GUEST EDITORIAL

Eighth Annual Scientific Conference on Lyme Borreliosis
Satyendra N. Banerjee, PhD

ORIGINAL ARTICLES

Detection of Borrelia burgdorferi-DNA in Urine From Patients With Lyme Borreliosis
Bruno L. Schmidt, PhD; E. Aberer, MD; C. Stoczenhuber; Ch. Wagner; H. Klade, MD; F. Breier, MD; and A. Luger, MD

Intracellular Morphological Events Observed by Electron Microscopy on Neutrophil Phagocytes of Borrelia garinii
Dagmar Hulinska, PhD; Jiri Basta; Rossella Murgia; and Marina Cinco, MSc

IgG Antibodies to Borrelia burgdorferi in Raccoons in Tennessee
Thomas M. Kollaris, Jr., PhD; Donald D. Oorith, PhD; and Timothy D. Lockey, PhD

REVIEW ARTICLES

Cardiovascular Manifestations of Lyme Disease
Bruce M. McManus, MD, PhD; and Shelina Babul, BSc

Acrodermatitis Chronica Atrophicans: Historical and Clinical Overview
Rudolph J. Scrimieri, MD
The *Journal of Spirochetal and Tick-borne Diseases* is published quarterly by SLACK Incorporated, 6900 Grove Road, Thorofare, New Jersey 08086. Dates of publication are: March, June, September, and December, on the third week of the publication month.

**POSTMASTER:** Send change of address to: *Journal of Spirochetal and Tick-borne Diseases*, SLACK Incorporated, 6900 Grove Road, Thorofare, New Jersey 08086. Third class postage paid at Sheperdsville, KY 40165.

**Change of address notices,** including both the old and new addresses of the subscriber, should be sent at least one month in advance.

**Yearly Subscription Rates:**
- Physician: $75.00 year
- Institution: $95.00 year
- Single copies: $25.00 each
- Students, fellows, and residents: $45.00 year
- Foreign: add $20.00 for postage ($10.00 for Canada)

To receive student/resident rate, orders must be accompanied by name of affiliated institution, data of term, and signature of program/residency coordinator on institution letterhead. Orders will be billed at single rate until proof of status is received.

**Back issues** can be ordered at a cost of $25.00 per issue. Back issues sold in conjunction with a subscription are on a prorated basis. Requests for orders should be sent to the *Journal of Spirochetal and Tick-borne Diseases*, SLACK Incorporated, 6900 Grove Road, Thorofare, New Jersey 08086.

**Letters to the editor** in the form of correspondence related to material published in the Journal or some aspects of spirochetal and tick-borne diseases may be submitted. Such letters, if related to work previously published in the journal will be referred to the author of the original work for a response.

Correspondence regarding subscriptions or change of address should be addressed to SLACK Incorporated, 6900 Grove Road, Thorofare, New Jersey 08086.

**Editorial correspondence** should be addressed to: Martina H. Ziska, M.D., Managing Editor, *Journal of Spirochetal and Tick-borne Diseases*, One Financial Plaza, Hartford, CT 06103-2610.
- Telephone: (203) 525-2000
- FAX: (203) 525-8425
- e-mail: jstd@connix.com

**Editorial content:** Topics relating to understanding disease mechanisms and the application of better diagnostic techniques and treatment strategies for all individuals suffering from spirochetal and tick-borne diseases.

**PUBLISHER:**
SLACK Incorporated, 6900 Grove Road, Thorofare, New Jersey 08086.

**SUBSCRIPTIONS:**
SLACK Incorporated, 6900 Grove Road, Thorofare, New Jersey 08086.

**EDITOR-IN-CHIEF:**
Willy Burgdorfer, Ph.D., R.M.L., 903 S. 4th St., Hamilton, MT 59840.

**ADVERTISING:**
SLACK Incorporated, 6900 Grove Road, Thorofare, New Jersey 08086.

Although all advertising material is expected to conform to ethical standards, acceptance does not imply endorsement by the Journal.
Guest Editorial
Eighth Annual Scientific Conference on Lyme Borreliosis
Satyendra N. Banerjee, PhD

Original Articles
Detection of Borrelia burgdorferi-DNA in Urine From Patients With Lyme Borreliosis
Bruno L. Schmidt, PhD; E. Aberer, MD; C. Stockenhuber; Ch. Wagner; H. Klade, MD; F. Breier, MD; and A. Lugner, MD

Intracellular Morphological Events Observed by Electron Microscopy on Neutrophil Phagocytosis of Borrelia garinii
Dagmar Hulinska, PhD; Jiri Basta; Rossella Murgia; and Marina Cinco, MSc

IgG Antibodies to Borrelia burgdorferi in Raccoons in Tennessee
Thomas M. Kollars, Jr., PhD; Donald D. Ourth, PhD; and Timothy D. Lockey, PhD

Review Articles
Cardiovascular Manifestations of Lyme Disease
Bruce M. McManus, MD, PhD; and Shelina Babul, BSc

Acrodermatitis Chronica Atrophicans: Historical and Clinical Overview
Rudolph J. Scrimenti, MD

Correspondence
Malarialotherapy for Spirochetosis Full Circle (Modulation of susceptibility of Borrelia burgdorferi to antibiotics by elevated temperature)

Departments
Information for Contributors
Lyme Disease Foundation Prize Winners
The Journal of Spirochetal and Tick-borne Diseases does not hold itself responsible for statements made by any contributors. Statements of opinions expressed in the Journal reflect the views of the author(s) and not the official policy of the Lyme Disease Foundation.

Copyright© by Lyme Disease Foundation Inc. All rights reserved. No part of this publication may be reproduced or transmitted in any form without written permission by the Executive Director of the Lyme Disease Foundation, One Financial Plaza, Hartford, CT 06103-2610.
INFORMATION FOR AUTHORS AND EDITORIAL POLICY

The following guidelines are in accordance with the “Uniform Requirements for Manuscripts Submitted to Biomedical Journals” and the International Committee of Medical Journal Editors (the “Vancouver Group”) statement, agreed upon at the January 1993 meeting.

The Journal of Spirochetal and Tick-borne Diseases publishes quarterly reviews and original-work studies about any aspect of spirochetal and tick-borne diseases. The primary purpose is to broaden our understanding of spirochetal and tick-borne diseases. Special focus is given to Lyme borreliosis (also known as Lyme disease), as the most prevalent spirochetal and tick-borne disease. The clinical topics may involve all medical disciplines, nursing, and pharmacy, as well as the social, ethical, and biological features of spirochetal and tick-borne diseases.

The Journal is composed of two major sections. One section is devoted to the review of a specific topic that is established by the Associate Editors, and a special guest editor is invited to coordinate the development of up to six manuscripts relating to the specific topic. The second section of original works is composed of unsolicited manuscripts that are subsequently reviewed by the Review Board, as well as external reviewers, depending upon the potential for conflict of interest within the editorial panel and the potential interest by the readership.

Expeditious review of all manuscripts is carried out with a projected response time of not more than six weeks. Rejected manuscripts are returned to authors usually within six weeks. Decisions about potentially acceptable manuscripts may take somewhat longer.

The Journal will publish material defined within the categories described below.

Reviews
Each issue includes a series of state-of-the-art articles on a topic related to spirochetal and tick-borne diseases. The articles represent invited presentation by authorities in the field on topics related to spirochetal and tick-borne diseases, with an emphasis on Lyme borreliosis.

Each manuscript should present a comprehensive state-of-the-art analysis and should be accompanied by an abstract of 300 words or less summarizing the major points.

Peer Review Articles
Original articles of 5000 words or less may be submitted to the editorial office. Each article should be accompanied by an abstract of 300 words or less describing the findings of the original research. All articles will be peer reviewed within a 3-week period with subsequent notification to the authors within 5 weeks of submission. Submitted articles may relate to any area of spirochetal and tick-borne diseases.

Case Reports
Specific clinical case reports describing a unique approach to Lyme disease and other related disorders in the area of diagnosis or treatment may be submitted for review. An abstract of 250 words or less should accompany the text.

Correspondence
Letters to the Editor in the form of correspondence related to material published in the Journal or some aspect of Lyme borreliosis and other spirochetal and tick-borne diseases may be submitted. Such letters, if related to work previously published in the Journal, will be referred to the author of the original work for a response. Letters to the Editor should be submitted in duplicate, typewritten and double-spaced, not exceeding 400 words of text and a maximum of five references. Letters should have no more than three authors, and should be signed by all the authors. Please include a word count. Receipt of letters is not acknowledged, but correspondents will be notified when a decision regarding publication is made.

Editorials
Editorials may be published, usually at the solicitation of the Associate Editors, but unsolicited submissions that relate to an unusual topic of interest exceeding the usual designation of correspondence, i.e., 1000 words or less will be considered.

Photographic Section
The topical photographic section will be a regular feature. Photographs pertinent to articles presented in the Journal, as well as other photographs related to any aspect of spirochetal or tick-borne diseases will be considered for the publication. The guidelines for the submission are designated in Illustrations.
Conflict of Interest

The Journal asks authors of research articles to disclose at the time of submission any financial or other arrangements they may have with a company whose product figures in the submitted manuscript or with a company making a competing product. Such information will be held in confidence while the paper is under review and will not influence the editorial decision, but if the article is accepted for publication, the editors will usually discuss with the authors the manner in which such information is to be communicated to the reader.

Because the essence of reviews and editorials is selection and interpretation of the literature, the Journal expects that authors of such articles will not have any financial or other interest in a company (or its competitor) that makes a product discussed in the article. Potential authors who have questions about these issues should contact the Managing Editor.

Submission of Manuscript

An original and three copies of the manuscript should be submitted to:

Martina Ziska, M.D.
Managing Editor
Journal of Spirochetal and Tick-borne Diseases
Lyme Disease Foundation
One Financial Plaza
Hartford, CT 06103
Telephone: (203) 525-2000
FAX: (203) 525-8425

Manuscripts containing original material are accepted with the understanding that neither the article nor any part of its essential substance has been or will be published or submitted for publication elsewhere before appearing in the Journal.

All manuscripts should be accompanied by a letter of copyright transmittal. This must be signed and dated by all authors. The letter is required before any manuscript can be considered for publication and should contain the following wording:

"In consideration of the Lyme Disease Foundation taking action in editing my (our) submission, the author(s) undersigned hereby transfers, assigns, or otherwise conveys all copyright ownership to the Lyme Disease Foundation. The copyright so conveyed includes any and all subsidiary forms of publication, such as electronic media. The author(s) declares that the manuscript contains no matter that is, to the best of the author's knowledge, libelous or unlawful, or that infringes upon any U.S. copyright."

All manuscripts should be submitted with a cover letter indicating the category for which the manuscript should be reviewed. Copies of any closely related manuscripts should be submitted to the Editor along with the manuscript that is to be considered by the Journal. A cover letter, signed by all authors, should identify the person (with the address and telephone number) responsible for negotiations concerning the manuscripts; the letter should make it clear that the final manuscript has been seen and approved by all authors and that they have taken due care to ensure the integrity of the work. Manuscripts should include a title page, abstract, and text with tables, illustrations, and references below. For the integrity of the published material, manuscripts describing clinical aspects of Lyme borreliosis must disclose: criteria for patient enrollment into the study and criteria for defining "successful" or "nonsuccessful" Lyme borreliosis treatment. Manuscripts without these requirements will be automatically rejected.

Titles and Authors' Names

With the manuscript, provide a page with the title of the article; titles should be concise and descriptive (not declarative). Also include a running head of fewer than 40 letter spaces; the name(s) of the author(s), including the first name(s) and academic degree(s); the name of the department and institution in which the work was done; the institutional affiliation of each author; and the name and address of the author to whom reprint requests should be addressed. Any grant support that requires acknowledgment should be mentioned on this page.

Abstract

Provide on a separate page an abstract of no more than 300 words (original and review articles) or 250 words (case report). This abstract should consist of four paragraphs, labeled Background, Methods, Results, and Conclusion. They should briefly describe the problem being addressed in the study, how the study was performed, the results, and what the authors conclude from the results.

Text

All material should be typed and double-spaced. Standard sequence of methods and materials, results, and discussion should be employed with tables and figures numbered in the order in which they are cited in the text.

Tables

Submit tables typed and double-spaced and provide a heading for all columns with a comprehensive title on separate sheets.

