Journal of Spirochetal and Tick-borne Diseases

Volume 1 September 1994 Number 3

Journal of the Lyme Disease Foundation, Inc.

Journal of
Spirochetal and
Tick-Borne Diseases

Volume 1, Number 3
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Dedicated to:
"Science and Art in Spirochetal and Tick-Borne Diseases"

Information for Authors and Editorial Policy

The following guidelines are in accordance with the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" and International Committee of Medical Journal Editors (the "Vancouver Group") statement, agreed 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 four weeks. Rejected manuscripts are usually returned to authors 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 articles on the state of the art 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 three-week periods with subsequent notification to the authors within five 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.

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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 all should sign a letter. Please include a word count. Receipt of letters is not acknowledged, but correspondents will be notified when a decision regarding publication is made.

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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 Editor-in-Chief.

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

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Editor-in-Chief
Journal of Spirochetal and Tick-Borne Diseases
Lyme Disease Foundation
1 Financial Plaza
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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 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 Author's Names

With the manuscript, provide a page giving 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 acknowledgement should be mentioned on this page.

Abstract

Provide on a separate page an abstract of not 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 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 double spaced and provide a heading for all columns with a comprehensive title on separate sheets.

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Photographs and figures should be submitted as glossy prints 5 × 7 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.

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References should be numbered in order of citation in the text. The standard form of INDEX MEDICUS should be followed. Numbered references to personal communications, unpublished data, and manuscripts either ''in preparation'' or ''submitted for publication'' are unacceptable.

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

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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.

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Journal of Spirochetal and Tick-Borne Diseases

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wants to encourage potential authors
to submit manuscripts
to all sections of the Journal.
Manuscripts without requirements, designated in the ''Information to Contributors,'' will be automatically rejected.

CALL FOR PHOTOGRAPHS

The *Journal of Spirochetal and Tick-Borne Diseases*
is now accepting photographs related to any aspect of spirochetal and tick-borne diseases
for publication in the Photographic Section of the Journal.
The Editor-in-Chief should be contacted for further details.

Journal of Spirochetal and Tick-Borne Diseases

Topic review for upcoming issues:

December 1994

Clinical Manifestations of Lyme Borreliosis: An Enlarging Spectrum?
Guest Editor: *Kenneth B. Liegner, M.D.*

March 1995

Focusing on the Tick-Borne Disease in North America:
Relapsing Fever, Ehrlichiosis and Babesiosis
Guest Editor: *Tom G. Schwan, Ph.D.*

June 1995

Treponemal Biology and Pathogenesis at the Cellular and Molecular Level
Guest Editor: *James N. Miller, Ph.D.*

September 1995

Spirochetal Diseases of Domestic Animals
Guest Editors: *Sandra L. Bushmich, M.S.DVM and Edward M. Bosler, Ph.D*

Journal of
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Lyme Disease Foundation Prize

To be awarded for an original paper, contributing significantly to the understanding of spirochetal and tick-borne diseases, published in *JSTD* the first 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 $5000, the value of the second prize is $2500**; the winners will also receive a certificate. The current competition period begins with the June 1994 issue and ends with the June 1995 issue. Funding for this award is a gift from an anonymous donor.

Original research, developments, or clinical observations, which include original research 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, 1 Financial Plaza, Hartford, CT 06103-2610, and **must be received on or before July 31, 1995.** Final selection will rest with a panel of distinguished scientists appointed by the Editor-in-Chief of *JSTD*.

The award will be presented at the 1995 Physician Conference. In cases of multiple authorship, the prize will be divided equally between or among the authors.

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JOURNAL OF SPIROCHETAL AND TICK-BORNE DISEASES

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Guest Editorial

To paraphrase Mark Twain . . . Reports of the eradication of disease-causing microbes have been greatly exaggerated!

Not so long ago the prevailing view was that, due to our understanding of the disease process and the effectiveness of weapons such as vaccines and antibiotics, bacterial infections were no longer a serious threat to modern society. Somehow, that view does not appear to square well with current concerns about Lyme disease, several venereal diseases, multiply drug-resistant tuberculosis, tissue-necrotizing Group A streptococcus, Legionaries' disease and others. In addition to new infections, bacterial killers of past history seem to be re-emerging with renewed virulence and tenacity.

