# Journal of Spirochetal and Tick-borne Diseases

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## GUEST EDITORIAL



## ORIGINAL ARTICLES




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## ERRATUM

In the article entitled "The Rabbit as a Model for the Study of Lyme Disease Pathogenesis and Immunity—A Review," by James N. Miller, PhD, et al.,which appeared in the March 1996 issue, we inadvertently omitted the acknowledgements. The acknowledgments should have read: Studies in the authors' laboratories were supported by NIH grant AI-37312 and a gift from Dr. Lin Yeiser Coonan to J.N. Miller, NIH grant AI-29733 to M.A. Lovett, NIH Training Grant 2-T32-AI-07323 to D.M. Foley and J.T. Skare, and NIH Postdoctoral Fellowship Grant 1-F32-AI-09117 to J.T. Skare. The editors apologize for the omission.

# Journal of Spirochetal and Tick-borne Diseases

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# *Guest Editorial*

# Going for the Gold

*Jonathan A. Edlow, MD, FACEP*

In 1996, exuberant athletes from around the world flocked to Atlanta to vie for the gold. No matter who won, each took home some valuable lesson from participating in the games, and each knew what the gold standard was; the competition ended in a measurable, defined endpoint. For those who attended the Ninth Annual International Conference on Lyme Borreliosis and Other Tick-borne Disorders in Boston last year, the excitement and the learning were equally abundant, but that gold standard remains elusive.

I come to Lyme disease with a different perspective than most. My interest began as an outgrowth of my writing medical epidemiologic stories for lay magazines. Because the history of Lyme disease is the medical detective tale par excellence, I started researching it, and the more I learned, the more fascinated I became. I also practice medicine—not neurology or rheumatology or infectious diseases—but emergency medicine. I see patients presenting to the Emergency Department with rashes, facial palsies, swollen joints, erratic heartbeats, and fevers with headache mixed in with colds, heart attacks, and appendicitis.

Nevertheless, the basic science sessions in Boston were especially exciting; in part, I think, because the issues are cleaner. Working with T cells and immunoglobulins lends itself to open-minded application of the scientific method. One makes observations at the bedside, constructs a hypothesis, tests that hypothesis with a carefully designed controlled experiment, and then analyzes the data. The sessions on animal models and pathogenesis were made not only accessible but downright fascinating, and the session on the emerging tick-borne pathogens was especially relevant to me as a generalist. Ehrlichiosis will forever change my approach to the "summer flu."

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During the pathogenesis session, the first of two themes I observed at the conference began to emerge—strain variation. The session on laboratory diagnosis began to suggest the second theme—lack of a reliable diagnostic gold standard. The treatment sessions were full of pearls and observations by clinicians with an enormous collective experience treating Lyme borreliosis. They provoked a great deal of conjecture, and I daresay, a bit of frustration at our current limitations battling this well-adapted spirochete, *Borrelia burgdorferi*.

The issue of strain variation was a recurring theme. How far does this concept go in explaining the situation in Missouri, where patients with an illness clinically indistinguishable from Lyme disease are being diagnosed but from whom *B. burgdorferi* has not been isolated?[1-3] Just as many different relapsing fever-causing borrelia have been found, perhaps we will ultimately learn that many different borrelia cause Lyme disease as well. Preliminary evidence suggests that this hypothesis could be true.[4]

Strain variation implies biologic and genetic diversity, which translates into heterogeneous borrelial proteins and cell-surface markers. This hypothesis too has supporting observations in the literature.[5-8] How does all of this relate to chronic Lyme disease?

Although we always talk about the sensitivity and specificity of diagnostic tests, what the clinician really wants to know is the positive or negative predictive value. How helpful is a positive test for ruling in a given diagnosis or a negative result in ruling it out?

The predictive value of any test is inextricably linked to the pretest probability that the patient has the disease in question. Perhaps the best studied example is in diagnosing pulmonary embolism (PE).[9] We know that in interpreting the ventilation/perfusion lung scan in the case of PE, we *must* factor in the clinical context. Let us look at two patients, each with an identical "low probability" lung scan. The first patient takes estrogens, just slept through a 6-hour flight from Paris to Boston, and has pleuritic chest pain with hypoxia. The second is an active athlete with no risk factors for thromboembolic disease, a dry cough, and left-sided positional chest pain. On clinical grounds alone, the first patient is far more likely to have a PE than the sec-

ond. This first patient, despite the "low probability" scan, still has about a 35% likelihood of having an embolism, whereas the second patient has about a 4% chance.[9] The predictive values of high probability scan results are similarly influenced by the clinical suspicion that the patient has or does not have an embolism. Although this diagnostic process is one of logic, the assignment of the clinical likelihood is part of the "art" of medicine.

The analogy between diagnosing Lyme disease and PE breaks down here because we have a gold standard for PE (the pulmonary arteriogram) to resolve any uncertainty, whereas serologic tests for Lyme disease leave us with a greater degree of uncertainty. Nevertheless, the guiding principle is the same, which brings us back to the discussions at the conference about the interpretation of what constitutes a positive Western blot test.[10,11] It seems to me that one must factor in the clinical (pre-test) probability of Lyme disease (geography, history of tick bite, prior erythema migrans rash, how suggestive the patient's symptoms are, and the season) along with the time factor (is this early or late disease?), before knowing how to interpret a Western blot result for a given patient. One must examine the specific results of the blots, not just the "result" that most laboratories render. Although specific criteria are useful for surveillance and research so that we can compare apples with apples, the clinician must interpret the data in the clinical context.

For example, in a patient presenting with a seventh nerve palsy in August on Nantucket island, the presence of a single significant band on an IgM Western blot would mean more to me than the identical blot in a patient presenting with arthritis in Montana. Similarly, even a positive blot by more strict criteria would carry less significance if the patient had a low pretest likelihood of having Lyme disease; you can't have it both ways.

How will strain variation affect serologic testing? One only finds, as Dr. Liegner pointed out in his presentation at the conference, what one looks for. Variability in the structure of *B. burgdorferi* proteins is well established.[11,12] To the extent that newer strains or species (like *Borrelia lonestari*, sp. nov.)[4] have novel antigenic profiles, we need to readjust the lens through which we look to bring them clearly into focus. Highly specific recombinant antigen from one strain may lead to a better blot for that strain but could yield misleading results for another more genetically diverse strain.

In the history of science, fundamental discoveries are often preceded, not by the small incremental evolution of knowledge, but by a revolutionary new way of thinking, a quantum leap, sometimes afforded by a new instrument or a fresh perspective.[13] After all, isn't that what led Mrs. Murray and Mrs. Mensch to spark Steere's original investigation of a statistically unlikely cluster of childhood arthritis in Lyme, Connecticut, 20 years ago?[14] Isn't that partly responsible for Burgdorfer's discovery of the Lyme disease spirochete 7 years later when he was examining the same ticks everyone else had been intensely scrutinizing for years?[15]

We need the vision, the tenacity, and the wisdom to pursue the basics: open-minded observation of clinical reality, application of the scientific method to test our hypotheses, and rigorous adherence to the logic and art of differential diagnosis. Only then can we forge ahead and try to see things without the blinders of either conventional (or unconventional) wisdom.

Undoubtedly, that is when we will find the gold.