Illustrations

Photographs and figures should be submitted as glossy prints 5x7 in., with one copy of each print for each copy of the manuscript. Figure legends should be provided on a separate sheet with identification of the figure. The back of the glossy print should indicate the number of the figure used.
References

References should be numbered in order of citation in the text, following the American Medical Association guidelines for references. The standard journal abbreviations from Index Medicus should be followed. Numbered references to personal communications, unpublished data, and manuscripts either "in preparation" or "submitted for publication" are unacceptable.

Drug Names

Generic names should be generally used. When proprietary brands are used in research, include the brand name in parentheses in the Methods section.

Units of Measure

Authors should express all measurements in conventional units, with Systeme International (SI) units given in parentheses throughout the text. Figures and tables should use conventional units, with conversion factors given in legends or footnotes.

Permissions

Materials taken from other sources must be accompanied by a written statement from both author and publisher giving permission to the Journal for reproduction. Obtain permission in writing from at least one author for articles still in press, unpublished data, and personal communication. Contributors are obligated to disclose that submissions constitute their own work unless otherwise specified. Contributors shall indemnify the Journal for any claims or actions instituted against the Journal by individuals claiming that the contribution is not the work of the contributor.

Acknowledgment of Receipt

An acknowledgment, with a reference number for future inquiries, is dispatched immediately (this does not apply to letters).
The Lyme Disease Foundation, Inc. is pleased to announce the winners of the Lyme Disease Foundation Prize

Each honoree authored an original paper, published in the *Journal of Spirochetal and Tick-borne Diseases*, contributing significantly to the understanding of spirochetal and tick-borne disease. The value of the first prize is $5,000.00 and a certificate of excellence; the value of the second prize is $2,500.00 and a certificate of excellence.

* First publication year (Vol. I/No. 2 to Vol. II/No. 2)

**FIRST PRIZE WINNER:**
*Induction of B-Cell Mitogenesis by Outer Surface Protein C of Borrelia burgdorferi*
W.W. Whitmire, PhD
(Vol. I, No. 3)
and
*Canine Lyme Borreliosis I.*
J. Robertson, PhD
(Vol. II, No. 2)

**SECOND PRIZE WINNER:**
*Bacteriophages and Ultrastructural Alterations of Borrelia burgdorferi Induced by Ciprofloxacin*
M. Schaller, MD
(Vol. I, No. 2)
and
*Analysis of Relapsing Fever Spirochetes from the United States*
T. Schwan
(Vol. II, No. 1)

**SELECTION COMMITTEE MEMBERS**

Richard Bader, MD, Mt. Sinai Hospital, NY
Willy Burgdorfer, PhD, NIH-RML, MT
Fred S. Kantor, PhD, Yale University School of Medicine, CT
Russel C. Johnson, PhD, University of Minnesota, MN
Patricia Rosa, PhD, NIH-RML, MT
Richard C. Tilton, PhD, BBI-NAACL, CT
Guest Editorial
Eighth Annual Scientific Conference on Lyme Borreliosis
Satyendra N. Banerjee, PhD

The Eighth Annual Lyme Disease Foundation International Scientific Conference on Lyme Borreliosis and other spirochetal and tick-borne diseases, held in Vancouver in April 1995, was a great success. The Lyme Disease Foundation and the Lyme Borreliosis Societies of British Columbia, Alberta, Manitoba, and Ontario deserve congratulations for sponsoring a great scientific program in such a picturesque setting as Vancouver, British Columbia.

The conference was also the occasion to celebrate the early birthday of Dr. Willy Burgdorfer, the discoverer of Lyme disease, who turned 70 on June 27, 1995.

This International Convention was attended by scientists, health care professionals, physicians, veterinarians and microbiologists from countries such as Australia, Austria, Belgium, Canada, Czech Republic, Finland, Germany, Japan, Switzerland and the United States.

The medical-scientific sessions were well coordinated by Martina Ziska on the chair. The co-chairs for the conference, S.N. Banerjee, Willy Burgdorfer and Bettina Wilske, as well as the Poster Session chair, Craig Cleveland, contributed greatly to make this event successful.

The main theme—the mechanisms of Lyme Borreliosis Persistency—was timely for the Canadian audience, because many of the health care professionals and provincial governments in Canada continue to deny the presence of Lyme disease in their areas. The symposium was divided in eight oral sessions and one poster session.

The epizootiology-epidemiology session dealt with

From the Tick-borne Diseases Research Laboratory, Provincial Laboratory, BC, Centre for Disease Control, Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada.

Address reprint requests Satyendra N. Banerjee, PhD, Project Director, Tick-borne Diseases Research Laboratory, Provincial Laboratory, BC, Centre for Disease Control, Department of Pathology and Laboratory Medicine, University of British Columbia, 828 West 10th Avenue, Vancouver, BC, V5Z 1L8, Canada.

The vaccine progress session included studies on induction of Lyme arthritis and role for *B burgdorferi* specific T lymphocytes (Schell), efficacy of recombinant outer surface protein A (OspA) (Phillipp), and an update on recombinant Lyme disease vaccine development (Barbour). Schell demonstrated that *B burgdorferi* specific T-lymphocytes were responsible for the adoptive transfer of severe destructive arthritis. The studies of three recombinant OspA vaccine formulations on Rhesus monkey (Phillip) showed that the vaccinated monkeys were protected against tick challenge. However, the presence of low spirochete burden or a transient infection was noticed at post-mortem analysis.

In the pathogenesis and dissemination session, Wilske discussed outer surface protein antigenic variations and their implications for pathogenesis, diagnosis, and prophylaxis.

Virulent *B burgdorferi* was shown to attack and kill TIB-215 human B lymphocytes (Dorward). *B turicatae* an agent for relapsing fever was shown to induce neuroborreliosis in mouse model (Barbour). This study showed that serotype A infects the brain, but serotype B produces polyarticular arthritis. Thus, Lyme disease manifestations may be due to the characteristic of infecting organisms.

The innovative biology session had three papers. Altamirano presented DNA sequencing studies of 16S RNA of *B burgdorferi* from Canada and USA and showed most of Canadian isolates as *B burgdorferi*. However, many of relapsing fever Borrelia species of the United States showed over 95% similarity suggesting these are variants only and not separate species.

The evidence for a spirochetal etiology of Alzheimer's disease was shown by Miklossy. The presentation on the controversial "Coll/Ag-30 (Mild Silver Protein) The Silver Bullet" was presented by Burgdorfer.

In the therapeutic approaches session, Garon discussed *B burgdorferi* surface DNA network as the possible target
for a new category of antimicrobial agents. The intracellular localization of Borrelia species in human fibroblasts, Langerhans' cells and leukocytes were shown by Hulinska. Once inside the cells the spirochetes became resistant to antibiotic treatment and could be cultured from these cells.

Donta discussed OspA the intracellular invasion of borreliae as a strategy to escape antibiotic treatment.

The diagnostic advancement sessions included tests with chimeric proteins containing peptides from OspA, OspB, OspC, P41 and P93 (Luft), PCR capture assay for blood, urine and other biological fluids (Manak), detection of B burgdorferi-DNA in urine (Schmidt), a solid-phase ELISA-antigen detection system for Lyme disease (Tilton), the immune response and its application toward diagnosis (Schutzer), new tools in the diagnosis of neurologic Lyme disease (Coyle), and morphological heterogeneity of B burgdorferi (MacDonald).

The clinical manifestations of Lyme disease session started with master's presentation of erythema migrans rashes of patients from Missouri and recent isolations of spirochetes from the same region. Price described the first locally acquired case of Lyme arthritis in B.C. The arthritis responded to treatment with doxycycline with no recurrence after 2 years. McManus spoke on cardiac abnormalities in association with acute or chronic borreliosis. Murakami described a new method for the removal of attached ticks with an injection of dilute Xylocaine below the area of the attached tick. The accompanied video showed that tick withdrew itself within 45 seconds.

Scrimenti discussed ACA. ACA is common in Europe and the diagnosis based on the presence of predominant CD4 lymphocyte infiltrate, lymyphatic telangiectasis, and lymphoedema. The symptoms and characteristics of chronic Lyme disease patients were described by Smithson. His analysis of more than 1000 patients showed that patients with Lyme, who are diagnosed early and treated aggressively, become symptom-free in a short time.

The chronic persisting Lyme borreliosis session started with Davies discussing antibiotic resistance in spirochetes. Liegnr presented cases of antibiotic-responsive meningocerephalomyelitis. One sero negative case met all clinical and diagnostic criteria for multiple sclerosis and improved after intravenous cefotaxime treatment. Burrascano discussed the management of chronic Lyme disease. Katzell discussed the diagnosis and treatment of disseminated Lyme disease. In the last oral presentation, Fallon discussed the difficulty of psychiatric assessment of non-responsive patients with persistent Lyme encephalopathy. In some cases, antibiotic therapy offers great relief to the patient.

There were 20 posters on various aspects of Lyme disease in humans and animals. This session generated much enthusiasm as each of the participants was requested to evaluate the merit of the poster presentation.

The public forum was moderated by J.J. Burrascano. In the opening session, Karen Vanderhoof-Forschner presented an overview of Lyme disease. The questions were presented to the panel from the day. Questions on whether other tick species or even non-ticks, such as mites, fleas, and other insects that parasitize rodents, could be responsible for transmitting Lyme disease were asked. New tick species, i.e., Ixodes angustus, Ixodes dentatus, Amblyomma americanum, and Haemaphysalis leporispalustris, were mentioned as carriers of Lyme spirochetes. The possibility of a human vaccine for Lyme disease was discussed. The experts were not sure about the time when such vaccines could be released.

The conference ended with a vote of thanks to the sponsor of the conference, The Lyme Disease Foundation, Inc. and the host, British Columbia Lyme Borreliosis Society.

---

Erratum

Due to publisher's error, references were incorrectly numbered in the Vol. II, No. 3 article "Persistent PCR Positivity in a Patient Being Treated for Lyme Disease" by K. Keszler, MD. We apologize to the author.

JSTD
Detection of *Borrelia burgdorferi*-DNA in Urine From Patients With Lyme Borrelia

Bruno L. Schmidt, PhD; E. Aberer, MD; C. Stockenhuber; Ch. Wagner; H. Klade, MD; F. Breier, MD; and A. Luger, MD

**ABSTRACT**

*Background:* Current diagnostic tests for Lyme disease are dependent upon the host’s serologic response. These antibody assays are insensitive early in infection, and can be problematic due to false positive results and interlaboratory variation. In vitro culture of *Borrelia burgdorferi* from clinical specimens is not practical for routine use and has a low diagnostic sensitivity. A sensitive and specific test for the detection of *B burgdorferi* in clinical specimens is needed not only for diagnosis of suspected early Lyme disease, but also for therapeutic monitoring.

*Patients and Methods:* A prospective, controlled, blinded study was performed with 92 patients with erythema migrans and 61 controls. A simple DNA-extraction procedure and a nested Polymerase Chain Reaction (n-PCR) was developed to detect Bb-DNA in urine of infected patients. As target for the nested PCR a specific part of the flagellin gene was chosen.

*Results:* *B burgdorferi*-DNA was detected in 80 out of 90 (88% to 89%) untreated patients with erythema migrans (EM). Two of the 10 nonreactive patients had re-infections; 3 patients had been examined during the first 2 days of the EM rash and in three patients, EM lasted for more than 7 weeks. In comparison, serology for IgG was reactive in 3% to 16.7%, for IgM in 25.6% to 40.5% of patients with EM patients, depending on different tests used.

Six months after treatment, all but nine patients were negative by urine PCR; however, these patients had ongoing arthralgias. In two of them a treatment failure due to a 2-day interruption could be demonstrated; in one patient, therapy was stopped after 5 days. After retreatment, patients became nonreactive and all specimens taken 1 year after therapy were negative.

*Conclusion:* Detection of *B burgdorferi* in patients with EM can be improved dramatically by examining urine for the presence of Bb-DNA. In addition, this test can be a reliable marker for the efficacy of treatment.

**Key words:** *Borrelia burgdorferi*, nucleic acid, polymerase chain reaction, urine

**INTRODUCTION**

The laboratory confirmation of the clinical diagnosis erythema migrans (EM) is based on the measurement of a *Borrelia burgdorferi* (Bb) specific antibody response. Although these tests have been improved, the diagnostic value is still poor due to low sensitivity in early disease,12 serological cross-reactions,3 and inability to distinguish between actual and past infection because of antibody persistence after adequate therapy.4 These limitations may only be circumvented by a reliable assay for the direct detection of Bb or of Bb-specific components in samples from patients.

