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Demonstration of *Borrelia burgdorferi* DNA in Urine Samples from Healthy Humans Whose Sera Contain *B. burgdorferi*-Specific Antibodies

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Since the possibility of asymptomatic infection with *Borrelia burgdorferi* has been suggested by a positive serology found in healthy subjects, we hypothesized that these subjects might excrete borrelial DNA sequences in urine as happens in patients with Lyme borreliosis. We found borrelial sequences by nested PCR in the urine samples from 3 of 13 healthy *B. burgdorferi* antibody-positive adults but not in urine samples from 79 antibody-negative healthy controls. After therapy with doxycycline, the urine samples were repeatedly negative for *B. burgdorferi* DNA. We conclude that urinary excretion of borrelial DNA sequences may occur in seropositive healthy subjects during asymptomatic infection. Demonstration of such sequences in urine must be interpreted cautiously and may not necessarily prove a borrelial cause of disease.

Borrelia burgdorferi is the causative agent of Lyme borreliosis, a tick-borne disease with a broad array of clinical manifestations including erythema migrans, lymphocytic meningoradiculoneuritis, carditis, arthritis, encephalopathy, and acrodermatitis chronica atrophicans in adults and children (1, 17). Laboratory confirmation of infection with *B. burgdorferi* is usually obtained by serological methods (1, 17). In addition, isolation of the organism from body fluids or demonstration of specific DNA by PCR in patients with Lyme borreliosis has been used and considered to be more specific than serology (4, 5, 7, 10, 11, 14, 15). In contrast to serology, which might indicate immune memory of past events, direct demonstration of the organism in patient material is considered to be indicative of active infection. Even several years after the onset of symptoms, *B. burgdorferi* could be demonstrated by these methods in patients with Lyme borreliosis, thus showing the remarkable capacity of the organism to maintain a persistent infection (7, 10, 13–15). Screening healthy humans for antibodies to *B. burgdorferi* in the United States and Europe has shown a high rate of seropositivity, ranging from 5 to 10%, which could indicate asymptomatic infections (1, 2, 17). We therefore investigated whether it was possible to detect *B. burgdorferi* DNA not only in urine samples of patients with Lyme borreliosis but also in seropositive healthy humans.

We tested urine samples for the presence of *B. burgdorferi* DNA in 13 healthy adults (aged 19 to 42 years) who were shown to have specific antibodies to *B. burgdorferi* by enzyme-linked immunosorbent assay, indirect immunofluorescence, and immunoblot analysis (2) as well as urine samples of 79 seronegative healthy controls at the same age range. The initial urine samples were obtained in winter. The 13 seropositive subjects investigated here included 11 blood donors (2) and 2 healthy laboratory workers. None of these 13 adults had an erythema migrans. They were carefully examined, including physical examination, complete blood count and laboratory chemistry including liver function tests, and chest X ray. Examination did not reveal clinical manifestations compatible with Lyme borreliosis. All were negative for antibodies to human immunodeficiency virus types 1 and 2 and hepatitis C virus. Hepatitis B virus surface antigen could not be detected. In addition, we investigated urine samples from 24 adult patients with Lyme arthritis and from 35 patients with neuroborreliosis. All patients and controls lived in Franconia, a region in northern Bavaria known to be endemic for Lyme borreliosis (8). Specific detection of *B. burgdorferi* DNA was performed by nested PCR with primers from the *fla* gene as described previously (7, 10). The extraction of DNA and the PCR conditions were as described previously (7). The PCR assay amplifies a portion of the flagellin gene highly specific for *B. burgdorferi* (7, 10). The specificity of the assay was shown by the failure to amplify DNA from various bacterial species (7, 10). Several controls were included in the PCR experiments. All amplifications were performed in parallel with a negative control (autoclaved water) to exclude spurious results due to trace contamination. In order to exclude inhibitory effects, urine samples were mixed with 100 *B. burgdorferi* B 31 cells and extracted as described previously (7, 10). The precautions used to prevent contaminations were those described by Kwok and Higuchi (12). To further confirm the identity of the DNA resulting from the *fla* PCR, these fragments were purified by the Prep-A-Gene purification kit, as described by the manufacturer (Bio-Rad, Munich, Germany). A total of 1 μg of the DNA was subjected to Taq-cycle sequencing reactions with the Prism Ready Reaction DyeDeoxy Terminator Cycle Sequencing kit (Applied Biosystems, Darmstadt, Germany). A total of 9.5 μl of terminator premix, template DNA (1 μg), and 3.2 pmol of the primers previously described (10) were mixed in a 0.6-ml reaction tube that was filled with distilled water to make a final volume of 20 μl. The tubes were placed in a thermal cycler preheated to 96°C and subjected to 25 cycles with the following parameters: 96°C for 15 s, 52°C for 15 s, and 60°C for 4 min. The cycle sequencing products were extracted with

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TABLE 1. Subjects tested for antibodies to *B. burgdorferi* and for borrelial sequences in urine

Source of urine samples	No. tested	No. with antibodies to *B. burgdorferi*	No. of subjects positive for borrelial DNA sequences in urine (%)
Seronegative healthy adults	79	0	0 (0)
Seropositive healthy adults	13	13	3 (23)[a]
Patients with Lyme arthritis	24	24	9 (37.5)[a]
Patients with neuroborreliosis	35	35	4 (11.4)[a]

[a] Statistical evaluation by U test of the shedding of borrelial DNA in urine showed no significance between the seropositive healthy adults and either patients with Lyme arthritis or those with neuroborreliosis.

phenol-chloroform and ethanol precipitated. The resulting DNA pellets were dissolved in 4 μl of a mixture of formamide and 50 mM EDTA (pH 8.0) at a ratio of 5:1. Separation of sequencing products was performed on 7% denaturing polyacrylamide gels in an A 737 automatic sequencer (Applied Biosystems). Sequence analyses of the positive and negative strands were carried out in duplicate. Comparison of the sequences obtained was performed with the HIBIO Mac DNASIS program, version 2.0 (Hitachi Software Engineering America Ltd., San Bruno, Calif.). The sequences were compared with those of the *fla* gene of the *B. burgdorferi* B 31 strain published by Gassmann et al. (3).