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# Human Ehrlichiosis in Texas

*Julie Rawlings, MPH*

**ABSTRACT**

**Epidemiologic data for 39 human ehrlichiosis cases have been collected. These cases occurred from 1986 to 1995 in Texas. Twenty-nine patients were men and 10 were women; ages ranged from 4 to 78. Most cases occurred in April, May, June, or July. Symptoms included fever, headache, malaise, myalgias, nausea and vomiting, and rash. Abnormal laboratory findings included thrombocytopenia, leukopenia, and elevated liver enzyme levels. Patients with more severe illness experienced severe respiratory symptoms, renal failure, clinical hepatitis, meningoencephalitis, confusion, and seizures. Twenty-eight persons recalled a tick bite prior to the onset of symptoms.**

**Key words:** ehrlichiosis

During the past 10 years, two types of human ehrlichiosis have been recognized in the United States: human monocytic ehrlichiosis (HME) and human granulocytic ehrlichiosis (HGE). HME is caused by *Ehrlichia chaffeensis*, a rickettsial organism with a tropism for monocytes and macrophages.[1,2] This organism, first isolated in 1990,[3] is closely related to *E. canis*, the agent of canine ehrlichiosis. Another species of *Ehrlichia*, related to *E. phagocytophila* (the agent of bovine tick-borne fever) and *E. equi* (the agent of equine ehrlichiosis) causes HGE.[4] This as yet unnamed organism infects granulocytes, primarily neutrophils.[5]

The first case of HME in the United States was reported from Arkansas in 1987.[6] Since then, the disease has been reported from at least 30 states; most of these have been southeastern or south central states where the Lone Star tick, *Amblyomma americanum*, is present.[7,8] More than 400 cases have been identified through laboratory-based surveillance.

The first cases of HGE were recognized in 1993 in Wisconsin and Minnesota.[4] Since then, cases have been reported from Arkansas, California, Connecticut, Florida, Maryland, Massachusetts, New York, Pennsylvania, and Rhode Island. Fewer than 100 cases have been confirmed. The primary vector for the HGE organism is *Ixodes scapularis*, the black-legged tick.[4]

Clinically, both forms of human ehrlichiosis are similar to Rocky Mountain spotted fever.[1,4,9] After an incubation period of 7 to 21 days, patients usually present with an acute febrile illness. Symptoms include fever, headache, malaise, myalgia, chills, nausea, and vomiting. In severe cases, patients may have renal failure, disseminated intravascular coagulation, and coma. A rash is seen in approximately 30% of patients. The mortality rate appears to be 2% to 5% for HME and 5% for HGE.

A compatible presentation with a history of antecedent tick bite is the basis for a clinical diagnosis. Supportive laboratory evidence includes leukopenia, thrombocytopenia, and elevated liver enzyme levels. Confirmation requires a fourfold increase or decrease in *E. chaffeensis* or *E. equi* antibody titer, usually by indirect immunofluorescence antibody (IFA) test or a positive test using polymerase chain reaction (PCR).[1]

Although human ehrlichiosis will not become a notifiable disease in Texas until mid-1996, epidemiologic data for 39 cases reported from 1986 to 1995 have been collected (Table). Of these, 29 patients were men and 10

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*Table*
*Human Ehrlichiosis Cases in Texas, 1986–1995*

| Patient | Onset | Collection | Titer | Exposure |
|---|---|---|---|---|
| JC | 4/86 | 4/24 | <1:40 | TX |
| | | 5/31 | 1:320 | |
| BR | 5/86 | 6/1 | 1:80 | TX |
| | | 6/16 | 1:1280 | |
| | | 4/87 | 1:640 | |
| CY | 7/86 | 7/31 | <1:40 | TX |
| | | 8/12 | 1:160 | |
| | | 4/87 | 1:40 | |
| KA | 7/86 | 7/19 | 1:20 | TX |
| | | 8/8 | 1:640 | |
| JS | 7/86 | 7/17 | 1:80 | TX |
| | | 7/24 | 1:20480 | |
| | | 3/87 | 1:320 | |
| GC | 7/86 | 7/9 | <1:40 | TX |
| | | 7/18 | 1:320 | |
| | | 3/87 | 1:160 | |
| EW | 4/87 | 4/17 | 1:80 | TX |
| | | 5/1 | 1:320 | |
| DS | 4/87 | 5/1 | 1:5120 | TX |
| | | 5/15 | 1:5120 | |
| | | 11/17 | <1:80 | |
| MW | 5/87 | 5/22 | <1:10 | TX |
| | | 6/30 | 1:640 | |
| HW | 6/87 | 7/1 | 1:1280 | TX |
| DC | 4/88 | 5/10 | 1:640 | TX |
| | | 5/18 | 1:5120 | |
| MA | 5/88 | 6/3 | <1:10 | TX |
| | | 6/15 | 1:1280 | |
| LJ | 5/88 | 6/5 | <1:40 | TX |
| | | 5/15 | ⩾1:320 | |
| JF | 6/88 | 7/6 | 1:640 | TX |
| | | 7/18 | 1:2560 | |
| JC | 5/89 | 5/18 | 1:512 | TX |
| | | 6/7 | 1:4096 | |
| RR | 11/89 | 11/30 | 1:512 | AR |
| | | 12/18 | 1:512 | |
| | | 4/90 | 1:32 | |
| RS | 10/89 | 10/17 | 1:1024 | TX |
| | | 11/2 | 1:1024 | |
| MH | 4/90 | 4/11 | <1:64 | TX |
| | | 5/29 | 1:1024 | |
| HC | 6/90 | 6/27 | <1:32 | TX |
| | | 7/31 | 1:512 | |
| OH | 6/90 | 6/26 | 1:512 | TX |
| | | 7/5 | 1:2048 | |
| VG | 4/91 | 4/24 | 1:2048 | TX |
| | | 10/31 | 1:128 | |
| RC | 5/91 | 5/13 | 1:16 | TX |
| | | 7/30 | 1:256 | |
| TM | 4/92 | ? | <1:16 | TX |
| | | ? | ⩾1:512 | |
| RH | 5/92 | 8/5 | 1:512 | TX |
| BB | 7/92 | ? | <1:16 | TX |
| | | ? | ⩾1:512 | |
| DH | 6/93 | 6/1 | ⩾1:512 | TX |
| | | 4/94 | <1:16 | |
| JC | 5/94 | 6/? | IgG >1:1024 | OK |
| | | | IgM >1:320 | |
| DS | 6/93 | 6/26 | 1:256 | TX |
| | | 7/16 | 1:512 | |
| | | 10/26 | <1:16 | |
| CR | 7/94 | 7/13 | 1:1024 | TX |
| | | 7/26 | 1:1024 | |
| | | 8/26 | 1:8192 | |
| VZ | 7/94 | 8/3 | 1:32 | AR |
| | | 8/12 | 1:512 | |
| JB | 4/95 | 5/23 | 1:10,000 | TX |
| PR | 4/95 | 5/22 | IgG 1:1024 | AR |
| | | | IgM 1:20 | |
| HB | 5/95 | 5/16 | 1:512 | TX |
| | | 8/3 | 1:128 | |
| AW | 5/95 | 6/5 | 1:1024 | TX |
| | | 6/30 | 1:1024 | |
| | | | IgM 1:80 | |
| LT | 6/95 | 6/30 | IgG >1:1024 | TX |
| | | | IgM >1:320 | |
| CH | 6/95 | 6/27 | <1:64 | TX |
| | | 7/3 | 1:512 | |
| AP | 6/95 | 7/9 | IgG >1 :1024 | AR |
| | | | IgM >1:320 | |
| BS | 9/95 | 9/21 | <1:64 | TX |
| | | 10/10 | 1:512 | |
| JR | 10/95 | ? | 1:1024 | TX |
| | | 11/7 | 1:4096 | |

were women; ages ranged from 4 to 78 years. Figure 1 shows the month of onset for each of the 39 cases. Twenty-eight (72%) persons recalled a tick bite prior to onset of symptoms. One person was bitten in Eastern Oklahoma and four were bitten in Arkansas; the remaining 34 were exposed in Texas. Figure 2 shows the county of tick exposure or, when patients were exposed outside of Texas, the county of residence for the 39 patients. Epidemiologic features for six cases that were confirmed in 1986 were previously described in detail.[10]

