responds promptly to antimicrobial therapy, most patients with Whipple's disease have an impairment of immune function, which is presumed to play a role in pathogenesis (3). As in AIDS, the immune defect in Whipple's disease consists of a decreased T-helper/T-suppressor (CD4/CD8) cell ratio during active disease, as well as other immunologic abnormalities (9).

At present, there is no convincing report of infection caused by the Whipple's disease bacterium in AIDS patients, although two previous reports suggested such an association (1, 6). The report by Aturan et al. (1) was later criticized, because retrospectively this case was likely the result of infection by *Mycobacterium avium* (13), and the report by Jankovic (6), although clinically and histologically suggestive, was not confirmed by electron microscopy. Instead, other opportunistic bacterial infections in AIDS, namely those with *Mycobacterium avium* complex and *Rhodococcus equi*, were reported to mimic the histologic appearance of Whipple’s disease by the presence of PAS-positive inclusions in macrophages (5, 13, 16).

We observed a 55-year-old Caucasian man with a history of homosexuality and human immunodeficiency virus infection diagnosed in 1987. Kaposi's sarcoma was diagnosed in 1991 (Centers for Disease Control and Prevention classification C2). As tumor progression was slow, he received no antineoplastic therapy. In 1992, he suffered from recurrent episodes of diarrhea, fever, and loss of appetite. Intestinal candidiasis and giardiasis were diagnosed and responded to therapy with nystatin and metronidazole. Since January 1993 he again suffered from watery diarrhea, low-grade fever, dysphagia, and weight loss. Stool examinations for *Giardia* spp., *Candida* spp., cryptosporidia, and nontuberculous mycobacteria and urine examinations for cytomegalovirus were negative. In July 1993, endoscopy was performed. At this time, the hematocrit was 33%, the leukocyte count was 2.96/nl, the CD4 cell count was 65/μl, the CD8 cell count was 310/μl, and the CD4/CD8 ratio was 0.2. Esophagoscopy revealed *Candida* esophagitis, gastroscopy showed normal findings, and duodenoscopy showed a whitish appearance of the mucosa. Histology of duodenal biopsies revealed some PAS-positive macrophages in the mucosa (Fig. 1). Ziehl-Neelsen stain for mycobacteria was negative. However, the cytologic features of the PAS-positive histiocytes were not diagnostic for sickleform-particle-containing cells, which are the hallmark of Whipple’s disease (15). Thus, the biopsy was subjected to PCR analysis.

A cryopreserved duodenal biopsy specimen obtained by endoscopy was processed for PCR analysis by standard techniques (11). Briefly, the biopsy specimen was digested for 2 h at 56°C in 40 μl of lysis buffer (50 mM KCl, 10 mM Tris, 1.5 mM MgCl2, 1% Triton X-100, 200 μg of proteinase K per ml) and boiled for 10 min after the addition of 20 μl of a 20% Chelex suspension (biotechnology-grade chelating resin Chelex 100, 100–200 mesh, sodium form; Bio-Rad, Richmond, Calif.). Ten microliters of the supernatant was added to the PCR mixture. The Whipple-specific primers pW3FE and pW2RB (12) were used in a modified version (without restriction endonuclease recognition sites) in a PCR mixture consisting of 100 μl containing 50 mM KCl, 50 mM Tris (pH 9.0), 2.0 mM MgCl2, each deoxynucleotide triphosphate at 200 μM, 50 pmol of each primer, and 2.5 U of Taq polymerase (AmpliTaq DNA polymerase; Perkin-Elmer, Norwalk, Conn.). After 70 μl of mineral oil was added, the PCR was performed in a Perkin-Elmer 480 thermal cycler using initial denaturation at 95°C for 3 min followed by 40 cycles of denaturation at 95°C for 45 s, annealing at 62°C for 55 s, extension at 72°C for 55 s, and final extension at 72°C for 2 min. The PCR products were analyzed...
by electrophoresis on 8% polyacrylamide gels as described previously (7) and subjected to direct nucleotide sequencing with the Perkin-Elmer AmpliTaq Cycle sequencing kit according to the instructions of the manufacturer.

The resulting PCR product had the expected length of the Whipple-specific DNA fragment (Fig. 2). The sequence was the same as that reported for the Whipple’s disease bacterium (12), with the exception of one nucleotide position (deletion of C at position 1160 of the Whipple-specific DNA nucleotide sequence). The same deletion had previously been found in another patient with confirmed Whipple’s disease (10). To confirm this finding, another cryopreserved biopsy specimen and a paraffin-embedded tissue section from the AIDS patient, which were obtained during the same endoscopy, were processed in separate experiments. Again, PCR products with the same nucleotide sequence were obtained.

The detected DNA fragment from the gene coding for 16S rRNA is considered to be unique to the Whipple’s disease bacterium (Tropheryma whippelii) (12). Therefore, this is the first confirmed report of the Whipple’s disease bacterium detected in an AIDS patient. However, interpretation of this finding is difficult. The symptoms of this patient, consisting of low-grade fever, weight loss, and diarrhea, occur both in classical Whipple’s disease (8) and in AIDS enteropathy (5), and in the latter they are associated with a variety of different pathogens (14). Also, the histologic features of the duodenal biopsy were not diagnostic, as the number of PAS-positive inclusions in histiocytes was not nearly as high as that usually observed in cases of Whipple’s disease (15). Therefore, two main explanations are possible. Either this patient had a mere coincidence of AIDS and infection with the Whipple’s disease bacterium, which should be a rare event, or Tropheryma whippelii has the ability to act as an opportunistic pathogen in immunodeficiency. The immune defect of AIDS could have allowed the bacterium to penetrate the epithelial barrier and multiply in the duodenal mucosa. The latter explanation is of interest, as the role of an immune deficit predisposing for

FIG. 1. Duodenal biopsy from the patient under study. Some histiocytes with PAS-positive material are present in the lamina propria mucosae (arrows). The epithelium is intact.

FIG. 2. Polyacrylamide gel electrophoresis after PCR amplification from duodenal biopsies with Whipple-specific primers. Lanes: 1, 100-bp DNA marker; 2, Whipple’s disease (positive control); 3, AIDS patient under study; 4, other AIDS patient infected with Mycobacterium avium complex; 5, normal duodenum. A PCR product of approximately 267 bp is detected in Whipple’s disease (lane 2) and in the AIDS patient under study (lane 3) (arrow).
Whipple’s disease has been discussed (4), specific immunologic abnormalities have been observed (3, 9), and Whipple’s disease associated with opportunistic infections has been reported (2, 8, 10).

**Nucleotide sequence accession number.** The GenBank accession number for the nucleotide sequence of the Whipple-specific DNA fragment is M87484 (12).

This work was supported by the Deutsche Forschungsgemeinschaft (grant Ma 1663/2-1).

**REFERENCES**