The recent introduction of in vitro amplification of DNA by polymerase chain reaction (PCR) might offer a solution. PCR enables a selected DNA sequence to be copied in vitro. With optimal conditions, a single strand of DNA can be copied over a million-fold and then may be detected by conventional methods like Southern blotting, enzyme-linked immunoassays, or chemiluminescence.

PCR assays have been reported for detection of Bb-DNA in cultured spirochetes,5 Ixodes dammini ticks,6 and infected animals.7 In humans, specific DNA sequences had been amplified in blood, cerebrospinal fluid, skin, skin cultures, and synovial fluid.8 A few reports only concern the application of PCR in urine from patients with Lyme borreliosis.9-14 Altogether, in these studies, 45 patients have been examined and sensitivity varied between 50% and 90%. Targets taken for PCR amplifica-
tion included the flagellin gene,9,12 the outer surface proteins (OspA and OspB)13,14 and the chromosomal Ly-gene.10

The aim of the study was to evaluate the diagnostic performance of PCR in patients with EM using urine as sample source; to develop a simple DNA-extraction procedure, which can be used routinely; and to investigate the duration of Bb-DNA excretion in urine after therapy.

METHODS

Patients

Ninety-two patients with clinically diagnosed EM (M:F=33:59, mean age 47.4 years) were seen at the Dermatological Department at the Allgemeine Krankenhaus of Vienna; 19 patients had EM accompanied by systemic symptoms, fever, chills, headache, vertigo, paresthesia, arthralgia, myalgia or diarrhea; 73 patients did remember the date of tick bite and the first appearance of the EM lesion. Duration of EM was from 1 to 100 days (mean=8±16.2); time interval between tick bite and examination was between 3 and 180 days (mean=15±30.6). Patients diagnosed with EM were treated with minocycline (100 mg twice daily) or doxycycline (100 mg twice daily) or phenoxymethylpenicillin (1.5 MU three times daily) or amoxicillin (750 mg three times daily) for 20 days or with ceftriaxone (2 g/day IM) for 15 days. Retesting was done immediately after end of treatment and 6 and 12 months after therapy. Sixty one patients with various dermatological disorders saved as controls.

Bacterial strains

Borrelia burgdorferi ssp. sensu stricto, strains B31, H3, HB1, B garinii, strain PBI, B afzelii, strain PKo, H1, H6, H10 and yet untyped strains H17, H18, H20, H21 and H22 were tested. Strains PKo and PBI were kindly supplied by Dr. Preac-Mursic, University of Munich, Germany. Strains starting with the capital letter H were isolated from lesionial skin of Lyme borreliosis patients in Vienna, strain HB1 was cultured from blood. The following spirochetes served as controls: B hermsii (ATCC 35209), Spirochaeta aurantiaca (ATCC 25082), Treponema denticola (ATCC 33520), T phagedenis and T pallidum (NICHOLS strain). In addition, specificity was tested with urinary tract pathogens: Candida albicans, Chlamydia trachomatis, Enterobacter aerogenes, Gardnerella vaginalis, Neisseria gonorrhoeae, Mycoplasma hominis, Pseudomonas aeruginosa, Staphylococcus aureus and Ureaplasma urealyticum, all clinically isolated.

DNA-extraction

Urine specimens were stored at 4°C and sent to the laboratory at ambient temperature within 3 days. DNA was extracted from urine by the following method: 8 ml of urine samples were centrifuged at 14,000 g for 30 minutes. The pellet was dissolved in 1 ml phosphate (PBS) buffered saline pH 8.0 and stored at -70°C. For analysis 300 µl were centrifuged at 14,000 g for 20 minutes and the resulting pellet was dissolved in 50 µl of PBS. 50 µl of a 5% Chelex-100 suspension (BIORAD, Richmond, Calif) was added to the sample before it was heated at 100°C for 5 minutes, centrifuged for 1 minute at 3,000 g and subsequently chilled on ice. Of the supernatant, 10 µl was used as template-DNA in the first PCR.

PCR

The DNA-preparation was added to 50 µl of a mastermix consisting of 10 mM TRIS (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 0.05% glycerol, 0.1% Triton X-100, 0.1 mM EDTA, 0.1% BSA, 2.0 units of Taq-polymerase (Amplitaq, Perkin Elmer, Cetus, CT), 50 pM of each outer primer BBSCH1: 5’ AGC ATC ACT TGC AGG GTC TC (483-502) and BBSCH2: 5’ TGT CAT TGT AGC ATC TTT TAT TT (881-906). Numbers are corresponding to the flagellin gene sequenced by Gassmann.15 One drop of mineral oil was overlaid to prevent contamination. Reaction was performed in 200 µl thin-walled tubes with attached caps in a PTC-thermocycler with hot bonnet for 25 cycles at temperatures of 95°C, 52°C, and 72°C for denaturation, annealing and elongation respectively. Thermocycling was preceeded by one minute at 95°C and followed by a ten minute extension at 72°C. After this first PCR, 3 µl were transferred with a positive displacement pipette with disposable piston into a mastermix for the second PCR, consisting of the same mastermix, but instead of outer primers 25 pM of each inner primer FL59: 5’ TTT CAG GGT CTC AAG CTT CTT (491-511) and FL7: 5’ GCA TTT TCA ATT TTA GCA AGT GAT G (743-767), modified after Picken (16) were used. Thermocycling was done with same temperatures.
Table 1

Reactivity of PCR in Urine Samples of EM Patients With Primers Published in the Literature

<table>
<thead>
<tr>
<th>PCR</th>
<th>Gene</th>
<th>Primers</th>
<th>Patients</th>
<th>Literature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nested</td>
<td>OSPA</td>
<td>PDO 1,2,3</td>
<td>13/18</td>
<td>Duray (^{17})</td>
</tr>
<tr>
<td>Nested</td>
<td>Ly1</td>
<td>679,680/Bor1 1,2</td>
<td>27/36</td>
<td>Liedtke (^{18})</td>
</tr>
<tr>
<td>Standard</td>
<td>Ly1</td>
<td>679,680</td>
<td>19/36</td>
<td>Goodman (^{10})</td>
</tr>
<tr>
<td>Standard</td>
<td>flag</td>
<td>F2,F3</td>
<td>18/35</td>
<td>Lebech (^{12})</td>
</tr>
<tr>
<td>Standard</td>
<td>flag</td>
<td>FL6,FL7</td>
<td>21/36</td>
<td>Picken (^{16})</td>
</tr>
</tbody>
</table>

and duration (all 30 seconds) as in first PCR, yielding a 277 bp amplicon.

Blank controls, containing 5 µl of water substituted for DNA in the same number as patient samples, as well as a positive control sample with 50 copies of total *B. burgdorferi*-DNA strain B31 and DNA-samples from uninfected as well as from two infected patients, were run in parallel with each amplification assay. If only one of the negative controls was found positive, the whole run was repeated. Positive results were verified by a repeated PCR starting with a second urine extraction. Negative results were checked for inhibition by a newly developed assay.

Extraction of DNA, preparation of PCR mixtures, amplification and analyzing of the ampiclons, were done each in separate areas. Filter barrier pipette tips and a dedicated set of pipettors were used to prepare all samples. All laboratory investigation were done without the knowledge of the clinical data of the patients, nor it was known if the urine was from a presumably infected patient or from a control person.

For preliminary studies, standard and nested PCR with primers PDO 1, 2, 3\(^{17}\), 679, 680,\(^{10}\) 679, 680/Bor1, 2,\(^{18}\) 2,9 F2,F3,\(^{19}\) and FL6,FL7\(^{16}\) were tested as described.

Amplified products (10 µl) were resolved by 2% agarose gel electrophoresis (NuSieve/SeaKem 3:1, FMC-Corporation, Rockland, ME) at 150 Volt for 25 minutes. The gel was then stained with ethidiumbromide and visualized under 254 UV illumination.

Inhibition assay

An internal standard was constructed with 40 mer primers whose inner 20 oligos were specific for the non-related V-erb gene and the outer 20 oligos were Bb-specific.\(^{20}\) All patients who failed to amplify this internal control were considered inhibitory and excluded from the study.

Serology

For specific IgG-detection the following kits were used: Immunowell (p39 recombinant) ELISA (Gen Bio, San Diego, CA), Lyme Borreliosis ELISA (DAKO A/S, Denmark), Borreliosis ELISA (Behring, Marburg, Germany) and Borrelia burgdorferi EIA IgG (Microgen, Munich, Germany). Specific IgM-antibodies were detected with: DAKO Lyme Borreliosis ELISA (DAKO A/S, Denmark), Borrelia burgdorferi EIA IgM (Microgen, Munich, Germany), Borreliosis ELISA (Behring, Marburg, Germany) and Lyme Borreliosis μ-Capture ELISA (DAKO A/S, Denmark).

RESULTS

Preliminary PCR-studies with primers from literature of EM patients yielded only a moderate sensitivity (Table 1). However, the nested PCR design had always a higher detection rate than a standard PCR. Therefore, newly designed outer and inner primers for a nested PCR were chosen in order to improve sensitivity. With primer pairs BBSCH1/BBSCH2 for outer PCR and FL59/FL7 for inner PCR, 80 out of 90 (88% to 89%) untreated patients with EM could be found reactive (Figure).

All *B. burgdorferi* spp. sensu stricte, *B. garinii*, *B. afzelii*, and yet untyped strains isolated from patients in Vienna with Lyme borreliosis (H1-H3, H16, H17, H19, H20-22) could be identified with a sensitivity of less than 5 organisms/PCR. Under experimental conditions, no reaction occurred with *T. pallidum*, *T. phagedenis*, *T. denticola*, *B. henselii*, *Spirochaeta aurantia*, *Candida albicans*, *Chlamydia trachomatis*, *Enterobacter aerogenes*, *Gardnerella vaginalis*, *Neisseria gonorrhoeae*, *Mycoplasma hominis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Ureaplasma urealyticum* (data not shown).

Inhibition of PCR was seen in 2 patients and these had to be excluded from the study. Two of 61 control patients with various dermatological disorders were reactive, yielding a false positive rate of 3%.

The 10 nonreactive EM patients were examined further: two had refections, both with high IgG titer despite only a 4 and 14 days duration of the EM rash, three patients had been examined during the first 2 days of the EM rash and in three patients EM lasted for more than 7 weeks. Six of the 10 nonreactive patients became reactive.
Table 2
Patients Reactive in Urine PCR 6 Months After Treatment

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age/Sex</th>
<th>Initial Treatment/Duration (days)</th>
<th>Clinical Syndrome 6 Months After Treatment</th>
<th>Retreatment Duration (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RK</td>
<td>17/M</td>
<td>minocycline/20; 2-day interruption (area of EM)</td>
<td>myalgias; vertigo; atrophy of quadriceps</td>
<td>ceftriaxone/20</td>
</tr>
<tr>
<td>HH</td>
<td>52/M</td>
<td>amoxicillin/20; 2-day interruption</td>
<td>arthralgia; myositis</td>
<td>ceftriaxone/15</td>
</tr>
<tr>
<td>WH</td>
<td>54/M</td>
<td>minocycline/20</td>
<td>arthralgia; sciatica; epicondylitis; carpal tunnel syndrome</td>
<td>ceftriaxone/20</td>
</tr>
<tr>
<td>AK</td>
<td>47/M</td>
<td>doxycycline/10</td>
<td>arthralgia; paresthesia</td>
<td>ceftriaxone/15</td>
</tr>
<tr>
<td>DH</td>
<td>60/F</td>
<td>ceftriaxone/5</td>
<td>arthralgia</td>
<td>doxycycline/25</td>
</tr>
<tr>
<td>RE</td>
<td>53/F</td>
<td>ceftriaxone/10</td>
<td>arthralgia; myalgia</td>
<td>—</td>
</tr>
<tr>
<td>RM</td>
<td>70/F</td>
<td>ceftriaxone/10</td>
<td>arthralgia</td>
<td>—</td>
</tr>
<tr>
<td>HH</td>
<td>68/F</td>
<td>doxycycline/10</td>
<td>arthralgia; carpal tunnel syndrome</td>
<td>—</td>
</tr>
<tr>
<td>SH</td>
<td>45/F</td>
<td>ceftriaxone/15</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

in the urine PCR when tested immediately after treatment.

Specific IgG-antibodies could be detected in 11.1% to 16.7% of untreated patients with ELISA, using B. garinii as antigen. With a recombinant p39 antigen, only two out of 66 (3%) patients were found positive. IgM sensitivity ranged between 25.6% and 36.7%; only with a μ-capture ELISA 32 of 79 patients (40.5%) were found reactive (Figure).