Twelve of the 39 cases occurred during 1994 and 1995. Of these, 11 patients were hospitalized, usually for 5 to 7 days. Persons who had more severe illnesses were hospitalized for longer periods. Symptoms and laborato-

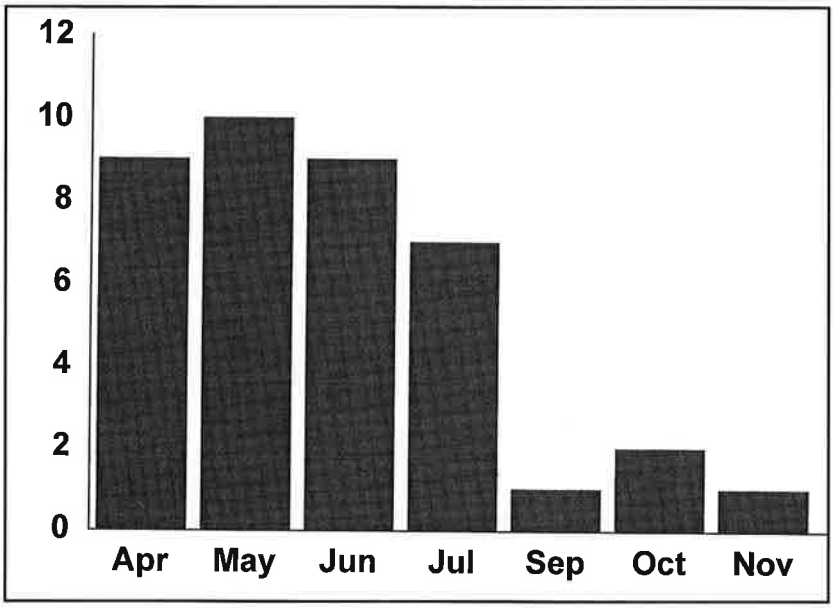


*Figure 1. Human ehrlichiosis cases in Texas by month of onset, 1986–1995.*

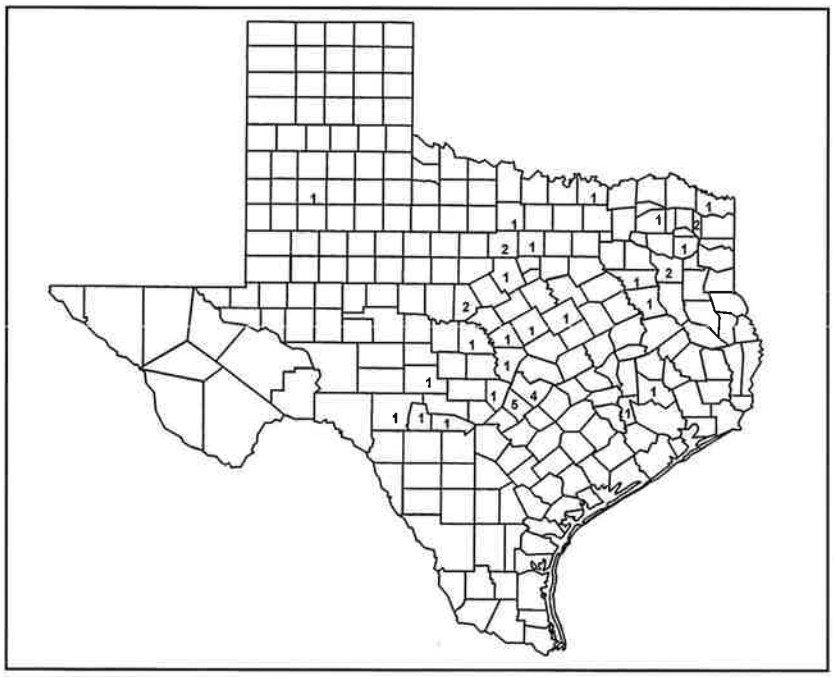

*Figure 2. County of tick exposure or residence for 39 patients with human ehrlichiosis, 1986–1995.*

ry findings included fever (12), headache (10), thrombocytopenia (10), malaise (9), myalgias (9), leukopenia (7), nausea and vomiting (7), elevated liver enzyme levels (5), and rash (5). The patients with more severe illness experienced adult respiratory distress syndrome (ARDS), renal failure, clinical hepatitis, meningoencephalitis, confusion, seizures, or a combination of these symptoms.

All Texas cases to date are considered to be HME. The Lone Star tick is abundant throughout the eastern two thirds of Texas[11] and readily bites humans and other mammals from late February through early October. The black-legged tick also is found in Texas, although it is active in cooler months and only bites humans on occasion. Immature *I. scapularis* prefer to feed on reptiles,[12] and adults feed on mid-sized or large mammals.[13] Further, for all cases reported to date have had positive antibody titers to *E. chaffeensis*, *E. canis*, or both. It should be noted, however, that Dawson et al[14] have shown that unidirectional cross-reactivity does occur. Sera from 34.6% of 26 mice infected with the HGE agent reacted with *E. chaffeensis*, whereas none of the sera from 16 mice infected with *E. chaffeensis* reacted with the HGE agent.

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# The Jarisch-Herxheimer Reaction in Patients with Erythema Migrans

*David J.M. Wright, MD; S.J. Cutler; Gary P. Wormser, MD; Steven W. Luger, MD; and Jeffrey J. Collins, PhD*


## ABSTRACT

**This study examined the relationship between the Jarisch-Herxheimer reaction (JHR) and the severity of early Lyme disease in antibiotic-treated patients. Patients were treated with 500 mg of cefuroxime axetil twice daily (n=182) or 100 mg of doxycycline three times daily (n=173), both for 20 days. Severity of disease was judged based on the duration of signs and symptoms and the number and size of erythema migrans (EM) lesions. The JHR was recorded in 50 of the 355 (14.2%) study patients. Clinically, neither number, size, nor duration of EM lesions predicted the occurrence of the JHR after treatment. The results of this study do not demonstrate a relationship between the development of the JHR and the severity of disease in patients with Lyme disease treated early with antibiotics. Disease severity was judged by various aspects of the EM lesion.**


