Borrelia burgdorferi Persists in the Gastrointestinal Tract of Children and Adolescents with Lyme Disease

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ABSTRACT

This study documents the persistence of *B burgdorferi* DNA in the gastrointestinal tract of pediatric patients who have already been treated with antibiotics for Lyme disease. Ten consecutive patients between the ages of 9 and 13 years presented with an erythema migrans (EM) rash, a positive western blot for Lyme disease, chronic abdominal pain, heartburn, or bright red blood in the stool. Endoscopy assessed the gastrointestinal (GI) mucosa for inflammation and biopsies were examined for *B burgdorferi* using a Dieterle stain and with polymerase chain reaction (PCR) to the outer surface protein A (Osp A) of *B burgdorferi*. As controls, 10 consecutive patients with chronic abdominal pain were also tested by GI biopsies and PCR. *B burgdorferi* persisted in the GI tract in all 10 patients with Lyme disease as shown by Dieterle stain of biopsies and with PCR. None of the control subjects' biopsies were PCR positive for *B burgdorferi*. Chronic gastritis, chronic duodenitis, and chronic colitis were found in Lyme disease patients and associated with the detection of *B burgdorferi* DNA in the GI tract despite prior antibiotic treatments. We have concluded that the DNA of *B burgdorferi* persisted in patients with Lyme disease even after antibiotic treatment.

Key words: Lyme disease, abdominal pain, heartburn, blood in the stool, *B burgdorferi*, gastritis, duodenitis, colitis, polymerase chain reaction

INTRODUCTION

Two previous studies have described the presence of *B burgdorferi* in the stomach, intestines, and colon of children.[1,2] To address the possibility of the persistence of *B burgdorferi* in the gastrointestinal (GI) tract, a prospective study was made of 10 consecutive patients who had a physician-documented erythema migrans (EM) rash followed by symptoms of Lyme disease that persisted for a year before diagnosis and antibiotic treatment was instituted. They had chronic GI symptoms that persisted during this same period and after completing antibiotic therapy. The purpose of this study is to address the possibility of persistence of *B burgdorferi* DNA after antibiotic treatment in patients with Lyme disease.

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MATERIAL AND METHODS

All of the patients included in our study had an EM rash with no prior history of GI complaints. They were referred to the Pediatric Gastroenterology and Nutrition Service of Jersey Shore Medical Center for evaluation of chronic abdominal pain, heartburn, or bright red blood in the stool that persisted for at least one year after the onset of the EM rash. In all cases, antibiotic therapy for the treatment of Lyme disease was instituted one year after the EM rash and initial symptoms of Lyme disease. Ten consecutive patients satisfying the above clinical criteria[3] were evaluated prospectively from January 1993 through July 2000. There were 5 boys and 5 girls evaluated (mean age 14±3.6 years, range 9-12). Each case included a history, physical examination, complete blood cell count, liver function tests, esophagoduodenoscopy (EGD), and/or colonoscopy. One year after the EM rash, a Lyme IgG western blot confirmed a *B burgdorferi* infection by using the commercially available Marblot strip test system (MarDx Diagnostics, Carlsbad, CA). A positive western blot contained the presence of 5 or more of the following Borrelia bands: 18, 23, 28, 31, 34, 39, 41, 45, 58, 66, 93kDa. The interpretation of the *B burgdorferi*

western blot satisfied the surveillance case definition of *B burgdorferi* infection of the Centers for Disease Control and Prevention. Ultrasonography of the abdomen was performed when the history suggested a diagnosis of biliary tract disease, gallstones, or pancreatitis. Stool samples were examined for occult blood, *Salmonella*, *Shigella*, *Yersinia*, *Campylobacter*, ova and parasites, and *Clostridium difficile* toxins A and B. GI biopsies assessed the mucosa by microscopy for *Helicobacter pylori* (on EGD) and for the presence of *B burgdorferi* by Dieterle stain.

Biopsy specimens were taken from areas of the GI tract that looked inflamed during EGD or colonoscopy. The biopsies were repeated on the dates shown in the **Table** because of the persistence of GI complaints despite the antibiotic treatment of Lyme disease. The biopsies were randomly assigned to five histopathologists who were blinded to the diagnosis of the specimens they received. Biopses were reported as acutely inflamed when polymorphonuclear cells were present in the mucosa and chronically inflamed if 6 or more plasma cells and lymphocytes were present in the gastric mucosa without polymorphonuclear cells. Chronic duodenitis or chronic colitis was diagnosed when greater than 6 intraepithelial lymphocytes per 100 surface absorptive cells were present in tissue biopsies in conjunction with a distortion in glandular architecture.

