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Species-Specific *Mycobacterium genavense* DNA in Intestinal Tissues of Individuals Not Infected with Human Immunodeficiency Virus

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Mycobacterium genavense **species-specific DNA was detected in intestinal tissues from two of nine individuals not infected with human immunodeficiency virus. This newly described microorganism is well documented as a causative agent of disseminated infections in AIDS patients. Our results suggest that it may colonize the guts of individuals not infected with human immunodeficiency virus.**

In developed countries, systemic nontuberculous mycobacterial infections are frequent in patients with AIDS, affecting up to 45% of those with end stage disease (1, 5). *Mycobacterium avium* complex (MAC) and occasionally other atypical mycobacteria are isolated (11). Portals of entry for MAC infections are probably gastrointestinal or respiratory (1, 5).

Recently, *Mycobacterium genavense* has been identified as a new organism causing disseminated infections in AIDS patients (2, 3, 6, 9). The clinical patterns are similar to those reported in disseminated MAC infections, including fever, weight loss, hepatosplenomegaly, anemia, and possibly earlier death (3, 17). The inability of *M. genavense* to grow on standard solid media delayed its identification (2, 6, 9, 16). This drawback can be overcome by amplification and analysis of species-specific DNA fragments (3). *M. genavense* has never been reported to have been observed in subjects not infected with the human immunodeficiency virus (HIV). Since *M. genavense* has been proposed to disseminate from the gut in HIV-infected patients (3), we tested intestinal biopsies of patients not infected with HIV for the presence of *M. genavense.*

Nine HIV-negative individuals (Table 1) were studied. They were free of infection at the time of biopsy and during the follow-up period. None of them was taking any antimicrobial chemotherapy. Biopsies were taken from grossly normal areas during colonoscopy performed for the excision of colonic polyps or during laparotomy for colonic cancer. Samples were immediately fixed in 10% formalin for histopathological examination or placed in sterile containers and stored at −20°C until processed with molecular biology techniques.

DNA was extracted from biopsies and resuspended in a final volume of 40 μl as previously described (8). Five microliters of DNA extract and 1 μl of first-run PCR product were amplified in a nested PCR for 40 and 30 cycles, respectively, with primers targeting areas of the 16S rRNA gene conserved at the genus level. The primer pairs used were (i) P1 (5′GAGAATTCGT GCTTAACACATGCAAGTCG, positions 43 to 64) in combination with P2 (5′ATGGATCCGTGAGATTTCACGAAC AACGC, positions 616 to 593) and (ii) P7 (5′CATGCAAG TCGAACGGAA, positions 54 to 71) in combination with P53 (5′TTTCACGAACAACGCGAC, positions 608 to 590). (All nucleotide positions correspond to the numbering of the 16S rRNA gene sequence in *Escherichia coli* [4]). P53 was biotinylated at its 5′ end. PCR conditions were as previously described (8) except for the deoxynucleoside triphosphate content of mixtures intended for oligonucleotide-specific capture plate hybridization (OSCPH) analysis. This was 50 μM dATP, dCTP, and dGTP, 35 μM dTTP, and 4 μM digoxigenin-11-dUTP (Boehringer Mannheim GmbH, Mannheim, Germany). The annealing temperatures of the first- and second-run PCRs were 56 and 52°C, respectively.

The method for OSCPH was adapted from that described by King and Ball (12). Briefly, the nested PCR products generated in the presence of digoxigenin-11-dUTP were allowed to hybridize with the 5′-end-biotinylated MycG (5′GGCCGGA CACCCTCTC, positions 296 to 311) and Gena (5′CACGGA ACGCATGTTTTG, positions 185 to 199) oligonucleotide probes attached to streptavidin-coated enzyme-linked immunosorbent assay plates. The MycG oligonucleotide probe was complementary to a region of the 16S rRNA gene conserved at the genus level as a positive control. The Gena oligonucleotide probe was complementary to the middle area of the *M. genavense* signature sequence (3) and allowed the identification of all 4 strains of *M. genavense* among 70 strains of 20 mycobacterial species (7). After washing, captured PCR products were detected with an alkaline phosphatase-conjugated antidigoxigenin antibody. All positive results were confirmed at least once. For positive cases detected with the Gena probe in OSCPH analysis, the nucleotide sequence of the nested PCR product was determined by direct sequencing performed on each strand of DNA with primers P7, P16 (5′ACGAACAA CGCGACAAACC, positions 585 to 604), and MycG by using the T7 sequencing kit (Pharmacia Biotech).