A total of 11 of the 13 seropositive individuals were found among 472 healthy blood donors (2), and a further 2 subjects were found among 31 healthy laboratory workers. *B. burgdorferi* DNA was detectable in urine samples from 3 of these 13 healthy subjects with *B. burgdorferi*-specific serum antibodies (Table 1). A 290-bp fragment was seen on agarose gel electrophoresis, and these amplification products were subsequently purified and subjected to Taq cycle sequencing. The sequences from a 240-bp stretch of the PCR products are shown in Fig. 1. When compared with published sequences for this region of

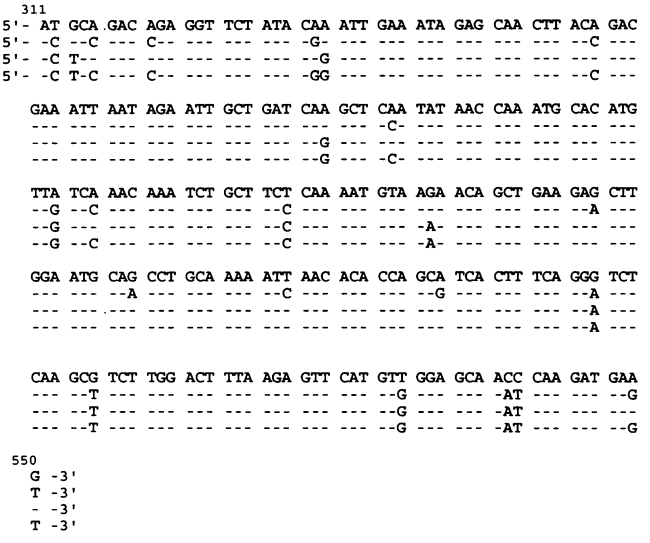

FIG. 1. Comparison of the nucleotide sequences of a 240-bp stretch from the *fla* gene of *B. burgdorferi* B 31 (upper row) with those found in urine samples from healthy subjects with *B. burgdorferi*-specific antibodies. Dashes indicate nucleotides identical to the *fla* sequences of strain B 31.

the *fla* gene from *B. burgdorferi* B 31, 95.8, 93.2, and 92.4% homologies were observed. Two of the three subjects whose urine samples were *B. burgdorferi* DNA positive were treated with doxycycline for 10 days. When the PCR was repeated 4, 6, and 36 weeks subsequent to antibiotic therapy, all urine samples were negative for *B. burgdorferi* DNA. One healthy subject who tested positive by PCR for borrelial sequences in urine refused therapy and tested positive in 12 of 18 urine samples obtained during the ensuing 9 months. During this period, at least one of the two samples tested each month was positive. No *B. burgdorferi* DNA was detected in urine samples from 79 seronegative controls. In addition, urine samples from patients with Lyme borreliosis were analyzed. The data revealed that 9 of 24 patients with Lyme arthritis and 4 of 35 patients with neuroborreliosis were positive by the *fla* PCR (Table 1).

Our results demonstrate persistent shedding of *B. burgdorferi*-specific-DNA in healthy humans whose sera contain *B. burgdorferi*-specific antibodies. Although persistent infection can be proven only by culture of the organism, the PCR detection of specific DNA sequences may suggest active infection. Therefore, these results might have important clinical and diagnostic consequences. Firstly, the question of whether *B. burgdorferi* can be transmitted by blood or blood products remains to be answered. A PCR positive for *B. burgdorferi* in urine might be taken as evidence for a possible spirochetemia in these subjects. Johnson et al. (9) demonstrated survival of *B. burgdorferi* in experimentally infected human blood conserved by routine blood processing and storage methods. However, transmission of *B. burgdorferi* by blood transfusion has not been reported to date (2, 6) and could be excluded for the recipients of blood from the two DNA-positive donors described in this study by lack of clinical manifestation and seroconversion (2). In addition, we could not detect *B. burgdorferi* DNA in the urine of these recipients. This might be due to either the absence or an insufficient infectious dose of *B. burgdorferi* in blood. It is also possible that *B. burgdorferi* is infectious in the presence of acarial products only. Secondly, demonstration of *B. burgdorferi* DNA in urine might not provide unequivocal proof that a patient indeed suffers from Lyme borreliosis, since the test may indicate only latent infection or shedding of DNA rather than disease and the patient may suffer from a different disease in the presence of asymptomatic infection with *B. burgdorferi*. For example, a patient with asymptomatic borrelial infection might be infected with an enterovirus and develop viral aseptic meningitis. Demonstration of borrelial sequences in urine might lead to the erroneous assumption that *B. burgdorferi* was the cause of the patient's meningitis. Although we have not shown this in the present study, such a scenario seems possible as *B. burgdorferi* has been found also in the cerebrospinal fluid without concurrent inflammatory signs (16).

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