The reason for the disparity is twofold. First, although great progress has been made in understanding contagion and the effects of hygiene and lifestyle on the infectious diseases, our understanding of the disease process at the molecular level is not, in every case, what we would wish it to be. What part of the disease process is contained solely in the invading microbe or its toxin? What part is contributed by the host's inappropriate response to it? Exactly what is it about the invading microbe that makes it pathogenic? How is the infectious agent delivered? How and where is it maintained in nature until delivery to its host? What is involved in the initial attachment and invasion of host cells by pathogens? Why did the body's defense mechanisms, at least in this case, fail to protect? Are arthropod vectors simply little, living syringes with legs or is there more involved? Is disease eradication, in the case of arthropod-borne diseases, best aimed at human host defenses, animal host reservoirs, or insect vectors? In fact, the remainder of this page could be filled with sentences ending with question marks—representing what we *do not* know about the process. Many aspects remain so mysterious and poorly understood that often we are not even sure we are asking the right questions.

The second reason for the disparity between prediction and reality is that whereas in the past we may have felt relatively powerful and perhaps a little superior given our armamentarium of antimicrobial agents, microorganisms, including the ones that cause disease, have during that period continued to do what microorganisms, snug in their various ecological niches, have always done extremely well, namely, adapt to prevailing conditions and survive. The widespread and effective use of antimicrobial agents in the past is precisely the reason that the survivors we now face are resistant to those agents and even to ones that they have never seen before but which work in a similar way.

The field of Microbial Pathogenesis has a deceptively simple mandate: What exactly (in biochemical or molecular terms) is it about the structure or function of the invading microbe that causes disease? Although that central question is relatively simple to ask, the answers are often difficult and resource consuming to collect. And the design and use of antimicrobial agents that exploit that new knowledge and work in completely different ways is even more long term and complex. However, both protection from emerging and re-emerging pathogens and future improvements in public health depend upon it! In order to effectively fill in the blanks in our knowledge, the best skills of clinical physicians and basic laboratory researchers are needed—working together. Although current economic conditions would prevent us from pursuing as many potentially productive avenues of inquiry as we are capable of pursuing, nevertheless, the diverse fields of clinical medicine, biochemistry, molecular biology, immunology, and medical entomology must still bear the problem of understanding microbial pathogens to the greatest degree possible. Furthermore, the breakthroughs in new information and understanding obtained in one field must be efficiently and effectively shared with others in other disciplines. It is the hope of many of us that vehicles such as the broad-based *Journal of Spirochetal and Tick-Borne Diseases* will provide such a format for that sharing.

Claude F. Garon

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JOURNAL OF SPIROCHETAL AND TICK-BORNE DISEASES

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Induction of B-Cell Mitogenesis by Outer Surface Protein C of *Borrelia burgdorferi*

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Department of Health and Human Services, Public Health Service, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Laboratory of Vectors and Pathogens, Rocky Mountain Laboratories, Hamilton, Montana

The mitogenic response to OspC in a murine splenic lymphocyte proliferation assay was significantly greater ($p < 0.01$) than the *Borrelia (B.) burgdorferi* flagellin-derived control. Fluorescence-activated cytometry identified the responding cells as B lymphocytes, a finding confirmed by use of specific markers. The 24-kDa borrelial surface protein OspC had been purified by two-dimensional (2D) gel electrophoresis and identified on immunoblots with OspC antisera raised in rabbits. These results indicate that like OspA and OspB, lipoprotein OspC is mitogenic for murine B cells. Sample processing did not appear to contribute to the effect.

Key words: Mitogen, Lipoprotein, Lyme disease, Spirochete

INTRODUCTION

The ability of *Borrelia (B.) burgdorferi*, the etiologic agent of Lyme disease, to cause mitogenesis of murine B cells has been described in several reports (1–4). Recently, we have shown that extracellular membrane blebs, which are shed from spirochetal surfaces, also possess significant B-cell mitogenic activity (5). All of these studies indicate that mitogenesis was not due to lipopolysaccharide (LPS). Moreover, de Souza et al. (3) demonstrated that two recombinant spirochetal lipoproteins, outer surface protein A (OspA) and OspB, were mitogenic. This latter finding was of particular interest since blebs contain significant amounts of OspA, OspB, and a protein of approximately 24 kDa that appears to be the OspC lipoprotein (5–9). It seemed possible that OspC might also contribute to the mitogenic effect of both blebs and whole spirochetes.

