Increased Frequency of Detection of *Ureaplasma urealyticum* and *Mycoplasma genitalium* in AIDS Patients without Urethral Symptoms

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The roles of *Mycoplasma genitalium* and *Ureaplasma urealyticum* in nongonococcal urethritis are not yet well established. The aim of this study was to determine the presence of these microorganisms in the urethral tracts of 187 human immunodeficiency virus type 1 (HIV-1)-infected male patients with no clinical signs of urethritis. The results indicate that the prevalence of *M. genitalium* and *U. urealyticum* was higher in AIDS patients than in asymptomatic, HIV-1-infected patients and in healthy individuals. The high rate of mycoplasmas and ureaplasmas detected in AIDS patients, in the absence of urethritis, argues against major roles in causing disease at the urethral mucosal level for these microorganisms.

The role of *Mycoplasma genitalium* and *Ureaplasma urealyticum* in acute nongonococcal urethritis (NGU) in humans is not yet established (6). The pathogenetic role of *M. genitalium* has been suggested because it is isolated with remarkable frequency from the urogenital tract of patients with NGU (3, 14, 17). Similarly, *U. urealyticum* is considered a pathogen of the urogenital tract because its isolation is more prevalent in patients with NGU than in asymptomatic subjects (9). However, the presence of *M. genitalium* (6, 7, 13, 18) and *U. urealyticum* (1, 12) in the urethras of healthy volunteers, who show no signs or symptoms of a urinary tract infection, urethritis, or prostatitis during the investigation, contradicts this hypothesis.

Human immunodeficiency virus type 1 (HIV-1)-infected patients are characterized by alterations in their T-cell immune functions long before the number of circulating CD4⁺ lymphocytes starts to decline (4). In the more advanced stages of the disease, these alterations are clinically reflected in an increased frequency of opportunistic infections (5, 10, 11).

The aim of the present study was to investigate the pathogenetic roles of *M. genitalium* and *U. urealyticum* at the urethral level by studying the presence of both microorganisms in urethral swabs taken from asymptomatic HIV-1-infected male patients and male patients with full-blown AIDS; neither group had symptoms of acute urethritis. The data show that *M. genitalium* and *U. urealyticum* can be detected in a larger proportion of AIDS patients and asymptomatic HIV-1-infected patients than in the other groups of subjects studied.

**Patients.** One hundred eighty-seven male heterosexual HIV-1-infected patients (all intravenous-drug abusers) were enrolled at the Infectious Diseases Unit of the Hospital of Lovere, Lovere, Italy. The ages of the HIV-1-positive patients ranged from 18 to 40 years (mean age, 28 years). The clinical status of the HIV-1-infected patients was defined according to the 1993 classification system of the Centers for Disease Control and Prevention (2): 115 were asymptomatic HIV-1-infected patients without AIDS (referred to herein as non-AIDS patients) (stage A1), whereas 72 patients had full-blown AIDS (stage C3) (Table 1). The control group consisted of 114 healthy male volunteers who either were attending the Institute of Microbiology for routine checkups or were recruited from the hospital staff. They ranged in age from 20 to 41 years (mean age, 27 years). Both the HIV-1-infected and the control groups consisted of sexually active Italian subjects. The samples from healthy subjects and from HIV-1-infected patients were completely randomized during their collection from 1995 to 1998. The Ethical Committee guidelines were followed throughout this study. Both patients and healthy subjects were evaluated by a standard protocol that included a sexual history, a genital examination performed by clinicians who confirmed the absence of inflammatory processes and pathological lesions, and two urethral swab specimens for the detection of *M. genitalium* and *U. urealyticum*. All samples were examined by microscopy to exclude the presence of other pathogenetic microorganisms, spores, or leukocytes.

**U. urealyticum growth conditions.** Urethral swabs were stirred into 2 ml of mycoplasma-carrying broth and tested for *U. urealyticum* by two complementary methods, conventional culture (Mycotrim GU; Irvine Scientific, Santa Ana, Calif.) and culture by biochemical testing (MYCOFAST; DID, Milan, Italy) (12). In the conventional culture method, each Mycotrim GU flask contained agar and broth specially formulated to support the growth of *U. urealyticum*; positive cultures raised the pH, caused a color change in the media, and were thus identified by low-power microscopic examination of the agar surface.

In the MYCOFAST biochemical testing, growth was first signalled by a color change from yellow to orange or red, and *U. urealyticum* was identified by determining its susceptibility to a range of antibiotics. Titration rates obtained with sample dilutions were defined in terms of CFU per milliliter.

**M. genitalium growth conditions.** Urethral swabs were stirred into 2 ml of mycoplasma transport medium (M.S. U.M.M.; International Mycoplasma, Signes, France). One milliliter of M.S. U.M.M. culture medium was added to 5 ml of SP-4 medium for *M. genitalium* isolation (17).

**M. genitalium DNA detection.** One milliliter of M.S. U.M.M. culture medium was centrifuged at 15,000 × g for 15 min at 4°C. The pellet obtained by centrifugation was resuspended in 200 μl of TE buffer (10 mM Tris hydrochloride, pH 8.0, and 1 mM EDTA), lysed by the addition of 1% sodium dodecyl sulfate (SDS), and incubated with 50 μl of proteinase K.