A polymerase chain reaction (PCR) to detect the DNA of *B burgdorferi* outer surface protein A (Osp A) was performed on all biopsies by Medical Diagnostic Laboratories, New Jersey.

DNA Extraction

As a target for DNA amplification, the gene coding for the Osp A of *B burgdorferi* was analyzed. The tissue was dissolved in 470 µL of tris-edetic acid (EDTA) buffer (10 mM tris-hydrochloride [pH, 8.0] and l mM EDTA), 25 µL of 10% sodium dodecyl sulfate, and 12 µL of freshly prepared deoxyonuclease-free proteinase K (10 mg/mL). The mixture was incubated at 55°C for 2 hours; DNA was extracted with phenolchloroform extraction and ethanol precipitation. The purified DNA was dissolved in pyrogen-free, double distilled water and quantified using a Genesys-5 spectrophotometer (Spectronic Instruments, Rochester, NY). The purified quantitated DNA was used as a template for *B burgdorferi* PCR analysis.

Primers

The PCR primers for the identification of *B burgdorferi*, as well as the sensitivity and specificity of the *B burgdorferi* primers are well described. The primers were synthesized by Research Genetics (Huntsville, AL) and purified by high-performance liquid chromatography.

Polymerase Chain Reaction

The PCR mixtures (50 µL) contained extracted DNA (5 µL, 2 µg/µL), P24E, and P12B primers (50 nM), 10 mM trishydrochloride (pH 8.3), 50 mM potassium chloride, 3 mM magnesium chloride, 0.001% (wt/vol) gelatin, the nucleotides dATP, dCTP, dGTP, and dTTP (each at concentrations of 200 mmol/L), and 2.5 U of Taq DNA polymerase (Perkin-Elmer, Foster City, CA). The PCR was carried out in 0.2 mL tubes. The thermocycler was a Perkin-Elmer Gene AMP PCR system 2400. The PCR program ran for 3 minutes at 94°C, followed by 40-one minute cycles at 94°C, 1 minute at 56°C, and 1.5 minutes at 72°C. The program finished with an additional 10-minute extension step at 72°C. A 30 µL sample of the final reaction product was run on 1% agarose gel containing 0.5 µg of ethidium bromide per mL, and the gel was photographed under ultra violet (UV) light.

Histone PCR

Alliquots (5 µL) of the newly extracted DNA were mixed in a 50 µL PCR reaction mixture containing 10X PCR buffer (Perkin-Elmer), 3 mM magnesium chloride, 200 mM dNTP, 2.5 µL of Taq DNA polymerase (5 U/µL), and 1 µL (8 pmol) of 5' and 3' histone amplifier primer set. The histone primers are complementary to the DNA of a constitutively expressed human histone gene H3.3 as described. The amplification process was subjected to 30 cycles of PCR (each cycle at 94°C for 45 seconds, 60°C for 45 seconds, and 72°C for 90 seconds) in a 2400 Perkin-Elmer DNA thermocycler. The histone primers served as internal controls for the sample's DNA integrity, presence of inhibitors, and intersample equivalency of total amount of DNA analyzed.

Precautions Against Contamination

The extraction of DNA and PCR were performed under sterile conditions and in separate rooms. All positive samples were confirmed by re-extraction from the original sample, followed by amplification in triplicate. DNA-positive status was defined as samples that were positive initially and in at least one of the replicates after re-extraction. Pyrogen-free water was used in the isolation of DNA from the biopsy specimens. The Eppendorif microcentrifuge tubes and the PCR tubes were sterilized in an autoclave and UV irradiated. New Finn pipettes were used solely with the filter tips for PCR. Disposable plastic trays were used to prepare PCRs in a UV irradiated PCR biohood. GI biopsy samples from 10 patients with chronic abdominal pain who had no history of tick-borne disease or antibiotic use in the year prior to endscopy were used in the PCR assays as negative controls. The laboratory performing the PCR analysis was blinded to the diagnosis of all specimens they received.