Immediately after therapy, 21 out of 66 tested patients (31.8%), having been found positive before treatment, were reactive in the urine PCR. Six months later, all but nine patients were nonreactive. As shown in Table 2, all these 9 patients were suffering from ongoing arthralgias; in two of them a treatment failure due to a 2 days therapy interruption could be verified; in an additional patient (D.H.) therapy was stopped after 5 days. Five patients could be retreated and became negative at a 6 months control. All specimens taken 1 year after treatment were negative.

**DISCUSSION**

Spirochetes occur in extremely low numbers in clinical specimens.\(^{21}\) Therefore, the recent introduction of in vitro amplification of DNA by PCR might offer a solution for the detection of Bb with high sensitivity. In order to circumvent amplification failures due to gene variations, known to be present in OspA genes of European strains,\(^{22}\) the flagellin gene was chosen as target.

The decision to use urine samples for investigations in this study was based on three considerations: *B. burgdorferi* has a high affinity to the urinary tract and Bb-antigens could be found in the urine;\(^{23,24}\) a Danish group has discovered a higher detection rate of *B. burgdorferi* in urine compared to the cerebrospinal fluid in patients with neuroborreliosis;\(^ {23}\); and tests of blood specimens did not render a sensitivity relevant for diagnosis (unpublished observations\(^ {25}\)).

Essential for a good PCR is an optimal extraction of spirochetal DNA out of clinical specimens. Generally DNA preparation includes the following steps: disruption of cells with chaotropic salt solutions, treatment with proteinase K to degrade proteins, phenol/chloroform extraction and precipitation with alcohols.\(^ {26}\) These labor-intensive and time-consuming methods cannot be adapted for routine work. Losses inherent to each reaction step can reduce yield of DNA to a high extent, resulting in false-negative PCR results, especially if only a few copies are present in specimens.

In our work, a simple procedure for DNA extraction was chosen. The urine samples were centrifuged, the pellet DNA was washed once and then boiled in the presence of a cation-exchange resin. Parallel experiments without the ion exchanger gave poor results (data not shown). How the mechanism of Chelex-100 works is not known, but it may be due to stabilizing the DNA structure.\(^ {27}\) With this extraction method, losses are negligible; however, DNA is not pure and the matrix is more complex (no protein degradation, no DNA precipitation). Therefore, amplification is not as effective as the use of DNA purified by precipitation.

This disadvantage can easily be compensated by performing a nested PCR. Recalculations have shown that
the second (inner) PCR is working well amplifying to $10^5$ to $10^6$, the amplification factor of the first PCR is generally not higher than $10^3$-$10^4$ in clinical specimens tested. Taken together, the amplification yield of the nested PCR was always higher than with the standard PCR (Table 1). In addition, the nested assay has further advantages: only a few microliters (generally 1 out of 50) of the first PCR are transferred to the second; therefore, inhibitors, presumably being present in clinical specimens, also are diluted out by the same extent (only 2 of 92 or 2.2% of patients specimens have been found inhibited compared to 70% in literature); nested primers generally do not only increase specificity, but can also replace hybridization with labeled probes; and due to the higher amount of generated amplicons, simple detection methods like ethidiumbromid-stained agarose gels can be used without losing sensitivity.

With this DNA extraction and by careful selection of primer pairs, 80 of 90 untreated patients could be found excreting Bb-DNA into the urine. Taking into consideration that out of the 10 nonreactive patients two were reinfections, where dissemination of spirochetes might be different, and three patients were examined at the very beginning of the EM rash (within 5 days after tick bite) or had long-lasting skin lesions of more than 7 weeks, the recalculated sensitivity of EM patients with a duration of the lesion between 5 and 50 days is 77 out of 83 (92.8%).

At the end of treatment, 31.8% of patients were still excreting Bb-DNA in urine. After 6 months, only nine patients with persistent complaints (Table 2) were reactive. After retreatment all 1-year controls were nonreactive, corresponding well with the improvement in clinical findings. These data suggest that the test may be a reliable marker for monitoring efficacy of treatment. However, further studies are needed to analyze excretion after treatment.

It is not known whether whole Bb-cells and/or components (DNA) are excreted with the urine, and with our method, no discrimination thereof is possible. However, the human urinary tract has been shown to harbor spirochetes and Bb-antigens have been detected in urine.

The validity of our PCR method was established by carefully selecting patients by an experienced physician (E.A.), by the blinded study design and the incorporation of fastidious methods to detect false-negative results due to inhibition and false positive results due to contaminations. Precautions to avoid contaminations (see Methods) were apparently efficient, as only 2 of 61 control samples were found false reactive.

Results of antibody testing are comparable to other studies. Comparing a sensitivity of about 15% with IgG tests and from 25% to 35% with IgM tests (only with a μ-capture assay a sensitivity of 40.5% was available), the laboratory confirmation of the clinical diagnosis erythema (EM) migrans can be improved dramatically by examining urine for the presence of Bb-DNA with PCR.

Evaluations of the diagnostic sensitivity of PCR in patients with late complaints of the disease and in patients suffering from suspected Bb-associated diseases, like Lichen sclerosus et atrophicus and Granuloma anulare, are presently under investigation.

REFERENCES

16. Picken RN. Polymerase chain reaction primers and probes derived from flagellin gene sequences for specific detection of the


20. Siebert PD, Larrick JW. PCR MIMICS: Competitive DNA fragments for use as internal standards in quantitative PCR. *BiTechniques.* 1993;14:244-249.


27. Walsh PS, Metzger DA, Higuchi R. Chelx 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *BiTechniques.* 1991;10:506-513.


Intracellular Morphological Events Observed by Electron Microscopy on Neutrophil Phagocytosis of *Borrelia garinii*

Dagmar Hulinska, PhD; Jiri Basta; Rossella Murgia; and Marina Cinco, MSc

**ABSTRACT**

Electron microscopy was used to present morphological events, which accompany the uptake of *Borrelia garinii* by polymorphonuclear leukocytes (PMNs) and intracellular events of phagocytosis throughout 2 hours of contact. Phagocytosis of borreliae proceeded very quickly, and seemed to be independent of opsonins. Opsonized borreliae emitted membrane-bound blebs, which were attached and engulfed by PMNs. Evidence of coiling and conventional phagocytosis were reported in the same cell. Coiling phagocytosis was time dependent (number of spirochetes internalized by this mechanism with time increased), but was not the preferential mechanism of engulfment by borreliae. Borreliae internalized by coiling phagocytosis were less morphologically altered, and their flagellae protruded into the host cytoplasm. The spirochetes were found discharged in PMN cytoplasm, and were not surrounded by a phagosomal membrane. This could be one of the possible mechanisms of persistence of *Borrelia burgdorferi* in the host organism.

**Key words:** *Borrelia burgdorferi*, phagocytosis, electron microscopy

**INTRODUCTION**

Several reports are concerned with the interactions between phagocytes and *Borrelia burgdorferi*, the etiological agent of Lyme disease. This organism is able to trigger an illness, which is characterized by multisystemic involvement and can progress and recur despite therapy. Spirochetes are thought to survive in privileged sites where they are not affected by the immune system, such as the CNS or within cells. Polymorphonuclear leukocytes (PMNs) and macrophages were found to ingest spirochetes and to be activated by them: recent observations under the electron microscope revealed that in addition to conventional internalization, phagocytes internalized borreliae by an unusual mechanism called coiling phagocytosis. Though there is evidence of some intracellular killing of the spirochetes, the molecular mechanisms underlying this event still are unknown and the majority of authors do not exclude the possibility of survival of spirochetes in some of these cells.

Most of this research was performed on macrophages. Because PMNs are the first line of defense against invading organisms, in the present study, we monitored the morphological events that accompany the uptake of *B burgdorferi* by PMN and the fate of this microorganism in the cells throughout 2 hours of contact.

**MATERIALS AND METHODS**

*Borrelia*. A high-passage strain named BITSS, belonging to *Borrelia garinii* was used in the study. It was cultivated in BSK medium up to the logarithmic phase of growth, harvested by centrifugation for 30 minutes at 10,000 g, washed twice, and resuspended in serum-free BSK medium at $10^7$ organisms/mL.

*Polymorphonuclear leukocytes*. Human PMNs were obtained by heparinized venous blood by Dextran sedimentation of red cells and centrifugation of the leukocyte-rich fraction on Ficoll-Paque (Pharmacia, Uppsala). Cells were free from erythrocytes by a brief hypotonic shock and suspended in serum-free BSK medium. Cell suspensions were composed of 90% to 95% neutrophils.

*Phagocytosis assessment*. Mixtures of PMN $2\times10^5$/mL and borreliae $5\times10^7$ cells (ratio 1/20) were incubated in BSK medium for 120 minutes at 37°C under shaking and at times 5, 30, 60, and 120 minutes. Samples were fixed in Sabatini solution for electron microscopy observations processing.

*Electron microscopy*. Double fixation with glutaraldehyde followed by osmium tetroxide was used: 6% glutaraldehyde in 0.2 M cacodylate buffer, pH 7.2, at 4°C, washed in cacodylate buffer, pH 7.2 (3x15 min),
postfixed in 1% osmium tetroxide in the same buffer—1 hour at 4°C—dehydrated in a graded series of ethanol solution and embedded into Lowicryl K4M medium (Che Werke Lowi, Germany) at 23°C. Serial optical sections, 0.5 mm thick were stained with 1% toluidine blue and ultrathin sections on nickel grids were stained with uranyl acetate and lead citrate. Sections were prepared with a Reichert ultramicrotome and examined with Jeol 100Cx electron microscope.

RESULTS

To elucidate the interaction between spirochetes and PMNs, all samples were examined by thin (80 nm) and thick (250 nm) serial sections. Electron microscopy results showed many PMNs associated with spirochetes in all experiments. Phagocytosis of the strain BITS proceeded very quickly. Most of the borreliae seemed to be phagocytosed without the mediation of opsonin.

At 5 minutes, large numbers of the nonopsonized borreliae were seen in close association with or adhering to the PMN plasma membrane (Figure 1A). The PMN membrane fuses with the borreliae surface membrane at any single point. The borreliae could be differentiated easily from the PMNs’ pseudopods with the electron microscope. Of the advancing pseudopods only one fused with the spirochete at a single point (Figure 1B).

After cultivation of PMN with the opsonized borreliae at 5 minutes, spirochetes appeared to be invaginated either by conventional or coiling phagocytosis. A representative cross section from the surface of PMN shows the long finger-like pseudopods bent around the two spirochetes (Figure 1C). Coated vesicles were seen in the cytoplasm of the PMN pseudopods. Opsonized borreliae emitted blebs (Figure 1D); these membrane-bound blebs were either attached to the PMN membrane or engulfed in endocytic vesicles. These vesicles and forming phago-
somes were surrounded by a thick electron-dense membrane (Figure 2A). Larger vesicles were located intracytoplasmically and contained altered borreliae.

At 10 minutes into progression of phagocytosis, a phagosomal activity in both opsonized and nonopsonized borrelial preparations was observed; in fact, Figure 2B shows membrane-lined bags (primary granules) discharging material into the large vesicle-forming phagosome; borreliae inside appeared damaged. In addition to conventional phagocytosis, there also was evidence of coiling phagocytosis: an internalized Borrelia was loosely surrounded by a distinct host cell-derived membrane forming a channel (Figure 2C).

Later, at the 30-minute contact, more borreliae were internalized in the phagosomes, which move from the surface toward the PMN interior (Figure 2D). The smaller vesicles still were lined by an electron-dense (indicated by arrow) plasma membrane. The fusing of the primary granules into ever greater vesicles filled with wastage caused thinning or dissolving of a part of the plasma membrane. Some PMN continuing to accumulate the wastage had both morphologically changed and unchanged borreliae inside one phagosome (Figure 3A). More disrupted borreliae could be found when they were opsonized. Protruding flagellae and blebs were seen in close contact of borreliae with a phagosomal membrane in tangential, as well as in cross sections (Figure 3B). The aggregate of the densely coated pits bordered some phagocytic vesicles in close contact with borrelial flagellae (Figure 3C).

After 60 minutes, in addition to evidence of destruction of borreliae inside phagosomes, some morphologically unchanged spirochetes still were found in the same PMN cell before its death (Figure 3D). Interestingly, these intracytoplasmic borreliae were found free in the cytoplasm after opsonization, and were not surrounded by a phagosomal membrane (Figure 4A). Features of both conven-
tional and coiling phagocytosis could be seen in the same PMN. The borreliae inside the channel appeared intact. The electron-dense material was between the spirochete and coated pits (Figure 4B).