MATERIALS AND METHODS

Bacteria and antigen preparations. Low-passage (P6 to P8) strain Sh-2-82 of *B. burgdorferi*, which originated from adult *Ixodes scapularis* (*I. dammini*) (10) ticks (Shelter Island, NY), was grown in BSK II culture medium (11) at 34°C. Spirochetes were isolated from the medium after centrifugation at 10,400 g for 30 minutes at 25°C and washed and resuspended in 0.15-*M*phosphate-buffered saline (PBS, pH 7.2) made with pyrogen-reduced water (<1 EU/mL; Milli Q, Millipore Corp., Bedford, MA).

Antigen preparations were produced by subjecting bacterial suspensions described above to three cycles of freeze-thawing at −80°C and sonication for six 15-seconds cycles (at a setting of 4) with a Branson sonicator (Branson Sonic Power Co., Danbury, CT). The resulting sonicate was centrifuged at 12,100 g for 20 minutes at 4°C. Supernatant fractions from the extracts were retained, filter sterilized (0.22-μM porosity), and assayed for total protein (BCA Protein Assay Reagent; Pierce, Rockford, IL). Final preparations were frozen at −80°C until used. Rabbit anti-OspC antiserum was produced by hyperimmunizing a rabbit with SDS-polyacrylamide gel-resolved 24-kDa protein from strain Sh-2-82 of *B. burgdorferi*. The specificity for OspC was confirmed by the reactivity of this antiserum with recombinant OspC (6, 9).