**Key words:** Jarisch-Herxheimer reaction, erythema migrans lesions

## INTRODUCTION

Jarisch-Herxheimer reactions (JHR) are encountered in 10% to 14% of patients treated for Lyme disease. They frequently occur within 2 to 4 hours of starting therapy and are more common in severe disease.[1-4] The characteristic feature of this reaction is intensification of signs and symptoms that were present before therapy.[5] What remains unknown is which patients are likely to react in this way. If syphilis is a paradigm, the JHR occurs most frequently after the early disease has been present between 30 and 90 days and maximally during the secondary stage, when the disease is the most florid,[6] although JHRs occur in all stages of the disease. Similarly, in other spirochetal diseases, the frequency of JHR is related to the severity of infection. In louse-borne relapsing fever, the JHR occurred in 43% of 389 cases,[7] whereas in leptospirosis, it was reported in up to 80% of patients.[8]

By analogy, it would be logical to examine the relationship of JHR to the severity of early Lyme disease, as determined by the duration of signs and symptoms and the size and number of lesions, and to investigate whether the occurrence of JHR affects patient prognosis. To ensure that only early Lyme disease was examined, physician-diagnosed erythema migrans (EM) was taken as the criterion for inclusion of patients in the analysis of JHR, which is the focus of this report.

## METHODS

### Patients

Patients were enrolled between June and September 1989, and between May and November 1990, into two independent, randomized, investigator-blinded, multicenter trials.[9,10] Outpatients, 12 or older, weighing at least 45 kg (100 lbs), who were diagnosed with early Lyme disease confirmed by the presence of physician-documented EM (with or without systemic manifestations of infection) were candidates for enrollment. The number of EM lesions was recorded, and the primary lesion was measured and (in nearly all cases) photographed.

The exclusion criteria used in these studies have been

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*Table 1*
*Relationship Between Jarisch-Herxheimer Reactions and Various Aspects of Erythema Migrans Lesions*

| Observation | No. patients (%) | No. with observation and JHR (%) | No. with observation without JHR (%) | *P* value |
|---|---|---|---|---|
| Total patients | 355 | | | |
| Patients with JHR | 50 (14) | | | |
| Patients with JHR with fever | 22 (6) | | | |
| Single EM lesion | | 43 (86) | 243 (80) | 0.295 |
| Multiple EM lesions | | 6 (12) | 43 (14) | 0.690 |
| Small EM lesions (<100 cm²) | | 33 (66) | 197 (65) | 0.847 |
| Large EM lesions (>100 cm²) | | 15 (30) | 93 (30) | 0.944 |
| EM duration ≤1 week | | 43 (86) | 241 (79) | 0.252 |
| EM duration 1 to 2 weeks | | 5 (10) | 41 (13) | 0.502 |
| EM duration 2 to 3 weeks | | 1 (2) | 14 (5) | 0.705 |

described in detail.[9,10] The study protocols were approved by the institutional review board at each center, and written informed consent was obtained from all patients. Demographic data for these patients have been published.[9,10]

### Treatment

The study treatments compared in the two clinical trials have been described.[9,10] Briefly, patients were randomly assigned to receive either 500 mg of cefuroxime axetil (Ceftin, Glaxo Wellcome Inc., Research Triangle Park, NC) twice daily or 100 mg of doxycycline (doxycycline hyclate, E.R. Squibb and Sons, Inc., Princeton, NJ) three times daily, according to a computer-generated randomization scheme. Each patient received a 20-day supply of drug.

### Efficacy assessment

A complete medical history and physical examination were done at the time of patient enrollment and 8 to 12 days after initiation of treatment. Electrocardiographic assessments were done at enrollment and 1 to 5 days after completion of therapy. A JHR was defined as an intensification of symptoms within the first 24 hours of antimicrobial use. A stricter assessment requiring the presence of fever was also applied.[6] A two-tailed chi-squared test was used throughout for determination of statistical significance.

## RESULTS

Of the 355 patients enrolled in the two trials, 182 were given cefuroxime axetil and 173 doxycycline. The clinical outcomes in these patients, which have been reported,[9,10] were not significantly different between the two groups. In summary, satisfactory clinical outcomes (cure or improvement) were achieved in 137 of 151 (91%) and in 133 of 144 (92%) of clinically evaluable patients treated with cefuroxime axetil or doxycycline, respectively.

Similarly, no difference was noted in the number of patients having JHR in the two treatment groups. Therefore, in subsequent analyses, all patients have been considered as a single group (Table 1). The total number of patients with JHR was 50 of 355 (14.2%), of whom 18 (10.4%) received doxycycline and 32 (17.8%) received cefuroxime axetil. If fever were taken as an objective sign of JHR, only 22 (6.2%) patients experienced this reaction, 9 (5.2%) in the doxycycline group and 13 (7.1%) in the cefuroxime axetil group. No changes were seen in the electrocardiographic pattern after treatment in any of these patients.

Clinically, 43 of 286 (15.0%) patients had a single EM lesion and a JHR, compared with 6 of 49 (12.2%) with multiple lesions ($P$=0.6; Table 1). When the area within the margin of the sole or primary EM lesion was 100 cm² or less, 33 of 230 (14.3%) patients had JHR, in contrast with 15 of 108 (13.9%) patients with larger lesions ($P$=0.8). The JHR appeared to become less frequent with increased duration of EM (43 of 284 [15.1%] patients with EM duration of <1 week, 5 of 46 [10.9%] with EM duration of 1 to 2 weeks, and 1 of 15 [6.6%] with EM duration of 2 to 3 weeks), although this trend was not statistically significant ($P$=0.50). As expected, the EM lesions were larger the longer they had been present (Table 2). Of 285 patients with lesions larger than 100 cm², 56 (19.6%) had an EM lesion duration of up to 2 weeks, as compared with 11 of 27 (40.7%) such patients who had EM lesions for longer than 2 weeks ($P$=0.011).

*Table 2*
***Relationship Between Duration and Size of Erythema Migrans Lesions***

| | Number of patients by duration (weeks) | | |
|---|---|---|---|
| Size ($cm^2$) | 1 (%) | 2 (%) | >2 (%) |
| <100 | 199 (82) | 30 (70) | 16 (59) |
| >100 | 43 (18) | 13 (30) | 11 (41) |

## DISCUSSION

Our inability to relate the development of the JHR with any clinical feature of EM in early Lyme disease patients was disappointing, particularly in light of reports that cultures of *Borrelia burgdorferi* contain endotoxin-like substances that stimulate the release of cytokines,[11,12] which in turn presumably mediate the JHR.[13] Although the relation in vitro between the amount of endotoxin and the quantity of cytokine released is direct (D. Kwiatowski, personal communication, 1995), the quantities of endotoxin measured are at picogram levels. Others, however, have questioned the role of endotoxin in the development of the JHR.[14]

The lack of a relationship between the JHR and duration of disease before treatment, assuming a constant bacterial multiplication rate, may imply differences in inocula size rather than virulence. This difference, in turn, may be reflected by variation in the size of the primary EM lesion. If the size and duration of the lesion are really based on a quantitative difference in the number of spirochetes resulting from the inoculum dose and duration of infection and thus the time the bacteria have had an opportunity to multiply, then the expectation would be for an increased prevalence of JHR the larger the lesion and the longer they had been present. This expectation was not confirmed. The non-parametric distributions of size of EM lesions with regard to duration and differences in size of various lesions of the same duration may alternatively reflect differences in virulence. Whether this difference in virulence is a reflection of differences in subtype of *B. burgdorferi* or of host recognition or response is still an open question.