After 120-minute contact, the events described at 60 minutes were observed again. The PMN continuing in phagocytosis contained different stages of Borrelia alteration: borreliae inside vesicles surrounded by a distinct plasma membrane appeared intact, but had protruding flagella and blebs. Borreliae inside channels, mostly with a thinner membrane, were surrounded with a dense fibrillar material, and their flagella protruded into host cell cytoplasm (Figure 4C). Borreliae inside bigger phagosomes appeared damaged.

At this time, it was evident that vesicles frequently contained more than one Borrelia with different morphological features. It could be the result of a fusion of borreliae-containing vacuoles with each other, rather than due to the moving of these organisms or their division, inside the vacuoles. All borreliae found inside channels, being formed during coiling phagocytosis, were less altered and were sometimes found discharged into the PMN cytoplasm (Figure 4D).

**DISCUSSION**

Lyme disease is caused by different species of *B. burgdorferi* sensu lato, like *B. burgdorferi* spp, *Borrelia garinii*, *Borrelia afzelii* and others. The present electron microscopy study deals with the uptake of the high-passage strain, named BITS, of *B. garinii* by human PMN. Results revealed that borreliae were internalized very quickly by both conventional and coiling phagocytosis. Pre-opsonization of *B. garinii* enhanced engulfment but did not influence the uptake mechanism, as has been shown by Rittig et al.

Coiling phagocytosis was time dependent, in fact after incubation for 30 minutes to 60 minutes, the number of borreliae taken up via coiling phagocytosis increased. The extracellular, finger-like pseudopods, which were formed from the peripheral hyaline cortex of the phagocyte, were predominant at 5 minutes and 10 minutes of our experiment. Later, at 30 minutes, we observed intact spirochetes wrapped up by pseudopods, forming a channel, thus protecting them from the action of the azurophilic granules. The borreliae engulfed by coiling phagocytosis were surrounded by a fibrillar material. The process of the degradation of borreliae engulfed by coiling seems to occur at a slower rate than by conventional phagocytosis. In contrast to this coiling, many borreliae engulfed by conventional phagocytosis appeared morphologically altered after 120 min. Rittig et al. suggested that the coiling uptake of *Borrelia* was predominant and behaved like a basic event during phagocytosis by leukocytes taken from various mammals; the report of Rittig, however, did not comment on the morphology of borreliae internalized by this mechanism.

The appearance of borreliae in the cytoplasm not surrounded by a phagosomal membrane in the late stage of phagocytosis, could be interpreted as an escape mechanism of borreliae from the channel into the PMN cytoplasm. Such a hypothesis was already suggested by Montgomery to explain the location, observed by confocal fluorescence microscopy, and survival of borreliae in macrophages. Our findings, that borreliae internalized by the coiling mechanism are less damaged morphologically than by conventional phagocytosis, and that they appear without phagosome membranes in cytoplasm, suggest the possibility of intracellular survival. This would enable borreliae to evade the immune system and to persist in host cells, thus causing a future chronic infection.

Borreliae were seen emitting blebs primarily in the presence of specific antibodies; these vesicles were received on the surface of PMN and internalized by endocytosis. The production of blebs by borreliae has been observed both in vitro (in stationary phase culture) and in vivo, in infected tissues, such as epidermis, cerebrospinal fluid, and blood.

The emission of blebs from borreliae magnifies the amount of surface antigens in the close environment and consequently could enhance the stimulation of PMN.

Regarding the mechanism of recognition of borreliae by phagocytes, which proceeds phagocytosis, Hochmey observed that adherence and entry in Vero cells appeared to be in association with clathrin-coated pits and vesicles. In fact, receptor-mediated endocytosis, which involves the attachment of a small particle or molecule (ligand) to a specific receptor, occurs at specialized regions termed coated pits with clathrin. We found only individual vesicles inside pseudopods and some rare pits in close contact with the wall of the channels. Therefore, we suggest that borreliae do not have to bind to the surface receptors of PMN within these specialized clathrin-coated pits. The other receptors, which are likely to bind a lot of microorganisms via their lectin domains are collagenous C-type lectins (collectins). The lectin domains bind carbohydrates on the microorganism while the collagenous regions are ligands for the collectin receptor on the phagocyte and also mediate C1q-independent activation.

In previous papers, we found different types of glycan structure on the surface of *B. burgdorferi*, spp strain B31, and *B. afzelii*, strain K5, using the blotting technique and immunocytocchemistry with lectins. Patterns of the lectin reaction indicated the presence of mannose, galactose, GalNAc, GlcNAc in the borrelial envelope. We suggest that receptors specific for mannose-binding protein or
other collectins cause the phagocytosis of *Borrelia* by PMN. Immunocytochemical studies are now being undertaken to determine whether collectins are involved in the coiling phagocytosis of *B. afzelii* and *B. garinii* by human fibroblasts (D.H. unpublished data 1995) and PMN.

**Acknowledgment:** This research was supported by a grant from the Ministry of University and Scientific Research (MURST 60%).

**REFERENCES**


IgG Antibodies to *Borrelia burgdorferi* in Raccoons in Tennessee

Thomas M. Kollars, Jr., PhD; Donald D. Ourch, PhD; and Timothy D. Lockey, PhD

**ABSTRACT**

Goat-anti raccoon (*Procion lotor*) IgG was used in an enzyme-linked immunosorbent assay (ELISA) and a Western blot assay to test for borrelial antibodies in sera from raccoons captured in Tennessee. Using the ELISA, 55 of 118 (47%) tested had antibodies to *Borrelia burgdorferi* at a titer >1:160; whereas, by Western blot, 12% of individuals were positive with the presence of 31 Kd and/or 34 Kd band present with other bands (15, 21, 26, 39, 41, 66 or 83 Kd). It appears that a high titer by ELISA is associated with a positive Western blot, but a high ELISA titer is not associated with the presence of a 31 Kd or 34 Kd band. The immunologic evidence found in this study and the recent isolations of *B burgdorferi* in the southern United States indicates that further research on Lyme borreliosis in Tennessee is necessary, including isolation of the spirochete.

**Key words:** Borrelia, Lyme disease, antibodies, enzyme-linked immunosorbent assay, Western blot, raccoon

**INTRODUCTION**

*Borrelia burgdorferi*, the causative agent of Lyme disease, has been isolated from many wild mammals. It was first isolated from white-footed mice and raccoons. Since then, a number of different wild mammals, birds, and ticks have been tested for presence of the spirochete or antibodies to *B burgdorferi* using enzyme-linked immunosorbent assays (ELISA) and immunofluorescent assays (IFA). Luttrell et al tested whether spirochetes could be isolated from deer after laboratory inoculation, with positive results. Magnarelli et al found ELISA to be more sensitive than IFA and more suitable for testing numerous serum samples for antibodies to *B burgdorferi*. ELISAs have been used to detect borrelial antibodies in sera from white-tailed deer (*Odocoileus virginianus*), cotton mice (*Peromyscus gossypinus*), white-footed mice (*Peromyscus leucopus*) and raccoons (*Procion lotor*) in the southern United States. Western blot analysis has been used to detect borrelial antibodies and confirm ELISA tests in humans and other mammals. No reports of borrelial antibodies in raccoons have been made in Tennessee.

The Memphis and Shelby County Health Department began a tick research project in 1990 to ascertain the risk of Lyme disease to the human population in western Tennessee. One of the aspects of this project was to determine if raccoons have antibodies to *B burgdorferi*. Sera from raccoons were tested for antibodies to *B burgdorferi* using the ELISA and Western blot methods to determine if the potential for Lyme disease is present in Tennessee.

**METHODS**

**Study sites and sampling**

Forty-nine one-acre sites were selected randomly for trapping raccoons from June 1990 through August 1991. Within each site, four to six raccoon-sized traps (Tomahawk Live Trap Company, Tomahawk, Wisconsin) were placed. Captured raccoons were anesthetized with a ketamine:rompun mixture in a 1:10 ratio (0.2 ml per 1 kg dosage). Raccoons were examined for ticks and prevalence of tick infestation is reported separately. Blood was collected by cardiac puncture and, after a short recovery period, the raccoons were released in the capture area. All serum samples were stored at -70°C until tested.

**Serologic tests**

The ELISA method was used, with modifications, according to Magnarelli et al. Microwell plates adsorbed with whole cell sonicated *B burgdorferi* (WCS) were provided by Zeus Laboratories, Raritan, New...
Jersey. Goat-anti raccoon (*Procyon lotor*) IgG-peroxidase conjugate (Kirkegaard and Perry Laboratories, Gaithersburg, Maryland) was absorbed with diluent containing *Treponema phagedenis* (MHA-TP) (Miles Laboratories, Elkhart, Indiana) to reduce nonspecific binding (1:100 dilution). Other researchers should note that the goat-anti raccoon conjugate cross-reacted with the WCS but with absorption with MHA-TP, all cross-reactivity ceased.

Dr. Louis Magnarelli (Connecticut Agricultural Research Station) provided a positive and a negative raccoon control sera. Sera were screened at a dilution (in PBS-Tween) of 1:160 also used by other researchers. If positive, sera were diluted serially to an end titer or estimated using a standard curve based on positive controls. The substrate used was ophenylenediamine (1,2-benzendiamine). The positive control serum from the raccoon was found to have a titer of 1:5120 as previously found by Dr. Magnarelli. Nine sera from raccoons testing negative by Western blot were used in addition to the negative control provided by Dr. Magnarelli. A net optical density (O.D.) \( \geq 0.05 \) was considered positive for the diluted sera from raccoons based upon the mean optical density of negative controls (0.02) plus three standard deviations (0.03). The positive control serum from the raccoon was found to have a titer of 1:5120 as previously found by Dr. Magnarelli. All sera testing positive by ELISA were then tested by Western blot analysis.

Western blot analysis was conducted to confirm all of the serum samples that tested positive using ELISA. Western blot analysis was conducted using test strips from the Lyme Disease MARBLOT strip test system (Mardx Diagnostics Inc., Carlsbad, California). Test strips were blocked and washed in a nonfat dry milk buffer (NFDM), and then incubated with diluted sera in sealed pipettes overnight at room temperature. Serum was diluted to 1:50 in an antibody dilution buffer with NFDM. The strips were then washed as above and incubated with diluted conjugate at room temperature for two hours. Goat-anti raccoon conjugate was diluted to 1:200 in antibody dilution NFDM. The strips were washed as above and rinsed with Tris-buffer and horseradish peroxidase color reagent was added. Strips were then rinsed with deionized water after color development. A serum sample was considered positive using the Mardx criteria 1993, (31 Kd and 34 Kd bands occurring together or one of these two bands occurring with at least one of the following bands: 25, 39, 41, or 83). Sera from the same positive and negative controls used in the ELISA tests were used as controls for Western blots. Statistical comparisons between the percent positive by ELISA and Western blot were conducted using Chi-square analysis and stepwise Bonferroni adjustment.

**RESULTS**

Sera from 55 of 118 raccoons (47%) tested positive for borreliai antibodies using ELISA. Titters ranged from 1:160 to 1:1280 for borreliai antibodies. Number of raccoons and reciprocal titers (in parentheses) for the ELISAs were: 29 (160), 15 (320), 8 (640), 3 (1280). Positive individuals were then tested by Western blot analysis.

Seventy-five percent of positive sera from ELISA were negative by Western blot analysis. The 41 Kd band was the most commonly found band using positive ELISA but negative Western blot serum data (Table). Fourteen raccoons tested positive for borreliai antibodies both by ELISA and Western blot analysis (Table).