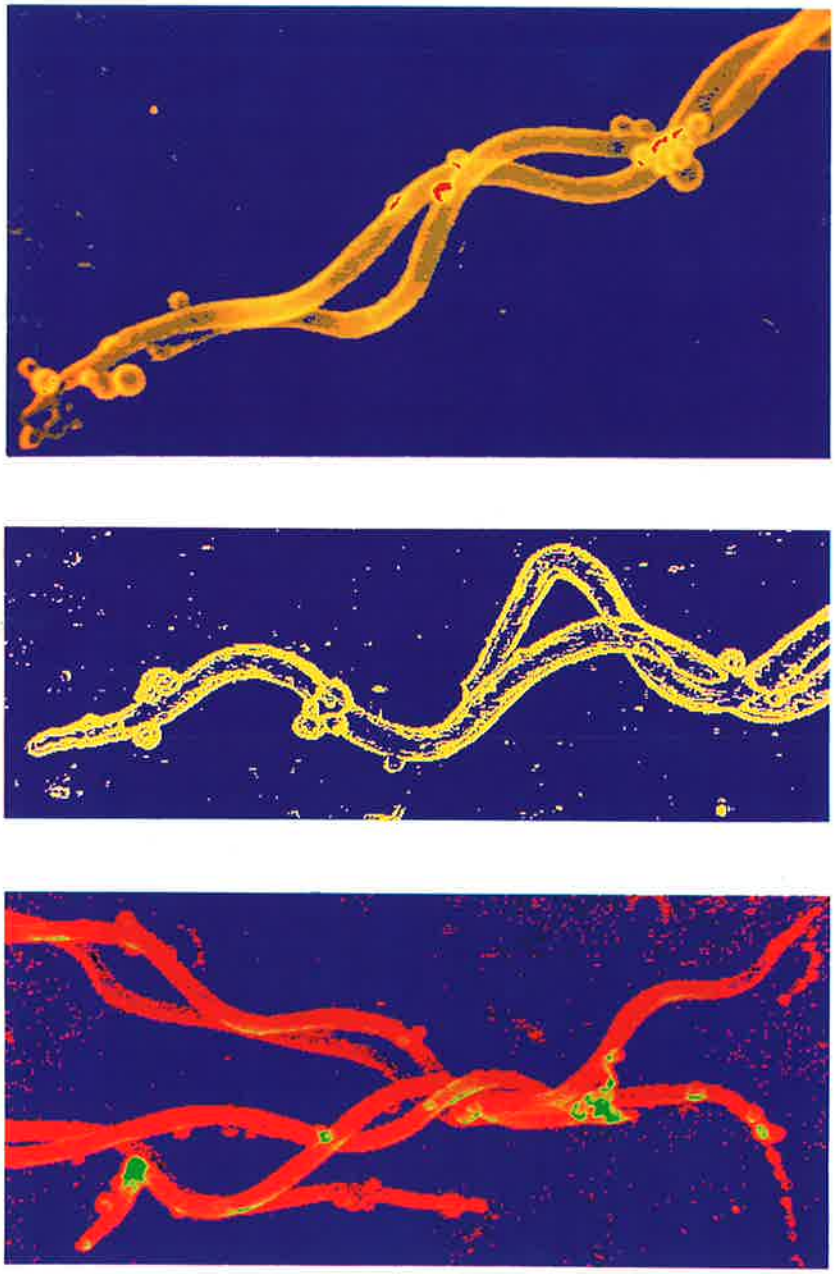
Polyacrylamide gel electrophoresis. Antigen preparations for one-dimensional (1D) analysis were diluted at a 1:1 ratio in double-strength sodium dodecyl sulfate (SDS) electrophoresis treatment buffer and heated for 5 minutes at 95°C, as previously described (5). For two-dimensional (2D) analysis, antigen preparations were solubilized in first dimension solubilizing solution (9.5-*M* urea, 2.0% Triton X-100, 5% beta-mercaptoethanol, 1.6% Bio-Lyte 5/7 ampholyte [Bio-Rad Laboratories, Richmond, CA], and 0.4% Bio-lyte 3/10 ampholyte [Bio-Rad]) for 2 hours at room temperature. The preparation was then centrifuged at 100,000 g for 2 hours at 4°C, and the supernatant fraction (approximately 30-μG total protein per tube) was subjected to electrophoresis for 3.5 hours in first dimension isoelectric focusing (IEF) tube gels, as described by O'Farrell (12) using a Mini Protean II Tube Cell (Bio-Rad). After electrophoresis, tube gels were equilibrated with single-strength SDS electrophoresis treatment buffer solution for 10 minutes at room temperature and applied to the second dimension in 12% SDS-polyacrylamide slab gels. Further electrophoresis at 200 V was performed with a Mini Protean II gel apparatus (Bio-Rad) and the discontinuous buffer system described by Laemmli (13). Antigen preparations (30-μG total protein per lane) that had been solubilized with electrophoresis treatment buffer as well as molecular size standards (Bio-Rad) were added to some slab gels along with tube gels. Following electrophoresis, proteins were visualized by staining with Coomassie blue or immunoblotted.

Immunoblotting for identification of OspC and flagellin. Spirochetal proteins were electrophoretically transferred from 2D slab gels to nitrocellulose sheets (0.1−μM porosity; Schleicher and Schuell, Inc., Keene, NH) in a Mini Trans-Blot Cell (Bio-Rad) for 1.5 hours at 100 V (5). Following transfer, nitrocellulose sheets were incubated in PBS with 0.05% Tween 20 (blocking buffer) overnight to block nonspecific binding sites. The sheets were then reacted with rabbit anti-OspC antiserum or monoclonal antibody H9724 (antiflagellin) diluted 1:500 and 1:25 in blocking buffer, respectively, for 2 hours at room temperature. After exposure to horseradish peroxidase-conjugated goat anti-