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# Gastrointestinal Pathology in Children With Lyme Disease

*Martin D. Fried, MD; Paul Duray, MD; and Dorothy Pietrucha, MD*

## ABSTRACT

**Ten children between the ages of 8 and 19 with Lyme disease presented with chronic gastrointestinal symptoms. Biopsy evidence of inflammation was found in the stomach, duodenum, and colon. Pathologies included gastritis, duodenitis, gastric ulcer, colitis, and a histopathology resembling Crohn's disease. Spirochetes with the microscopic appearance of *Borrelia* were found in five patients with chronic inflammatory conditions of the gastrointestinal tract. The inflammation may have been due to the spirochete itself, a reactive product related to their presence in the gastrointestinal tract, or a consequence of medications used to treat Lyme disease.**

**Key words:** Lyme, abdominal pain, gastritis, duodenitis, colitis, ulcer

## INTRODUCTION

Lyme disease can affect a wide range of organ systems, producing dermatologic, musculoskeletal, neurologic, genitourinary, respiratory, cardiovascular, and ocular manifestations.[1] Reports of gastrointestinal (GI) manifestations have been limited to the liver and the spleen thus far.[2,3] To address the possibility of direct involvement of the GI tract, a retrospective study was made of children who had Lyme disease and chronic abdominal pain.

## METHODS

All patients included in our study had a physician-documented erythema migrans rash and a positive Western blot test[4] and were referred to the pediatric gastroenterology and nutrition service of Jersey Shore Medical Center for evaluation of chronic abdominal pain. The records of 400 children were examined, and 10 patients satisfying the listed criteria were retrospectively identified (2 boys, 8 girls; mean age: 15 ± 4 years, range 8 to 19). Each case record included a history, physical examination, complete blood cell count, liver function tests, ultrasonography of the abdomen, upper gastrointestinal radiograph series with small bowel follow through, esophagogastroduodenoscopy (EGD), and colonoscopy. Stool samples were examined for occult blood, *Salmonella*, *Shigella*, *Yersinia*, *Campylobacter*, ova, parasites, and *Clostridium difficile* toxin. Gastrointestinal biopsy specimens were reviewed to assess the GI mucosa by microscopy and whether *Helicobacter pylori* (on EGD only) or spirochetes were present. Spirochetes were detected in tissue biopsy specimens using a modified Dieterle stain.[5]

Biopsy specimens were reported as acutely inflamed when polymorphonuclear cells were present in increased number and chronically inflamed if plasma cells and lymphocytes were present in increased numbers without an increase in polymorphonuclear cells. Depending on the depth of penetration of the inflammatory cells, severity of inflammation was graded as mild (superficial mucosa), moderate (lamina propria), or marked (deeper than the lamina propria).

## RESULTS

In all of the patients, abdominal pain began 1 month to 4 years before diagnosis (median, 6 months before diagnosis) and before the initiation of antibiotics or non-

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*Table 1*
*Severity and Distribution of Inflammation in the Gastrointestinal Tract of 10 Children and Adolescents With Lyme Disease*

| Patient | Gastritis | Duodenitis | Colitis |
|---|---|---|---|
| 1 | Mild chronic | — | Crohn's-like pathology |
| 2 | — | Mild chronic, spirochetes* | |
| 3 | — | Mild acute | |
| 4 | — | — | Mild chronic, spirochetes* |
| 5 | Gastric ulcer | Mild chronic | |
| 6 | — | Mild chronic, spirochetes* | Mild chronic |
| 7 | Moderate chronic, spirochetes* | Moderate chronic | |
| 8 | Moderate chronic | | |
| 9 | Mild chronic, *Helicobacter pylori* | Moderate chronic | Moderate chronic |
| 10 | Moderate chronic, *Helicobacter pylori*, spirochetes* | Moderate chronic | |

**Spirochetes seen had the microscopic morphology of* Borrelia.

steroidal anti-inflammatory drugs (NSAIDs). Ultrasonography of the abdomen did not reveal any gallstones or pancreatitis. Serum bilirubin, alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase and amylase levels were normal in all patients. Upper gastrointestinal series with small bowel follow through did not reveal a gastric or duodenal ulcer or terminal ileum disease. Endoscopy revealed evidence of inflammation in the stomach, duodenum, or colon in all of the patients (Table 1).

Spirochetes consistent with the microscopic appearance of *Borrelia burgdorferi* were identified in the antrum of the stomach, the duodenum, and the colon, and were associated with chronic inflammation at these sites (Figs 1 and 2).

Two patients with chronic gastritis had *H. pylori* identified in the gastric antrum on biopsy. In one of these, *H. pylori* and spirochetes were present in both the stomach and intestines. The latter patient had been diagnosed with Lyme disease after the occurrence of an erythema migrans rash 2 years before and had completed a 1-month course of intravenous antibiotics prior to EGD.

In four patients who had chronic diarrhea and chronic abdominal pain, no laboratory evidence of *Salmonella*, *Shigella*, *Yersinia*, *Campylobacter*, ova, parasites, or *Clostridium difficile* toxin was found in the stool. These patients had visibly evident blood on several occasions. In three patients, colonoscopy revealed mild to moderate chronic colitis. Spirochetes consistent with *B. burgdorferi* were present in the descending colon in one patient with colitis (Fig 2).

In the fourth patient with abdominal pain, Lyme disease, and chronic diarrhea, biopsies revealed areas throughout the entire colon with patchy active inflammation and cryptitis. Multiple lymphoid aggregates were seen, however, no granulomas were identified. These findings were believed to resemble active Crohn's disease (at another institution) and were treated with daily oral prednisone for 4 weeks. The gastrointestinal symptoms improved, but the patient developed Bell's palsy, chest pain, arthritis of the knee, heart palpitations, photophobia, tinnitus, stiff neck, an antalgic gait, and foot drop. One year after this presentation, blood reappeared in the stool. Colonoscopy biopsies that were independently evaluated by pathologists at Jersey Shore Medical Center and at Brigham and Women's Hospital revealed a chronic mucosal inflammation limited to the ascending colon, with no crypt abscesses, evidence of granuloma formation, or submucosal involvement.

## DISCUSSION

Abdominal pain and the associated GI pathology in children with Lyme disease has not been reported previously. Spirochetes that are not *Borrelia* have been detected in the GI tract previously.[6–8] Patients with gastric syphilis have presented with abdominal pain, vomiting, and weight loss,[7] and endoscopic findings range from minimal erythema to deep ulceration. In another report, colorectal spirochetosis was associated with chronic diarrhea without other intestinal symptoms.[8]

We found strong histopathologic evidence that the *Borrelia* organisms were present in our biopsy samples. In 5 of 10 cases, we found spirochete organisms with the microscopic appearance of *Borrelia* and a characteristic morphology enabling differentiation from other spirochetes that could have been present (Figs 1 and 2). In addition, we found an increased number of plasma cells and lymphocytes in the tissue biopsies, a pattern that is seen in other tissues infected by *B. burgdorferi*.[9,10] *B. burgdorferi* could contribute to GI symptoms by its presence in the GI tract or by its ability to elicit a humoral, inflammatory, or immune response anywhere along the entire GI tract.[11] Duray and Steere[9] have proposed that *B. burgdorferi* can elicit interleukin-1, collagenase, prostaglandin E2, and circulating immune complexes. An intermittent and varying degree of immune complex formation may continue long after an infection is gone,[12] raising the possibility of a spectrum of disease expression from mild chronic inflammation to what appears histologically to resemble Crohn's disease in the colon.