No significant differences were noted between the percentage positive by both tests with titers at 160 or 320 (\( P \geq 0.05 \)). Significant differences occurred between the percentage positive at a titer of 640 over 160 or 320 (\( P \approx 0.01 \)) and 1280 and 640, 320, and 160. A map of Shelby County showing sites from which raccoons tested positive using Western blot assay are shown in the Figure.
**Table**

Raccoons With Positive ELISAs and Negative Western Blots* and Individual Raccoons Positive by Both ELISA and Western Blot†

<table>
<thead>
<tr>
<th>Negative raccoons</th>
<th>Without Titors</th>
<th>Bands</th>
<th>15</th>
<th>18</th>
<th>21</th>
<th>31</th>
<th>34</th>
<th>39</th>
<th>41</th>
<th>60</th>
<th>66</th>
<th>75</th>
<th>83</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive raccoons</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>with identification number</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>502</td>
<td>160</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>526</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>549</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>934</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1395</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1396</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1400</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1408</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1422</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1432</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1475</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1477</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1497</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1562</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* individuals may have more than one antibody shown; † individual bands shown

**DISCUSSION**

Forty-seven percent of raccoons were positive for borrelial antibodies using ELISA at a ≥1:160 dilution. The ELISA results in this study were similar to results in other eastern states. Borrelial antibodies in raccoons ranged from 15% to 79% from various eastern states. Sera positive by the ELISA were confirmed using commercially available Western blot strips. Cross-reactivity of antibodies can occur in human disease, and this may be true for wild mammals as well. Serologic testing for *B burgdorferi* may give false positive results due to shared antigens with other spirochetes. A number of Western blot studies have been conducted to determine what bands are best in diagnosing the presence of infection by *B burgdorferi*. Fikrig et al set a criterion of 5 of 10 of the most common bands as a positive IgG serodiagnosis for borrelial infection. It appears that a high titer by ELISA is associated with a positive Western blot but that the ELISA titer is not associated with the presence of a 31 Kd or 34 Kd band.

Although rare, cross-reactivity to 31 Kd or 34 Kd bands (OspA and OspB, respectively) by serum antibodies to other diseases can occur in humans. However, reactivity to both 31 Kd and 34 Kd bands only occurred in positive control patients. In the present study, a Western blot was positive if a 31 Kd and 34 Kd band occurred together or if one of these two bands was present with at least one of the following bands: 25, 39, 41, or 83 Kd (Mardx standard). This standard is more conservative than the criteria used by Fikrig et al because a 31 Kd or 34 Kd band must be present. Using the Mardx criteria, the percentage of raccoons having borrelial antibodies was reduced (ELISA versus Western blot results) from 48% to 12% (Table). Use of these conservative criteria may also exclude some positive animals if, as in humans, antibodies to OspA and OspB are produced at later stages of infection or if antibodies to these two proteins are found in immune complexes. Although this conservative criteria may exclude detecting antibodies to non-*Borrelia* species, antibodies against another, or even multiple *Borrelia* species, may have been detected. A *Borrelia* species was isolated from dogs in Florida and a new species (*B andersonii*) has been described from rabbits collected in the eastern United States. In addition, phenotypic variation has been shown to occur in *B burgdorferi* isolates from Illinois and mixed infection of differ-
ent *Borrelia* species has been shown to occur in wild mammals.\(^{35}\)

Positive titers were found in raccoons from urban areas of Shelby County and areas within the city of Memphis and indicate that raccoons are coming into contact with vector tick species. *B. burgdorferi* has been isolated from the blacklegged tick (*Ixodes scapularis*), *Ixodes cookei*, *Ixodes denettii*, and the lone-star tick (*Amblyomma americanum*) in the southern United States.\(^{12,26-29}\) Of these four species, only *I. scapularis* and *I. denettii* have been shown to transmit *B. burgdorferi* in the laboratory\(^{30,32}\) and these tick species have been collected in Tennessee.\(^{16,33,34}\) Positive raccoon sera are associated with areas in Shelby County having substantial white-tailed deer (*Odocoileus virginianus*) populations. White-tailed deer are important in the maintenance of *I. scapularis* populations.

Human cases of Lyme borreliosis have been reported in Tennessee.\(^{35}\) Serologic testing of wild mammals, such as raccoons, can provide important information in surveillance programs\(^{10}\) and may indicate areas of increased risk for Lyme disease in Tennessee. Raccoons have become well adapted to the urban environment of Memphis. They often inhabit attics or chimneys and many people encourage raccoons to live in close proximity by feeding them. By encouraging raccoons to forage and live in their neighborhoods, people may increase their risk of coming into contact with vector ticks. We suggest that *B. burgdorferi* or another *Borrelia* species infects raccoons in Western Tennessee. The occurrence of *B. burgdorferi* in the southeastern United States has been documented\(^{26}\) and it has recently been isolated in southeastern Missouri about 150 miles north of Memphis.\(^{36}\) The isolation of *B. burgdorferi* from ticks or wild mammals is necessary to confirm the presence of this spirochete in Tennessee and is the focus of ongoing studies. Based upon the serologic evidence, further research of the interactions and ecology among borrelial spirochetes, ticks, and hosts is necessary in Tennessee.

**Acknowledgement.** Appreciation is expressed to Paul Peel, Ted Pierce, Daniel Markowski, Willie Crutcher, and Andrew Kuhn for their help in collecting ticks and sera from wild mammals. We also thank Zeus laboratories for providing ELISA titer plates. Funding of this research was supported in part by the Memphis and Shelby County Health Department, Department of Biology (University of Memphis), the Institute of Arthropodology (Georgia Southern University), and the Lyme Disease Institute (Cape Girardeau, Mo).

**REFERENCES**


21. Schutzer SE, Coyle PK, Brunner M. Identification of specific


Cardiovascular Manifestations of Lyme Disease

Bruce M. McManus, MD, PhD; and Shelina Babul, BSc

Key words: Lyme disease, cardiac manifestations, borrelial infections, myocarditis, vascular diseases

Lyme borreliosis is a globally occurring, multisystemic illness caused by the recently recognized spirochetal bacterium, *Borrelia burgdorferi*, and transmitted by the tick of the genus *Ixodes*. Since the late 1970s when cardiac abnormalities were first appreciated in association with acute or chronic borreliosis, approximately 200 patients with infection-related heart disease have been recognized worldwide (Table 1). Each year, the number of patients with borreliosis has been increasing steadily and recognition of the cardiovascular manifestations of Lyme disease has improved. This article will review the most common cardiac manifestations of Lyme borreliosis.

Generally speaking, this disease is contracted during the summer months, June through September, occurring most frequently in Northeastern, North-Midwestern, and Western regions of the United States, although it occurs in Canada, Europe, Australia, Soviet Union, China, Japan, and Africa (Figure 1). Lyme disease is the most frequently diagnosed of any zoonotic disease with the annual occurrence of newly affected patients being greater than the sum of all other tick-borne illnesses in the United States and appears to be spreading rapidly—from 59 reported cases in 1975 to 863 reported cases in 1985. In Delaware, reported cases of Lyme disease increased 246% between 1989 and 1992. Because Delaware does not solicit Lyme disease reports actively, even this level of disease occurrence probably reflects under-reporting. The reporting of cardiac involvement may be as unreliable.

Lyme disease develops in three approximate stages with an evolution of clinical manifestations that includes remissions. Disease onset is heralded by fatigue, fever, chills, and headache, at times in association with Erythema Chronicum Migrans (ECM) rash 2 to 30 days post-tick bite. About 25% to 50% of patients will have secondary skin lesions at various sites of the body during this stage.

Stage 2, weeks to months after ECM, is predominated by neurological complications and migratory musculoskeletal pain. It is at this stage that cardiac abnormalities may become prominent, ranging from a high frequency of electrophysiologic perturbations, with fluctuating degrees of atrioventricular block (Figure 2), intraventricular conduction defects and bundle branch blocks, sinoatrial block, and fluctuating dysrhythmias. Atrial fibrillation occurs occasionally and the degree of atrioventricular block may vary from one minute to the next. Perimyocarditis, left ventricular dysfunction, and congestive heart failure are also seen in about 15% of these patients with evidence of heart involvement.

The Centers for Disease Control and Prevention in Atlanta reviewed 84 patients presenting with Lyme carditis; 69% had palpitations, 19% had conduction abnormalities, 10% had myocarditis, 5% had left ventricular failure, and 21% required hospitalization. In another series, McAlister et al reviewed 52 patients with Lyme disease with cardiovascular manifestations; 87% had symptomatic atrioventricular block and 40% showed Wenckebach-type periodicity, while complete atrioventricular block was encountered in 50% (Table 2).

Lastly, stage 3, several months to years after ECM, is marked by the onset of arthritis, primarily affecting the knees and other large joints. These musculoskeletal features come to dominate as a basis for clinical complaints.

Early detection of Lyme disease is essential because of the potential development of chronic illness and life-threatening complications. In patients with Lyme disease, it has been estimated that 4% to 10% of all North American patients and 0.3% to 0.4% of all European patients will exhibit some form of cardiac involvement. A male predominance of approximately 3:1 in cardiac Lyme disease appears evident. Diagnosis of cardiac involvement in patients with Lyme disease depends on a degree of suspicion when other symptoms and signs of borreliosis in particular, neurological complications are present and when the
patient exhibits presyncope, syncope, palpitations, dizziness, shortness of breath, chest pain, or electrocardiographic abnormalities. Echocardiography is a powerful noninvasive tool to investigate possible cardiac involvement in the course of Lyme disease, allowing assessment of cardiac anatomy, chamber sizes, and myocardial and valvular function.\textsuperscript{14}

The presence and degree of cardiac dysfunction observed on echocardiography thus provide essential information for the management and treatment of affected patients. Exploration by magnetic resonance imaging (MRI) also has been undertaken in the assessment of patients with cardiac involvement.\textsuperscript{15,16} Serological tests that are both sensitive and specific may also aid in establishing the diagnosis of this disease.\textsuperscript{10}

In a study conducted by Mertz and associates,\textsuperscript{15} available diagnostic serological tests were reviewed. These included an indirect immunofluorescence assay and enzyme-linked immunosorbent assay (ELISA) for antibodies against \textit{Borrelia burgdorferi}, tools that detect the disease in 50% of patients in stage 1 and 100% of patients in later stages. The ELISA is more specific and reliable.\textsuperscript{6} Specific IgM antibody levels may be detected in most patients in the early phases of illness and specific IgG antibody levels may be detected in virtually all patients in the later stages.\textsuperscript{17}

Endomyocardial biopsies may sometimes be useful in showing the spirochetes and myocardial infiltration by lymphocytes, plasma cells, and macrophages.\textsuperscript{6} The organism of cause may be stained with silver stains, cultured, or detected by molecular means. Antimyosin and gallium scanning also have been suggested recently in the assessment of patients with Lyme carditis. Indium 111-monoclonal antimyosin antibody imaging in combination with MRI may provide important information in the assessment of Lyme carditis in patients presenting with clinical symptoms and positive serological findings; severe precordial pain of acute onset and arthralgias of the upper limbs, fever, headache, and no prior history of cardiac disease should evoke thoughts of Lyme disease.\textsuperscript{16}

Histologically, Lyme carditis commonly shows transmural inflammatory infiltration by lymphoid cells with small inflammatory clusters of neutrophils and macrophages prominent in hyperacute disease.\textsuperscript{18} Necrosis of myocardial fibers and evolution of endocardial and interstitial fibrosis have been demonstrated. The presence of spirochetes in the myocardium signals a direct pathophysiologic role for the \textit{Borrelia} organism in Lyme carditis.\textsuperscript{12}

Early diagnosis based on a combination of clinical findings, serologic testing, and endomyocardial biopsy results may be favorable in terms of acute management and full functional recovery from Lyme carditis. Cardiac involvement, however, sometimes may be clinically occult and fatal.

In one reported instance,\textsuperscript{9} a 66-year-old man died unexpectedly 8 hours after hospitalization for treatment of babesiosis. The autopsy revealed abundant Lyme spirochetes in the myocardium in the neighborhood of interstitial lymphoid cells, and in an intramyocardial blood vessel. Lymphoplasmocytic pericarditis also was demonstrated and subsequent serologic testing was positive for Lyme
disease. All layers of this patient's heart were inflamed and the presumable cause of death was complete heart block or ventricular arrhythmia. This patient's course indicates that early recognition of Lyme disease with or without cardiac manifestations is important because of the potential for life-threatening complications, to say nothing of chronic disease.

Similarly, an English farm worker with fatal Lyme carditis was reported by Cary et al. Postmortem examination of the heart revealed pericarditis, focal myocarditis, and prominent interstitial and endocardial fibrosis. Whether other patients with Lyme disease experience this evolution to chronic cardiomyopathy is unknown.

Therapy for Lyme disease, and more specifically, for cardiovascular manifestations of Lyme disease, include broad-spectrum antibiotics: penicillins, tetracyclines, erythromycins, or cephalosporins; possibly, corticosteroids. Oral administration of either tetracycline or intravenous penicillins are recommended for the treatment of Lyme disease with cardiac involvement.