*Corresponding author

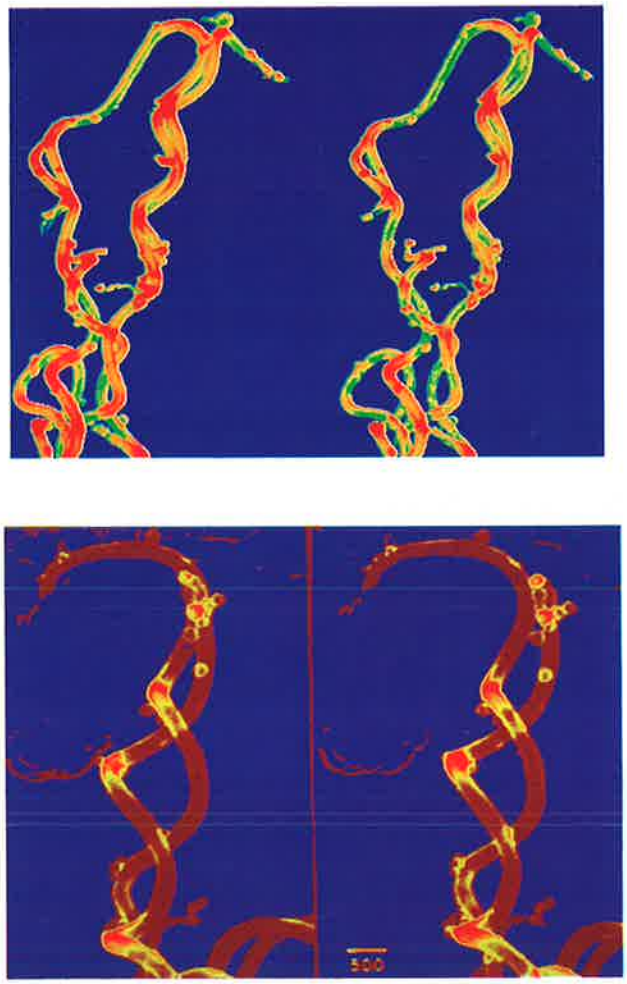
PATHOGENESIS OF LYME BORRELIOSIS

Colorized scanning electron micrograph of *Borrelia burgdorferi* strain Sh-2-82. Individual spirochetes are coiled together in a counter-clockwise fashion. Extracellular membrane vesicles (v) or "blebs" are evident on and extending from cell surfaces. Scale bar: 0.5 μm. Courtesy of David Dorward, Ph.D., Rocky Mountain Laboratory, Hamilton, Montana.