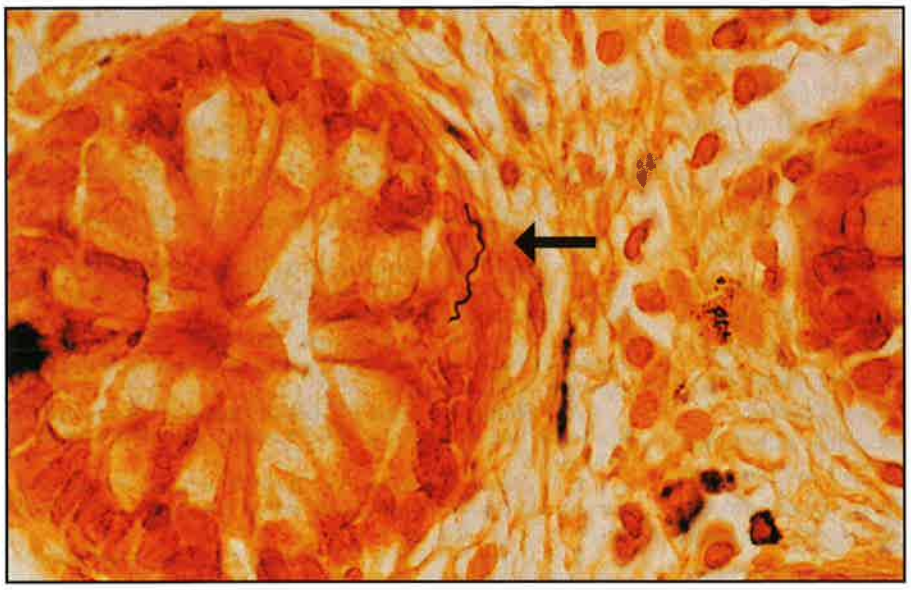

*Figure 1. Colon biopsy specimen from a patient with Lyme disease. Abdominal pain and blood in the stool were symptoms. Arrow shows typical helical-shaped spirochete characteristic of* Borrelia burgdorferi *(Dieterle stain-magnification ×522).*

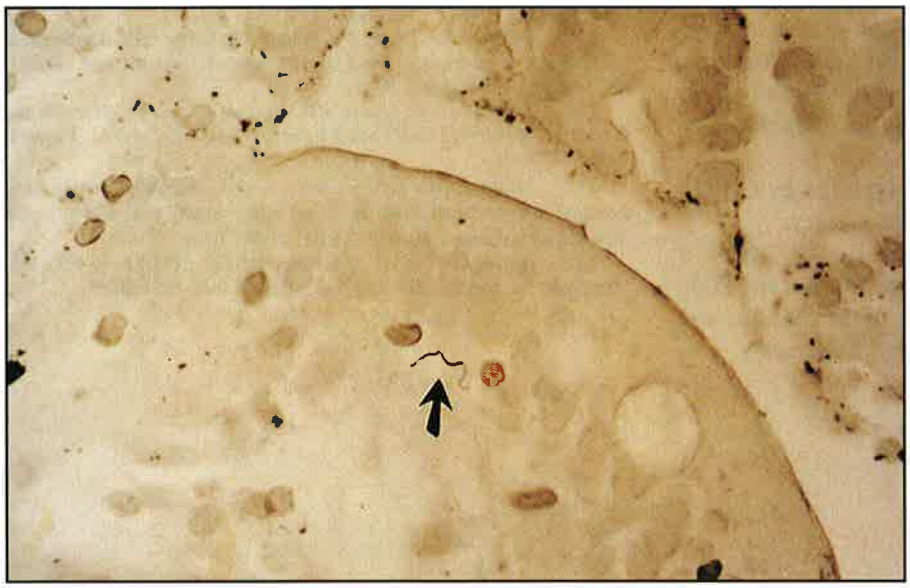

*Figure 2. A spirochete (*arrow*) consistent with the microscopic appearance of* Borrelia *is seen in the basal layer of the columnar epithelium from a duodenal biopsy specimen of a patient with Lyme disease and chronic abdominal pain (magnification ×790).*

One of our patients illustrates this possibility. Patient 1 was initially treated for Crohn's disease, although no clinical or laboratory evidence supported this diagnosis. In this case, treatment of the Lyme disease with antibiotics improved the condition of the colon from one involving its entire length with cryptitis to one described as superficial mucosal involvement restricted to the ascending colon and sigmoid colon. In cases like this one, we suggest that an inflammatory or immune reaction as a result of Lyme disease could injure the colon initially and, even with elimination of the organism, the injury may persist as a Crohn's-like condition because of circulating immune complexes, which have been found in other tissues of patients with Lyme disease.[12]

A diagnosis of Crohn's disease in a patient with a history of exposure to *B. burgdorferi* needs to be weighed carefully. If the inflammation in the colon is in fact an immune reaction to the organism, the use of steroids might be contraindicated because immune suppression might exacerbate the Lyme disease as may have occurred with patient 1. Another treatment of Crohn's disease, sulfasalazine, inhibits prostaglandin synthesis and might be a better choice of drug to ameliorate the inflammatory process of patients with Lyme disease in the colon without causing immune suppression.

We found the spirochetes to be associated with chronic inflammation in 5 of 10 cases. Other causes of inflammation also must be considered. Medications used to treat

Lyme disease also may contribute to GI manifestations. Patient 5 was taking naproxen, an NSAID, for 2 years prior to endoscopy. On endoscopy, a 5 cm × 5 cm gastric ulcer was found. NSAIDs recently have been reported to cause gastroduodenal injury in children with juvenile rheumatoid arthritis.[13] In patients with Lyme disease, colitis, and abdominal pain, the colitis also may have been related to intravenous use of cefotaxime. The *Physicians' Desk Reference*[14] reports a 1.4% incidence of colitis in patients using cefotaxime. NSAID and other medications used to treat Lyme disease pose a significant risk of GI complications and suggest a need for careful differential diagnosis in patients with Lyme disease and chronic GI symptoms.

In summary, all children and adolescents with Lyme disease and chronic abdominal pain had evidence of inflammation on biopsy in the stomach, duodenum, or colon. We found spirochetes in biopsy samples obtained throughout the GI tract that had a microscopic appearance consistent with *B. burgdorferi*. The inflammatory reaction we describe may have been caused by spirochetes, by immune system products elicited in response to spirochete presence, or by medications used to treat Lyme disease. A careful differential diagnosis is recommended in all patients with Lyme disease and chronic abdominal pain or persistent or bloody diarrhea.