Corticosteroids or salicylates have been the treatment of choice in individuals with a high degree atrioventricular block, PR interval prolongation greater than 0.30 seconds, or cardiomegaly. Prednisone has been recommended specifically in patients with associated meningocerehalitis, persistent complete heart block longer than 1 week, or cardiomegaly. Corticosteroids, however, appear to have more and more narrow indications in patients infected with B. burgdorferi.

When heart block occurs during the acute inflammatory phase, a temporary pacemaker has been found valuable for hemodynamic support in one third of patients. Early detection of the cause of heart block is imperative to prevent unnecessary permanent pacemaker implantation. Although rare, Nagi et al. recently described the insertion of a permanent pacemaker in a 42-year-old man with underlying persistent 2:1 heart block, 6 months after initial presentation.

To define the pathological and pathobiological nature, Lyme carditis should be a high priority, so that the manner in which the offending organisms or the immune responses that participate in injury of the heart muscle and induce chronic cardiac dysfunction might be documented.

Only systematic retrieval of biopsy material, explants, or autopsied hearts will allow the necessary insights to be achieved. Thus, registration of patients with overt cardiac involvement with Lyme disease and inclusion of a com-
Table 1
Historical Perspective on Cardiovascular Involvement in Lyme Disease

<table>
<thead>
<tr>
<th>Study</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steere</td>
<td>Lyme carditis first characterized in North America</td>
</tr>
<tr>
<td>Stewart et al</td>
<td>Lyme carditis case published in Australia</td>
</tr>
<tr>
<td>Houwerzijl</td>
<td>First European cases published</td>
</tr>
<tr>
<td>Cormau</td>
<td>Specific observations concerning electrophysiologic abnormalities and demonstration of inflammation and spirochetes in endomyocardial biopsies</td>
</tr>
<tr>
<td>Reznick</td>
<td>General overview of 54 cases of Lyme carditis</td>
</tr>
<tr>
<td>Linde van der</td>
<td>Synopsised 105 North American and European cases of Lyme carditis</td>
</tr>
</tbody>
</table>

Table 2*
Block, Arrhythmias, and Other Cardiac Findings† in Patients‡ With Lyme Disease

<table>
<thead>
<tr>
<th>Atroventricular Block (No. of Patients)</th>
<th>Other Cardiac Findings</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atroventricular block-(15)</td>
<td>Murmur</td>
<td>Prednisone</td>
</tr>
<tr>
<td>Wenkebach block-(2)</td>
<td>Cardiomegaly</td>
<td>Aspirin</td>
</tr>
<tr>
<td>Supra-His/Infra-His block-(8)</td>
<td>S3 (third heart sound)</td>
<td>Atropine</td>
</tr>
<tr>
<td>Complete heart block-(16)</td>
<td>Positive gallium scan</td>
<td>Penicillin</td>
</tr>
<tr>
<td>Left bundle-branch block-(5)</td>
<td>Pericardial effusion</td>
<td>Tetracycline</td>
</tr>
<tr>
<td>Right bundle-branch block-(5)</td>
<td>Supraventricular premature beat</td>
<td>Chloroquine</td>
</tr>
<tr>
<td>Sinoatrial block-(2)</td>
<td>Supraventricular tachycardia</td>
<td>Pacemaker, temporary or permanent</td>
</tr>
<tr>
<td></td>
<td>Atrial fibrillation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sudden death</td>
<td></td>
</tr>
<tr>
<td></td>
<td>T inversion</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ST depression</td>
<td></td>
</tr>
</tbody>
</table>

*Adapted from McAlister et al. (1989)
†Mean duration post-ECM rash=31 days; mean duration to resolution=27 days
‡54 patients, 42 men, 10 women (two not stated), with a mean age of 34.

prehensive clinical, serological, and microbiological database should be promoted as the standard of care.

REFERENCES

Acrodermatitis Chronica Atrophicans: Historical and Clinical Overview

Rudolph J. Scrimenti, MD

ABSTRACT

Acrodermatitis chronica atrophicans (ACA), a late manifestation of Lyme borreliosis, is an excellent example of chronic and latent spirochetosis. Indeed, it may serve as a model for investigation of chronic bacterial infection. Cases of ACA are extremely rare in the United States, despite the fact that they have been recognized in this country for at least a century. In Europe, where it is much more frequently encountered, its clinical features have been delineated carefully.

ACA consists of an early infiltrative and inflammatory stage and a late atrophic stage. It does not spontaneously abate. The histologic features are a lymphohistiocytic and sometimes plasmacytic dermal and subcutaneous infiltration and telangiectasia of blood and lymphatic vessels with lymphedema. Eventually, dermal and subcutaneous atrophy and disruption of collagen and, especially, elastic fibers ensue. Borrelia burgdorferi, the causative organism, may be cultured from even decade-old skin lesions. The skin eruption is characteristically accompanied by fibrotic bands, juxta articular nodules, a distinctive arthropathy and a peripheral neuropathy. Occasionally, pseudosclerodermoid skin changes appear. Marked differences in clinical manifestations are noted in recently reported cases from the United States. Perhaps this is due to genospecific and organotrophic differences between spirochetes on either side of the Atlantic.

Early skin changes usually respond to antibiotic treatment. Later changes, especially severe atrophy, may resist therapy. Elevated serologic titers of antibodies to Borrelia burgdorferi, a characteristic finding in virtually all European cases, may remain positive indefinitely, even after adequate treatment.

Key words: Acrodermatitis chronica atrophicans, borreliosis, review

BACKGROUND

Alfred Buchwald from Breslau described an unusual case of idiopathic skin atrophy in 1883. In so doing, the first clinical manifestation of what eventually became known as Lyme borreliosis (LB) about a century later was documented. In 1895, Pick described the early inflammatory phase of this disease process naming it erythromelie. He considered this a new disease, unrelated to others. In the same year, two New York city dermatologists, Bronson and Elliot independently published similar cases to the one described by Buchwald in immigrants living in that city. Bronson had read his case report before the American Dermatological Association the previous year. Presciently, Elliot mentions in his paper a purplish infiltration of the skin appeared to be a primary event preceding the skin atrophy, the latter being a secondary consequence. However, it remained for Herxheimer and Hartmann in Frankfurt, Germany to carefully study more fully a dozen patients and correctly conclude this disease had two stages, early infiltration and/or inflammation and, later atrophy. They renamed the entity with the title by which it is known today. Montgomery and Sullivan from the Mayo Clinic reviewed 45 cases of ACA in 1945, 39 of which occurred in immigrants. Recent reviews of old records showed some patients with ACA were treated at Mayo Clinic early in this century. DNA of Borrelia burgdorferi, the causative organism has been detected in museum specimens of white footed mice from Massachusetts dating from 1894.

Despite this early recognition of ACA in the United States, most detailed accounts are reported from highly endemic foci in Europe, especially from Germany and Sweden. The causative organism of ACA was first isolated in Stockholm from skin lesions in 1984. Very few case reports have appeared in the American literature since that time.

Consequently, most knowledge American physicians possess comes from this rich European experience.

CLINICAL MANIFESTATIONS

ACA is an outstanding example of prolonged latency and chronic infection. Indeed, it may serve as an ideal study model for persistent bacterial infection. Unlike erythema migrans, it does not spontaneously resolve. In
Europe, it appears to be more common in females, especially older ones. However, it may be seen at any age.

Early ACA usually begins in a subtle fashion with infiltration, inflammation, or doughy swelling of an extremity. Ordinarily mildly symptomatic, on rare occasions it may weep. It appears, at times, that a traumatic injury or surgical procedure elevates the inflammation to a level of clinical awareness where it had been unnoticed before. Characteristically, the poorly demarcated bluish-red to purplish eruption presents over extensor surfaces. Early predilection sites are the dorsal hands and feet, knees, elbows and posterior heels. At times, the discoloration may be striking (Figure 1). Lymphedema of the heel occurs frequently. Patients do not usually associate it with an earlier bite. In some patients, it may be preceded by signs and symptoms of early LB. However, such information is not volunteered readily by patients because a correlation is often not perceived between symptoms separated by long time intervals.

Slow, but definite progression, over months to years, even decades, is the usual course. Central progression toward the torso ensues. Although one extremity is initially infected, two or more extremities may eventually become involved. The buttocks often are involved, but rarely is the torso inflamed above the nates and Poupart’s ligament creating a clinical boundary line. The face rarely is involved and may resemble dermatomyositis, contact dermatitis or collagen disease. The palms and soles participate in this disease process as they do in syphilis, but the lesions are much more confluent.

Eventually, atrophy appears in the involved sites, subcutaneous fat is lost, and inflammation may subside. Now the skin becomes wrinkled, thin, scaling, dry, hypohidrotic and transparent. The underlying venous architecture is readily visible. Alopecia, hyperesthesia, hypo- and hyperpigmentation may occur. Atrophy also may involve the nasopharyngeal, lingual, and vaginal mucous membranes. Various stages of inflammation and atrophy may coexist at the same time.

A noteworthy feature of the atrophy is that it may take many years to occur and it is not an absolute requirement for the diagnosis. Also, atrophy may be diffuse or localized (Figure 2). Rarely, sac-like formations occur in areas of macular atrophy. Superinfection of erythema migrans in patients with preexisting ACA has been reported in Europe.

Other special distinguishing features noted in European patients are fibrotic bands, fibrous juxta articular nodules, a distinctive arthropathy and pseudoscleroderma skin changes. Such changes have not been noted in recently reported American cases. However, this pool of patients is too small from which to draw valid conclusions.

Reddish ulnar bands frequently appear in the early stages of infiltration and extend from the olecranon process to the wrist where they may be fastened to the ulna or fixed to the underlying skin. If they spontaneously disappear, they are usually replaced by atrophy. Much less frequently, similar bands appear on the pre-tibial area of the legs. Fibrous nodules appear painlessly over the elbows, knees, hands, fingers, and elsewhere. Ranging in size from one to three centimeters, they may be solitary, multiple and frequently grouped (Figure 3). Their color varies from yellow white to reddish blue.

Since shortly after the original description of ACA, European observers have reported a sclerosing process of connective tissue as an integral part of the syndrome of idiopathic atrophy. Such lesions vary from moderately infiltrated, yellow, stretched plaques of skin to firmly indurated marble white areas resembling idiopathic scleroderma.

Oppenheim was the first of several observers to distinguish this from true scleroderma. He maintained that sclerosis always appeared in preexisting areas of atrophy in ACA, a process foreign to idiopathic scleroderma. Recent studies indicate true scleroderma and lichen sclero-
sus et atrophicus (LSA) may be impossible to distinguish from some cases of sclerotic and atrophic ACA clinically and histopathologically.\textsuperscript{20}

Deforming arthritis with bone atrophy associated with ACA was first reported in 1924 by Jessner.\textsuperscript{21} Later, he and his associates more specifically associated this with areas of skin atrophy.\textsuperscript{22} This observation has been confirmed by others.\textsuperscript{23,25} Recent studies indicate subluxations, and luxations of small bones of the hands and feet are the most characteristic arthritic features.\textsuperscript{23} This contrasts with the features of typical Lyme arthritis seen in the United States popularized in the 1970s.\textsuperscript{24}

A mild, but chronic, motor and/or sensory axonal polyneuropathy is characteristic in a large percentage of patients with chronic borreliosis on both sides of the Atlantic. However, it has not been specifically associated with recently described American cases of ACA.\textsuperscript{13,14,16,25,26}

\section*{ASSOCIATED CONDITIONS}
Enthesopathies, periostitis, myositis, myalgias, fasciitis, localized and generalized lymphadenopathy, weight loss, fatigue, personality disorders usually with negative cerebrospinal fluid laboratory findings have been reported with ACA.\textsuperscript{15,26,27,28}

\section*{LABORATORY FINDINGS}
Increased erythrocyte sedimentation rates are noted regularly. Sometimes, abnormal liver function studies are found, but, in general, routine laboratory findings are normal. On the other hand, elevated titers of IgG antibodies to Bb, especially in the IgG1 and IgG3 subclasses are uniformly found in serum.\textsuperscript{29} The organism can be cultured slowly on modified Barbour-Stoenner-Kelly medium from skin lesions, even decade-old lesions at temperatures of 32° C to 33° C.\textsuperscript{12}