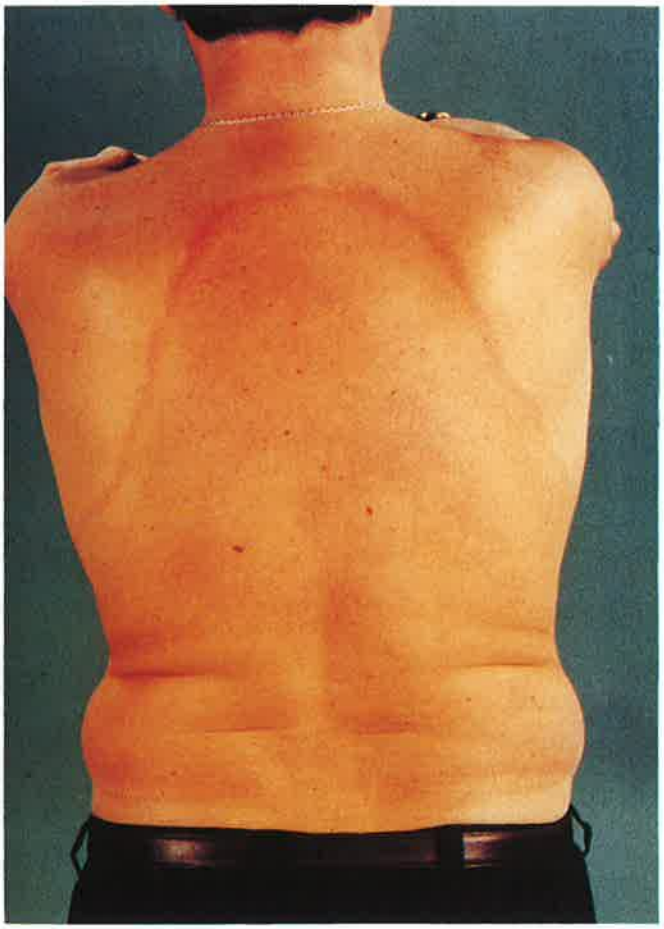
PATHOGENESIS OF LYME BORRELIOSIS

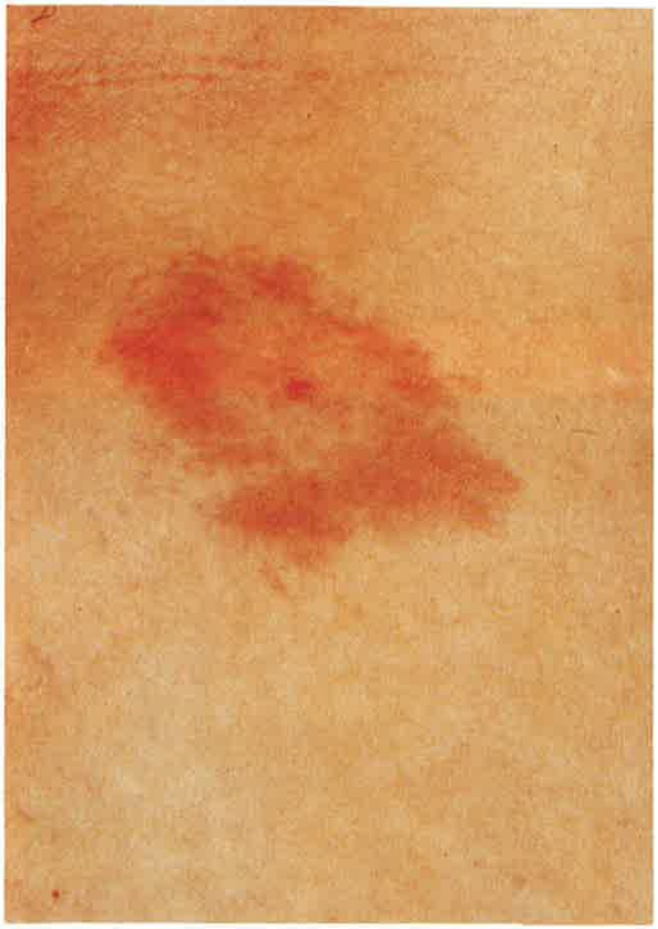
Scanning electron micrograph *stereo* pair of *Borrelia burgdorferi* strain Sh-2-82. Individual spirochetes are coiled together in a counter-clockwise fashion. Extracellular membrane vesicles (v) or "blebs" are evident on and extending from cell surfaces. Scale bar: 0.5 μm. Courtesy of David Dorward, Ph.D., Rocky Mountain Laboratory, Hamilton, Montana.



DO GENERAL PRACTITIONERS RECOGNIZE CASES OF LYME BORRELIOSIS IN THE NETHERLANDS?

The pictures used in the article are enclosed for the educational purposes.





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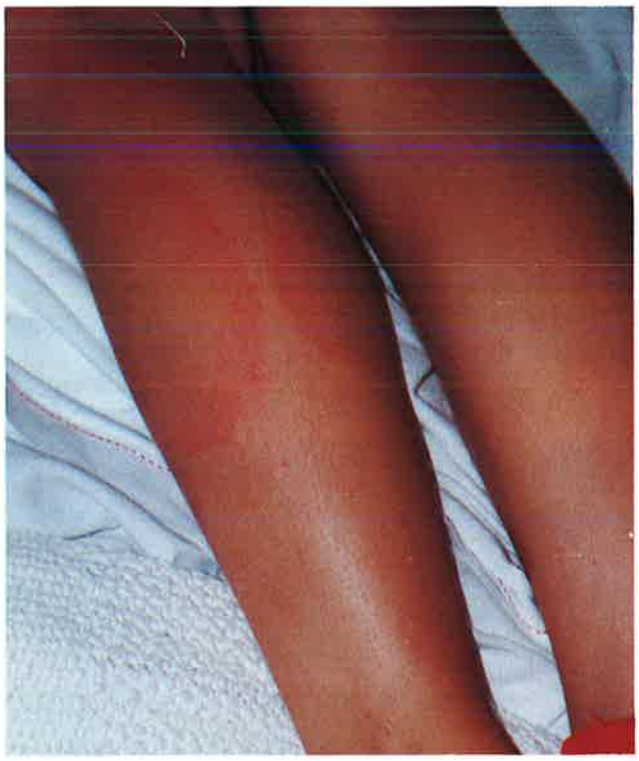