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(Boehringer, Mannheim, Germany) for 4 h at 55°C. DNA was extracted twice with an equal volume of phenol saturated with TE buffer and once with chloroform-isomyl alcohol (96:4, vol/vol). After extraction and precipitation with 3 M sodium acetate and 95% ethanol, the pellets were resuspended in 40 μl of TE buffer. M. genitalium ATCC 33530 was grown in SP-4 broth medium, collected by centrifugation for 15 min at 8,000 × g, and stored at −80°C until DNA was extracted as described above.

Primers MgPa 1 (5′-AGTTGATGAAACCTTAACCCCTTG-3′) and MgPa 3 (5′-CCGTGAGGGGTGTTCCATTTTG-3′) were used for specific genome amplification; they amplify a 281-bp segment of the 140-kDa adhesion protein gene. Amplification (Amplitaq; Perkin-Elmer Cetus, Norwalk, Conn.) was carried out for 35 cycles (1 min at 94°C, 1 min at 65°C, and 1 min at 72°C), with an automatic DNA thermal cycler (Perkin-Elmer Cetus).

Southern blotting was performed as previously described (13). Briefly, DNA transfer was carried out by the Southern blotting method on Zeta probe blotting membranes (Bio-Rad Laboratories, Richmond, Calif.). Prehybridization was carried out for 1 h at 42°C in Rapid-Hib buffer (Amersham, Little Chalfont, United Kingdom) (7). Hybridization was performed in Rapid-Hib buffer containing 30 pmol of the 5′-biotin-, end-labelled oligonucleotide MgPa 2 (5′-GACCATCAAGGTTATTCTCAACAGC-3′) for 2 h at 42°C. After hybridization, the filter was washed twice in 5× SSC (0.3 M NaCl plus 0.03 M trisodium citrate, pH 7.0) and 0.1% SDS for 15 min at room temperature and twice in 0.1× SSC and 0.1% SDS for 30 min at 42°C. Reagents for nonradioactive detection of the hybridization probe were purchased in a kit (PolarPlex detection kit; Millipore, Bedford, Mass.). DNA hybrids were revealed by exposing the filters to X-ray film.

PCR performed on samples of HIV-seropositive and HIV-seronegative individuals was considered positive if it revealed a band corresponding to the 281-bp fragment amplified in the M. genitalium ATCC 33530 control DNA specifically hybridized by the M. genitalium MgPa 2 oligonucleotide probe.

Results. U. urealyticum was found in the genital tracts of 23 (12.3%) HIV-1-infected patients and 8 (7.0%) healthy individuals; the number of positive specimens in non-AIDS patients totalled 10 (8.5%). Compared to healthy subjects and non-AIDS patients, a dramatic increase in the frequency of U. urealyticum colonization was observed in AIDS patients, with 13 (18%) samples testing positive (Table 2). The increase of U. urealyticum isolation between healthy subjects and AIDS patients and between non-AIDS patients and AIDS patients was statistically significant as assessed by Student’s t test (P < 0.05). No statistically significant difference was observed between healthy individuals and non-AIDS patients. There was no qualitative difference in the results obtained with the two culture methods for U. urealyticum detection. All positive cultures, regardless of their patient group source, grew >10⁴ CFU/ml after 24 h of incubation.

M. genitalium was detected in the genital tracts of 52 (27.8%) HIV-1-infected patients. The frequency of M. genitalium-DNA detection in non-AIDS patients was similar to that of healthy subjects; 8 (7.0%) urethral swabs obtained from the healthy subjects and 12 (10.4%) urethral swabs from non-AIDS patients tested positive for M. genitalium. A dramatic and statistically significant increase (P < 0.01) in the frequency of M. genitalium colonization was observed in samples from AIDS patients at stage C3. In fact, 40 (56.0%) specimens tested positive by PCR. M. genitalium was not isolated in SP-4 medium for any of the tested samples.

Discussion. M. genitalium and U. urealyticum are normally harbored in the urethral tracts of healthy individuals (1, 2, 6, 7, 12, 13, 18). In our study, we found that 7.0% of our samples were positive for both of these microorganisms. In non-AIDS patients, the frequency of M. genitalium and U. urealyticum colonization was comparable to that observed in healthy subjects. In contrast, the frequency of M. genitalium and U. urealyticum isolation increased in AIDS patients. The relative occurrences of these two microorganisms may be explained by two nonexclusive phenomena. On the one hand, such data could be the result of defective humoral and cell-mediated immunity typical of AIDS patients. On the other hand, it cannot be ruled out that colonization by antibiotic-resistant M. genitalium and U. urealyticum strains in the urethral tracts of AIDS patients may be linked to alterations in the urogenital environment following antibiotic therapy, which is so extensively used with these individuals. Indeed, differences in the frequency of M. genitalium and U. urealyticum isolation in AIDS patients versus non-AIDS patients can be ascribed to the differences in the use of antibiotics in the two groups of patients; in non-AIDS patients, an expanded-spectrum antibiotic (usually sulfamethoxazole plus trimethoprim) was commonly used for long-term prophylaxis, whereas AIDS patients received broad-spectrum prophylaxis. This hypothesis is also supported by our recent study of U. urealyticum resistance to different antibiotics, including quinolones and tetracyclines, in AIDS patients (12).

In summary, this study demonstrates that M. genitalium and U. urealyticum are found in the urethras of patients with AIDS and to a lesser degree in subjects with asymptomatic HIV infection as well as healthy individuals without signs of ure-
arthrits. Our observations argue against major pathogenetic roles at the urethral mucosal level for these microorganisms.

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