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# The Outer Surface Protein C (OspC) from *Borrelia burgdorferi* Can Appear as a Dimeric Molecule in Western Blot

*Sebastian Rauer, MD; Emile Schiltz, PhD; Christiane Rasiah, PhD; and Arnold Vogt, MD*

## ABSTRACT

**Antibodies against the outer surface protein C (OspC) of *Borrelia burgdorferi* are a specific and sensitive marker for Lyme disease, particularly in the early stages. When performing Western blots with native and recombinant OspC, a monoclonal antibody against OspC recognized a band in the range of 50 kDa, in addition to the expected band of 25 kDa. N-terminal amino acid sequence analysis of both the 25-kDa band and the 50-kDa band revealed identity to the N-terminal region of OspC. Because the 50 kDa-band vanished after reduction with 2-mercaptoethanol while the 25-kDa band increased in intensity, we inferred that the 50-kDa band was a thiol-linked dimer of the 25-kDa OspC band. The 50-kDa band was detected in Western blots with whole cell lysates of five different borrelial strains, representing three genospecies of *Borrelia burgdorferi* sensu lato.**

**Key words:** *Borrelia burgdorferi*, OspC-dimer, Western blot

## INTRODUCTION

Laboratory diagnosis of Lyme disease currently relies mainly on serologic testing.[1,2] Western blotting is a common method for the detection of antibodies against *Borrelia burgdorferi*.[3-6] Spirochetes contain proteins that crossreact with those of other bacterial species, like heat-shock proteins in the range of 60 to 70 kDa.[7,8] For a reliable interpretation of Western blots, it is important to identify and characterize bands corresponding to specific spirochetal antigens.[6] In early stages of Lyme disease, specific IgM antibodies are mainly directed against an outer surface protein C (OspC)[3,9-12] and the flagellin of *B. burgdorferi*.[9,13-15] OspC has been detected in Western blots in the range of 21 to 25 kDa.[3,16,17] Coding of the genes for OspC is heterogenous, according to the subspecies of *B. burgdorferi*.[17,18] We now show that OspC can appear as a dimeric molecule in Western blot.

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## METHODS AND MATERIALS

*B. burgdorferi* sensu stricto strains GeHo (provided by K. Pelz, Freiburg), B31 (ATCC35210) and IP1; *Borrelia garinii* strains IP3 (provided by G. Baranton, Paris) and ZQ1 (provided by M. Simon, Freiburg); and *Borrelia afzelii* strain Pko (provided by V. Preac-Mursic, Munich) were grown as previously described.[19] Spirochetes were harvested by centrifugation and washed twice with phosphate-buffered saline containing protease inhibitors (5 mM EDTA, 5 mM sodium tetrathionate, 5 mM benzamidine, 1 mM leupeptin). Cells were disintegrated by ultrasonication. For urea extraction, 8 M urea in phosphate-buffered saline and for Triton-X100 extraction, 1% Triton-X100 in phosphate-buffered saline were used. After extraction, cells were centrifuged (30,000 × gravity, 30 minutes), and the supernatant was used as antigen in Western blot.

Recombinant OspCs were constructed by methods previously described for other borrelial antigens.[19] Oligonucleotides for amplification of the entire open reading frame of OspC by polymerase chain reaction were derived from published sequences[16,17] (sense primer: 5´AGGCACAAATCCATGGAAAAGAATACA 3´, NcoI-site underlined, antisense primer: 5´CTTATAATATGGATCCTATTAAGGTTT 3´, BamHI-site underlined). Polymerase chain reaction products were ligated into the expression vector pJLA602 (medac).

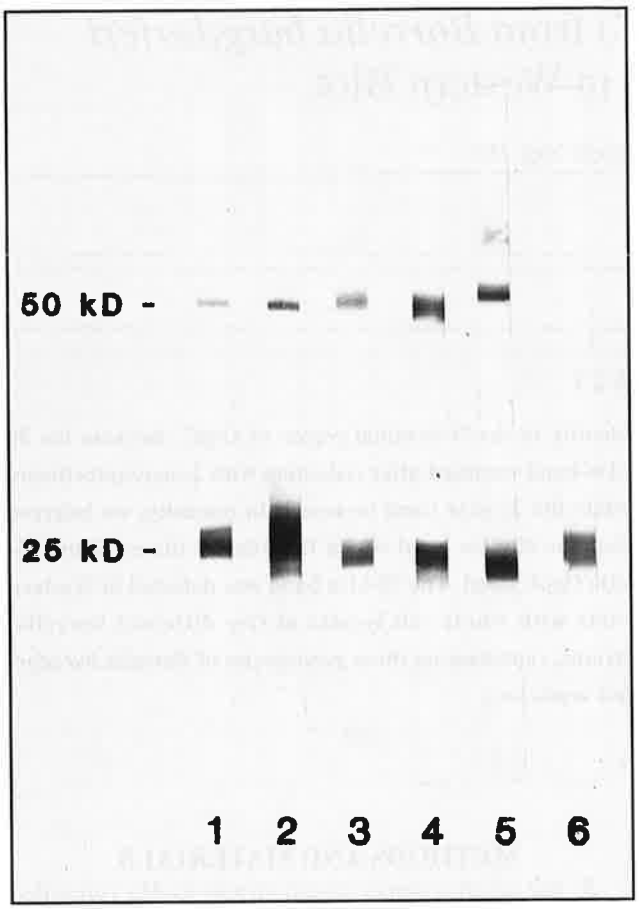


*Figure 1. Western blots with monoclonal antibody BBpCA3 and different antigen preparations. Lane 1: whole-cell sonicate from* Borrelia burgdorferi *sensu stricto; lane 2: recombinant OspC from* B. burgdorferi *sensu stricto; lane 3: whole-cell sonicate from* B afzelii*; lane 4: recombinant OspC from* B afzelii*; lane 5: urea extract from* B. burgdorferi *sensu stricto; lane 6: Triton-X100 extract from* B. burgdorferi *sensu stricto.*

*Escherichia coli* strain HB101 expressing recombinant OspC was grown in Luria broth medium (GIBCO) to a cell density of $10^5$ cells/mL. Expression of OspC was induced by raising the temperature to 42°C for 7 hours. Cells were washed twice in phosphate-buffered saline and extracted in phosphate-buffered saline containing 8 M urea and protease inhibitors. The cells were then stirred for 2 hours at room temperature. After centrifugation, enrichment of OspC was achieved by gel filtration of the supernatant on a Superdex 200 column (Pharmacia). Antigen preparations were subjected to electrophoresis on a 12.6% polyacryamide gel (loading capacity 200 µg) according to the method of Laemmli.[20]

For immunodetection and N-terminal sequencing, proteins were transferred after electrophoresis to a polyvinyliden-diflouride membrane (PVDF, Immobilon-P, Millipore) as described by Towbin et al.[21] The bands were cut out and sequenced in a pulsed-liquid gas phase sequencer equipped with on-line high-pressure liquid chromatography identification of the amino acid phenylthiohydantoins (Applied Biosystems Inc., models 477A and 120A) according to the manufacturer's instructions.

Immunodetection in Western blot was done with peroxidase-conjugated goat-anti-rabbit IgG (Dianova, diluted 1:3000) using diaminobenzidine (Sigma) as substrate.

IgG-Mabs were produced by immunization of 3 Balb/c mice with 80 µg enriched recombinant OspC from *B burgdorferi* strain GeHo in Freund's complete adjuvant. Mice were immunized three times at intervals of 3 weeks: Mab-producing clones were obtained from the mouse that had the highest IgG antibody titer against OspC; clone BBpCA3 had the highest antibody titer against OspC and was used for further investigations.