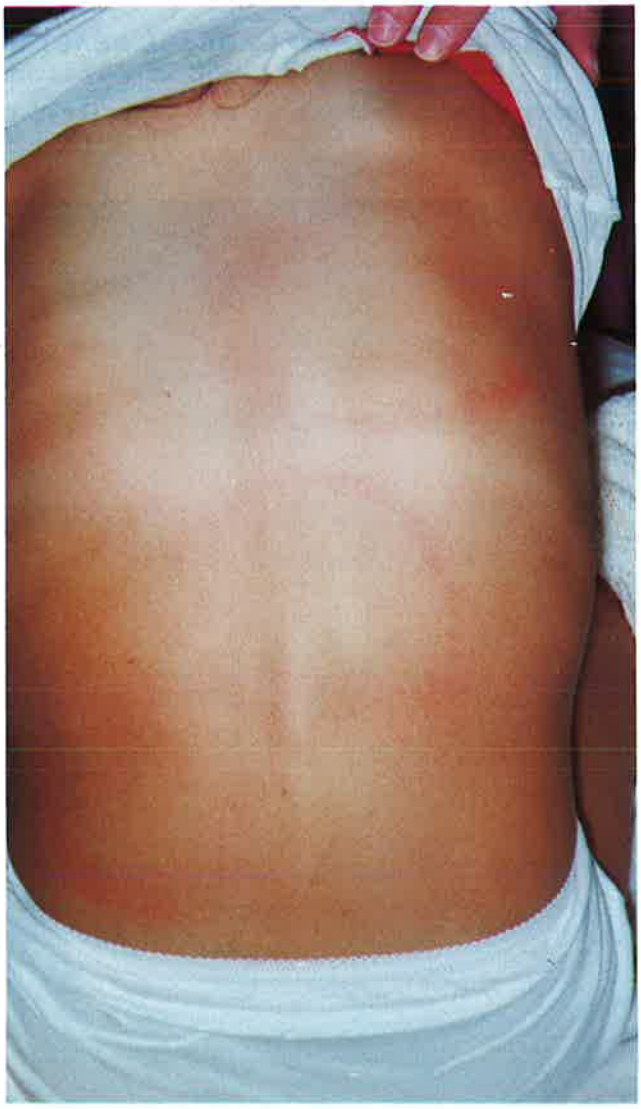

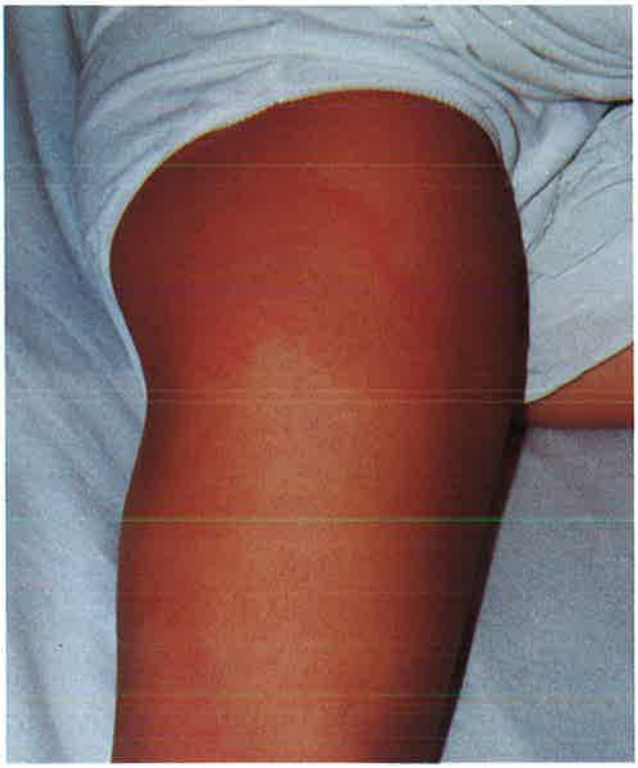

mouse or antirabbit IgG (heavy- and light-chain specific) diluted 1:2500 or 1:1000 in blocking buffer for 1 hour at room temperature, respectively, bound peroxidase activity was detected with peroxidase substrate solution, as previously described (5). The rabbit anti-OspC antiserum and monoclonal antibody H9724 were supplied by Dr. Schwan (Rocky Mountain Laboratories, Hamiton, MT).