Histopathology of early lesions manifests a lymphocytic dermal infiltrate of extremely variable size and character and a telangiectasia of blood and lymph vessels with lymphedema. Focal areas of plasma cells may be present in the deeper dermis. With progression, epidermal thinning, degeneration of elastic fibers, and collagen occurs. Eventually, after many years, advanced atrophy of the dermis, including all appendages, follows the inflammation. Later, the inflammation disappears, and a pronounced thinning of the entire dermis and subcutis develops. A rich mixture of plasma cells, if present, may be the only feature differentiating sclerotic and atrophic ACA from idiopathic scleroderma (morphea) and lichen sclerosus, except for the presence of the spirochete.\textsuperscript{17,20} Immunohistologic staining shows a predominance of CD4 lymphocytes.\textsuperscript{30} Fibrotic nodules display fibrosis of the deep dermis and subcutis leading to dermal thicken-

\begin{wrapfigure}[20]{r}{0.5	extwidth}
\centering
\includegraphics[width=0.5\textwidth]{Image1.png}
\caption{Acrodermatitis chronica atrophicans. Late cutaneous atrophy of legs revealing prominent venous patterns (Reprinted with permission from McGraw-Hill Publishing Company).}
\end{wrapfigure}

\begin{wrapfigure}[20]{r}{0.5	extwidth}
\centering
\includegraphics[width=0.5\textwidth]{Image2.png}
\caption{Acrodermatitis chronica atrophicans. Asymptomatic, juxta-articular, violaceous nodules occurring on elbow regions (Reprinted with permission from McGraw-Hill Publishing Company).}
\end{wrapfigure}

ing and the disappearance of fat lobules as well as the more typical features of ACA. In some cases, hyalinization of collagen bundles occurs deep in the dermis and subcutis.\textsuperscript{20}

Most, but not all, studies show no association between the development of ACA and HLA class II alleles.\textsuperscript{31}
Differential Diagnosis

The distinctive plasmacytic infiltrate (if present), unique clinical features and serologic findings differentiate ACA from arterial and venous insufficiency, acrocyanosis, livedo reticularis, vasculitis, contact dermatitis and collagen diseases. Fibrotic nodules histologically are distinguished from gouty tophi, calcinosis cutis, rheumatoid nodules and xanthomas.

MANAGEMENT

There are no comparative studies on therapy of ACA. However, the effectiveness of penicillin had been demonstrated in the 1940s and confirmed in the 1980s. At this time the regimen favored by Asbrink is as follows: Doxycycline 100 mg twice daily for 21 days for cases of uncomplicated ACA. If neurological or arthritic manifestations are present, benzyl penicillin intravenously (12 gm daily) for 14 days followed by 14 days of oral doxycycline (200 mg daily) is preferred.

The inflammatory changes and edema respond early as do the fibrotic lesions but, in general, the response is slow but progressive. Understandably, some advanced atrophic changes may not respond at all. Serologic tests may remain positive for life, a characteristic of chronic spirochetal disease.

REFERENCES

Malariotherapy for Spirocheto-
sis Full Circle (Modulation of
susceptibility of Borrelia burg-
dorferi to antibiotics by elevated
temperature)

To the Editor:
Poor responsiveness of especially
late Lyme borreliosis to antimicro-
bial treatment has been reported
repeatedly. In particular, neuroborre-
liosis often constitutes a major ther-
apeutic challenge and symptoms may
persist despite repeated courses of
penicillin or cephalosporines. Thus,
in a Letter to the Editor in the New
England Journal of Medicine,1
Heimlich drew attention to the possi-
bility of raising the body temperature
by malariotherapy in order to treat
persisting neuroborreliosis. This self-
duced type of therapy already has
been used by a patient in Texas.2
Malariotherapy has been intro-
duced here by Wagner v. Jauregg in 1917* and is still recommended for neu-
rosyphilis that persists after repeated
courses of antibiotics.3,4 The mecha-
nism underlying the antispirochetal
effect of malariotherapy has, how-
ever, not been completely elucidated in
its nature so far.

In this in vitro study we now
ed temperature enhances the efficacy
of antibiotics against Borrelia burg-
dorferi exponentially. Using
Borrelia burgdorferi strain B 31
(type ATCC 35210), cultured in a
modified Barbour-Stoenner-Kelly
medium (BSK II) at 36°C in a BD 53
incubator (Binder, Germany) we
assessed MIC values for penicillin
and ceftriaxone of 0.062 μg/ml and
0.015 μg/ml, respectively. When the
same cultures were incubated at
38°C, the MIC of penicillin and cef-
triaxone decreased to 0.004 μg/ml
and 0.002 μg/ml, respectively. In
other words, at 38°C, 16 times less
penicillin is needed in order to pro-
duce the same antimicrobial effect as
seen at 36°C. However, in cultures
incubated at 39°C or above without
antibiotics borreliae were no longer
detectable after 5 days.

Our data are in agreement with
Heimlich’s suggestion to treat per-
sisting late Lyme disease with
benign tertian malaria, possibly in
combination with penicillin or
cephalosporines. In the pre-penic-
cillin era fever induced by benign
malaria in combination with sal-
varsan or bismuth was reported to
be highly successful.5 In con-
clusion, one question emerges: is it
worth trying again?

Acknowledgment: We would
like to thank Professor G. Stanek
from Vienna for providing Borrelia
burgdorferi strain B 31.

REFERENCES
1. Heimlich HJ. Should we try malario-
1990;322:1234-1235.
2. Centers for Disease Control and
Prevention. Self-induced malaria associated
with malariotherapy for Lyme disease—
3. Wagner V, Jauregg R. Über die
Einwirkung der Malaria auf die progressive
Paralyse. Psychiatrisch-Neurologische
Wochenschrift. 1917;21:132-134.
4. Chemin E. The malariotherapy of neu-
5. Grant AR. The treatment of general
paresis by malariotherapy. Br Med J.
1923;2:698-700.

*Wagner Jauregg was Professor of
Medicine in our Department at the University
of Graz and later in Vienna. In 1927, he
received the Nobel Prize for successful treat-
ment of neurosyphilis by inducing elevated
body temperature by infecting patients with
benign tertian malaria (Lit).

E. Reisinger MD,
I. Wendelin PhD,
R. Gasser MD, PhD
The Borreliosis Study Group
Medizinische Universitätsklinik
Graz, Austria
On October 17th, 1989,
Allstate Insurance gave away
free coverage to thousands.

In light of employee efforts to collect and
distribute clothing, canned food, and bottled water
for San Francisco earthquake victims, perhaps
"The Good Hands People" should be called
"The Good Hearts People."

Chairman Wayne E. Hedien has
always been very pleased with
his company's responsiveness to claims, but he's
equally proud of their responsiveness
to serious social problems, such
as homelessness, teen pregnancy,
substance abuse, illiteracy, AIDS, and youth at risk. Helping Hands, the name of the Allstate
employee volunteer program, works by matching the talents of employee volunteers with the specific needs of the community.

It's this kind of corporate activism the Points of Light Foundation would like to promote. The Foundation is a non-profit, non-partisan organization founded in 1990 to encourage community

service. Like Allstate Insurance, we are committed
to solving social problems on a local level — with innovative solutions.

And although employees often give time to these social programs during business hours, companies have reported only positive effects on their businesses, such as enhanced employee self-esteem and morale, and improved leadership and teamwork.

Of course, without the support of people such as Allstate's chairman Wayne E. Hedien, programs like these would never be possible.

It takes the kind of power only our nation's business leaders can provide to solve serious community problems.

For more in-depth information on corporate involvement in community service, contact the Points of Light Foundation at 1-800-888-7700.

But please call soon. Because the involvement of businesses like yours could be the only insurance we have for a better America.
Topic review for 1996

MARCH
Animal Models in Lyme Disease
Guest Editor: Mario T. Philipp, PhD

JUNE
IX Annual Scientific Conference on Lyme Borreliosis
Guest Editor: Jonathan A. Edlow, MD

SEPTEMBER
Lyme Disease Surveillance System in the United States
Guest Editor: Julie Rawlings, MPH

DECEMBER
Lyme neuroborreliosis
Guest Editor: Patricia K. Coyle, MD
The Lyme Disease Foundation Inc.
is pleased to announce the . . .

IX Annual International Scientific Conference

CHRONIC LYME BORRELIOSIS:
BASIC SCIENCE AND
CLINICAL APPROACHES

With a Panel on Emerging Tick-borne Diseases

BOSTON, MASSACHUSETTS
APRIL 19 & 20, 1996

The Wyndam Copley Plaza Hotel

Conference Chair:
Martina Ziska, M.D. Lyme Disease Foundation

Conference Co-Chairs:
Sam T. Donta, M.D. Boston University Hospital, Boston VA Medical Centers, Boston, MA
Elisabeth Aberer, M.D., University of Graz, Austria

Poster Session Chair:
Jonathan A. Edlow, M.D. Mount Auburn Hospital, Cambridge, MA
About the IX Annual International Conference on Lyme Borreliosis

GENERAL INFORMATION

The IX Annual Scientific Conference on Lyme Borreliosis is an interdisciplinary scientific forum designed to stimulate the exchange of new information among those interested in tick-borne diseases.

Lyme Borreliosis poses an increasing health risk to residents of endemic areas in the U.S. and elsewhere. Chronic Lyme Borreliosis is currently the subject of intensive research and discussion. The purpose of this conference is to provide a forum for the discussion of basic science and clinical approaches to chronic Lyme Borreliosis and to facilitate medical care to chronic sufferers. Due to emerging information on other tick-borne diseases, a panel discussing this topic will be scheduled.

CME Credits:
CME accreditation, Category I., will be provided by Mount Auburn Hospital, Cambridge MA. Nursing credits will be offered, too. The exact number of credits will be announced in the final conference program.

Conference Program:
The program will consist of oral and poster presentations, with two roundtable discussion sessions scheduled. A reception and other social events will also be included.

TRAVEL AND ACCOMMODATIONS

Carlson Wagonlit/Ultimate Travel has been selected as the official travel management company for the conference. This company will be the exclusive contact for room reservations in the host hotel, the Wyndham Copley Plaza. The company will also offer significant savings on airfare to Boston for those who are interested. Call 1-800-899-2717

---

Registration Form

Last Name __________________________ First Name __________________________ Designation __________________________

Name for Badge (if different from above) ____________________________________________________________

Affiliation __________________________________________________________

Address ________________________________________________________________

City __________________________ State/Province __________________________ Postal Code __________________

Country __________________________ Soc. Sec. (for CME's) __________________________

Phone __________________________ Fax __________________________

---

Payment in US FUNDS ONLY!
Registration fee includes: scientific program, book of proceedings, lunches, refreshments, evening reception.

No discounts. No sharing attendance. No refunds.

$100 Full-time medical students, Post Doc's, public health officials

$175 Poster Presenters

$250 Physicians/Researchers/General Public payment on or before 3/15/96

$300 Physicians/Researchers/General Public payment after 3/15/96 until 4/18/96

$350 On-site registration fee (4/19-4/20)

$50 Reception fee (Non-registrants)

Credit Card Charges

☐ Master Card ☐ Visa ☐ American Express

Expiration date _____/_____

Card # __________________________

Cardholder's signature __________________________

☐ Payment by Check/Money order # __________________________

☐ Credit card charge

Total Payment Enclosed $ __________________________

Mail to:
LDI, One Financial Plaza, Hartford, CT 06103-2610

or Fax credit card payment to 860-525-7149(8425)
Lyme Disease Foundation Prize

To be awarded for an original paper, contributing significantly to the understanding of spirochetal and tick-borne diseases, published in the *JSTD* during its second and third year of publication.

The Lyme Disease Foundation Prize will be awarded to the author of an outstanding original research, development, or clinical observation, published in *JSTD*. The value of the first prize is $5,000, the value of the second prize is $2,500; the winners will also receive a certificate. The current competition period begins with the September 1995 issue and ends with the December 1996 issue. Funding for this award is a gift from an anonymous donor.

Original research, developments, or clinical observations, which include original data and are fundamental contributions to the knowledge or understanding of spirochetal and tick-borne diseases, are all eligible for consideration for the prize. The paper must be a first-time publication of the author's own work. Reference to pertinent earlier work by the author may be included to give perspective.

Throughout the competition period, readers are invited to nominate papers appearing in *JSTD*. Nominations must be typed, and the following information provided: the title of the paper, issue in which it was published, author's name, and a brief statement of justification for nomination. Nominations should be submitted to the Editor-in-Chief, *JSTD*, One Financial Plaza, Hartford, CT 06103-2610, and must be received on or before January 31, 1997. Final selection will rest with a panel of distinguished scientists appointed by the *JSTD*.

The award will be presented at the 1997 Annual Scientific Conference. In case of multiple authorship, the prize will be divided equally between or among the authors.