## RESULTS

Fig 1 shows Western blots with Mab BBpCA3 specific for OspC. In lane 1, whole-cell sonicate of strain GeHo, and in lane 2, enriched recombinant OspC of the same strain are used as antigens. In both blots, a band of 25-kDa corresponding to OspC and a weaker band in the range of 50 kDa are visible. Three other Mabs (BBpCAl-2 and BBpCA4) gave similar results; the bands were weaker because of the lower antibody titers (data not shown). These data suggested that the 50-kDa band corresponds to a dimeric molecule of OspC. We tested this suggestion by N-terminal amino acid sequence analysis of both the 25-kDa band and the 50-kDa band of the recombinant antigen. The 50-kDa band showed the sequence Met-X-Lys-Asn-Thr-Leu-Ser-Ala-Ile-Leu. Lys2, as expected according to the findings of other authors,[16,17] was not detectable, possibly the result of a modification because Lys3 was unambiguously identified. This potential modification is not related to the dimerization because sequencing the 25-kDa band yielded the same result with a missing residue in position 2.

In lane 3 (Fig 1), a whole-cell sonicate of the *B. afzelii* strain Pko and, in lane 4, a recombinant OspC extract of the same strain were blotted with Mab BBpcA3. Both lanes reveal, in addition to the 25-kDa band, a 50-kDa band, indicating that in this subspecies, OspC may also dimerize. In addition, we tested whole-cell lysates of four further borrelial strains representing two genospecies (*B. burgdorferi* sensu stricto: strain B31 and IP1 and *B. garinii*: strain ZQ1 and IP3) with Mab BBpCA3 in Western blot. In strain B31, we could not detect a 50-kDa band, this strain almost did not express OspC (low expression of OspC by strain B31 also was reported by other authors).[17] The other strains revealed the putative OspC dimer in the 50-kDa range (data not shown).

Fig 2 shows an SDS-PAGE of recombinant OspC from strain GeHo. In lane 1, a sample buffer was used without a reducing agent. In lane 2, 2-mercaptoethanol was added to the sample buffer. It was observed that the 50-kDa band nearly vanished (arrowhead), and the 25-kDa band became stronger in lane 2. From this finding, one can assume that OspC is dimerized by disulfide bonds. OspC, in fact, contains two cysteine residues.[16,17]

In Figure 1, lanes 5 and 6, two different extractions from *B. burgdorferi* strain GeHo were blotted with Mab BBpCA3. Lane 5 shows an extraction with 8 M urea. Lane 6 shows an extraction with Triton X100. No 50-kDa band can be recognized in lane 6, whereas in lane 5, a distinct band is seen in the 50-kDa mass range, indicating that Triton X100 prevents the dimerization of OspC, whereas 8 M urea does not.

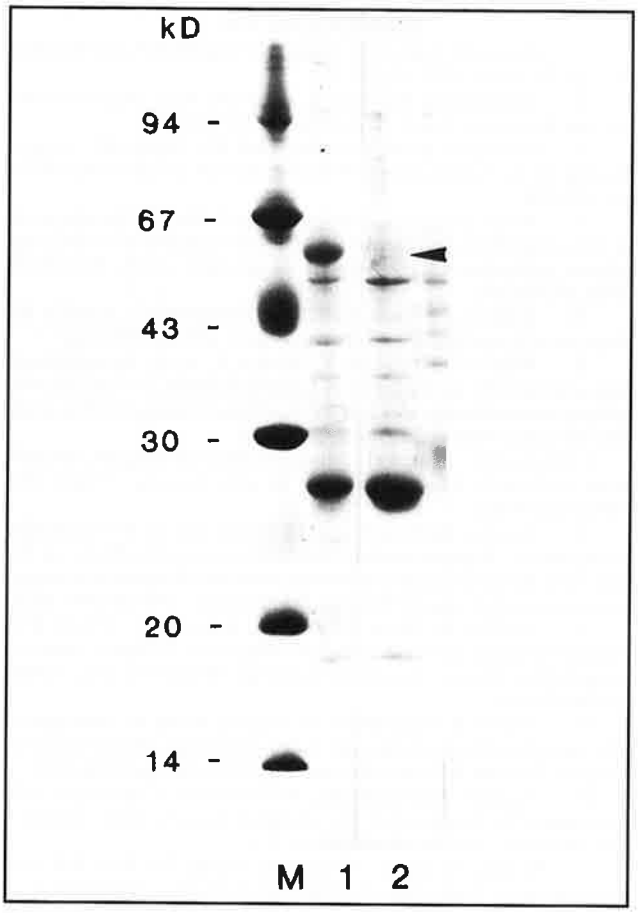


*Figure 2. SDS-polyacrylamide gel (12.6%) stained with Coomassie. Lanes: M, molecular mass markers; 1, enriched recombinant OspC without a reducing agent in sample buffer; 2, enriched recombinant OspC with 2% 2-mercaptoethanol in sample buffer. Arrowhead indicates the position of the vanished dimeric molecule.*

## DISCUSSION

In this study, we have shown that recombinant and native OspC from strain GeHo (*B. burgdorferi* sensu stricto) and strain Pko (*B. afzelii*) may form a thiol-linked dimeric molecule in the range of 50-kDa that can be detected in Western blot. Theisen et al[17] reported on three phenotypic groups of OspC according to three previously defined genospecies. On testing representatives of each of these groups, we observed the 50-kDa band in all of them (except B31; this strain expresses little, if any OspC). Thus, we suggest that other isolates may have similar properties. We suppose that OspC containing a cysteine residue usually may form a dimeric molecule if cell extraction is performed without a reducing agent like 2-mercaptoethanol. In seven of eight published amino acid sequences,[17,18] OspC revealed a cysteine residue.

Our findings may be helpful for interpretation of bands in the range of 50-kDa in Western blots.[3,4] Ma et al[4] tested sera of 186 patients with Lyme disease. They found a 22-kDa band and a 55-kDa band in 69 patients (37.1%). If the 22-kDa band corresponds to OspC, the 55-kDa band possibly could represent an OspC dimer; however, the authors give no further information about the identity of the 22-kDa band or the 55-kDa band,[4] nor whether they used any reducing agents in the sample buffer for Western blot.

Western blot is a nonquantitative method, and results are dependent on the observer, especially in cases of faint bands. Interpretation criteria for Western blots are somewhat controversial regarding the number and the pattern of bands for differentiating between positive and negative sera in Lyme disease.[3-5,22] Preventing the dimerization of OspC would result in a more intense 25-kDA band of the OspC monomer (Fig 2), whereas the 50-kDa band decreases. Because the dimeric 50-kDa band is of no additional diagnostic value compared with OspC, its prevention may improve standardization of Western blots. Therefore, we recommend addition of a reducing agent to the sample buffer prior to performance of SDS-PAGE with cell extractions of *B. burgdorferi*. Triton-X100, when used for cell extraction, also seems to eliminate the 50-kDa band; however, no reducing qualities about Triton-X100 are known, so that the molecular mechanism of this phenomenon is not yet clear.

In conclusion, this study may help interpretation of a possible dimeric 50-kDa band of OspC in Western blot. Prevention of this band may result in a more sensitive and reliable Western blot.

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