The presence of PCR inhibitors was tested for in each sample by spiking a reaction mixture with 100 fg of mycobacterial DNA. Standard recommendations for the prevention of contamination were meticulously followed, including the simultaneous processing of no more than six specimens as well as the exclusive use of disposable equipment and filter tips. Negative controls consisted of reagent-only tubes. They were subjected to each step of the procedure together with the samples in a 1-to-6 ratio.

PCR inhibitors were not detected in any sample. PCR revealed the presence of mycobacterial DNA in five of nine samples (55%) (Table 1). OSCPH analysis was positive in all

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TABLE 1. Characteristics of the specimens and patients

Patient no.	Age (yr)	Sex[a]	Sampling procedure[b]	Diagnosis	Result of:	
					PCR	OSCPH with MycG/Gena probes
1	66	M	C	Adenocarcinoma	+	+/+
2	85	F	L	Adenocarcinoma	+	+/+
3	49	M	C	Adenoma	+	+/−
4	66	F	C	Adenoma	−	−/−
5	55	M	C	Adenoma	−	−/−
6	74	M	C	Adenocarcinoma	−	−/−
7	83	F	L	Adenocarcinoma	−	−/−
8	70	M	C	Adenocarcinoma	+	+/−
9	76	M	C	Adenoma	+	+/−

[a] M, male; F, female.
[b] Biopsies were obtained during colonoscopy (C) or laparotomy (L).

five cases with the MycG probe and in two of them with the Gena probe. The species identity of *M. genavense* was confirmed in these last two cases by direct DNA sequencing (positions 44 to 583). The sequenced fragments included the *M. genavense* characteristic sequence signature and the short helix 18 (3). This was identical to the reference sequence (EMBL accession number 60070). These specimens were obtained during laparotomy (case 2) or endoscopy (case 1). Positive and reagent-only negative controls yielded appropriate results. At microscopy, Ziehl-Neelsen staining of specimen suspensions (9) did not disclose any acid-fast bacilli. Specimen sections were not used for this purpose. No attempt to culture mycobacteria from these smear-negative specimens was made. At pathology, hematoxylin and eosin staining of sections did not show any abnormality in any case.

The detection of *M. genavense* in intestinal samples from HIV-negative individuals presenting with colonic polyps or cancer supports an environmental origin of the species, as does the massive intestinal involvement in birds (10) and AIDS patients (3, 9, 15) infected with this organism. *M. genavense* probably colonizes the gut by ingestion. False-positive results could be attributed to endoscope-related pseudoinfections (14) or PCR contamination. We believe that this is unlikely for three reasons: (i) *M. genavense* was demonstrated in a surgical specimen; (ii) regular controls for possible contamination of endoscopes and cleaning devices were used, repeatedly revealing no evidence for mycobacteria; and (iii) all reagent-only controls were subjected to each step of the procedure and were always negative.

In conclusion, this study demonstrates the possible presence of *M. genavense* in patients not infected with HIV (at least in some of those affected with colonic polyps or cancer). Therefore, its detection in intestinal samples by PCR and DNA direct sequencing should not be considered to be indicative of disseminated infection in the absence of other evidence. These techniques will help in ongoing studies to establish the true prevalences of infection and asymptomatic carriage of *M. genavense*, both of which are probably underdiagnosed conditions (13). Like for MAC, the question of whether AIDS patients with gastrointestinal colonization by *M. genavense* are at a higher risk of disseminated infection than others should be addressed in future research.

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