Isolation and purification of OspC and flagellin. Two-dimensional gel-resolved protein spots that comigrated with OspC or flagellin bands in 1D gels and that reacted with anti-OspC antiserum or antiflagellin monoclonal antibody, respectively, were excised from 16 two-dimensional gels and pooled. Proteins in the excised spots were eluted with an Electro-Eluter (Bio-Rad), exhaustively dialyzed against 5-mM ammonium bicarbonate with 0.05% SDS and precipitated overnight at −20°C in acetone containing 1-mM hydrochloric acid. Precipitated proteins were then washed twice in cold acetone, vacuum dried, and stored at −20°C. The protein preparations were resuspended in PBS and assayed for total protein prior to use in the lymphocyte proliferation assay. An area on each stained gel, which contained no detectable protein, was also excised and processed in a similar fashion to serve as a background control.

Lymphocyte proliferation assay. Spleens were aseptically obtained from three naive 5-week-old C57BL/10 female mice obtained from a colony at Rocky Mountain Laboratories. Cell suspensions were washed and resuspended at a concentration of 2×10^6 viable cells/mL in RPMI 1640 culture medium supplemented with 20-mM glutamine and 200 U/mL of penicillin. Triplicate cultures were set up in 96-well flat-bottomed microtiter plates (Flow Laboratories, Inc., McLean, VA) by adding 0.1 mL of the cell suspension to wells containing RPMI medium with 20% (v/v) fetal bovine serum (Hyclone Laboratories Inc., Logan, UT) and either 50 μG/mL of LPS mitogen (LPS from *Escherichia coli* 0111:B4; Difco Laboratories, Detroit, MI), 15 μG/mL of purified OspC or flagellin, or 5 μL of background control (equal to volume of OspC preparation used per culture). After 2 days of incubation at 37°C in a humidified 95% air–5% CO_2 atmosphere, the lymphocyte proliferation assay was performed, as previously described (5). The incorporation of [methyl-^{3}H]thymidine (specific activity 6.7 Ci/mmol; NEN Research Products, Du Pont Co., Wilmington, DE) by the cultures was recorded as disintegrations per minute (DPM; counts per minute/counting efficiency). Results of lymphocyte proliferation assays were expressed as increased DPM, defined as test cultures DPM minus background control cultures DPM. Mean increased DPM ± standard error of the mean (SEM) of triplicate OspC- and flagellin-stimulated cultures were calculated. The results were subjected to the Student's *t*-test and single-factor analysis of variance.

Fluorescence-activated cytometric analysis. After 2 days of exposure to OspC or LPS, spleen cell cultures were centrifuged at 300 g for 10 minutes resuspended in 50 μL of fluorescein-conjugated anti-Thy-1.2 or anti-B220 monoclonal antibody in fluorescence-activated cell sorting (FACS) medium (PBS containing 5% [v/v] fetal bovine serum and 10-mM sodium azide), and incubated for 20 minutes on ice, as previously described (5). Cells were then washed twice, resuspending in 200 μL of FACS medium containing propidium iodide (5 μG/mL), and analyzed with a FACStar I fluorescence-activated cell sorter (Becton Dickinson Immunocytometry Systems, San Jose, CA). Fresh unstimulated spleen cells obtained from a naive mouse were treated with a monoclonal antibody against B220 to set limits (boxed area) for detection of blasting cells (data not shown).

RESULTS

Figure 1A shows that 2D gel electrophoresis resolved OspC (double arrows) and flagellin (single arrow) as two large distinct spots that shared alignment with OspC and flagellin bands, respectively, in 1D gels. Outer surface protein A and OspB were not resolved by the 2D gel electrophoresis system used in this study. The reactivity of rabbit anti-OspC antiserum against the 2D gel-resolved OspC on immunoblots is shown in Fig. 1B. The reactive spots immediately to the left of OspC may be due to peptides of OspC that were modified during sample preparation (12), since similar streaking is often noted when monoclonal antibodies to lipoproteins OspA and OspB are used in this fashion. Reactivity of the antiserum to a basic protein in the higher molecular weight region was also evident (Fig. 1B). Mono-

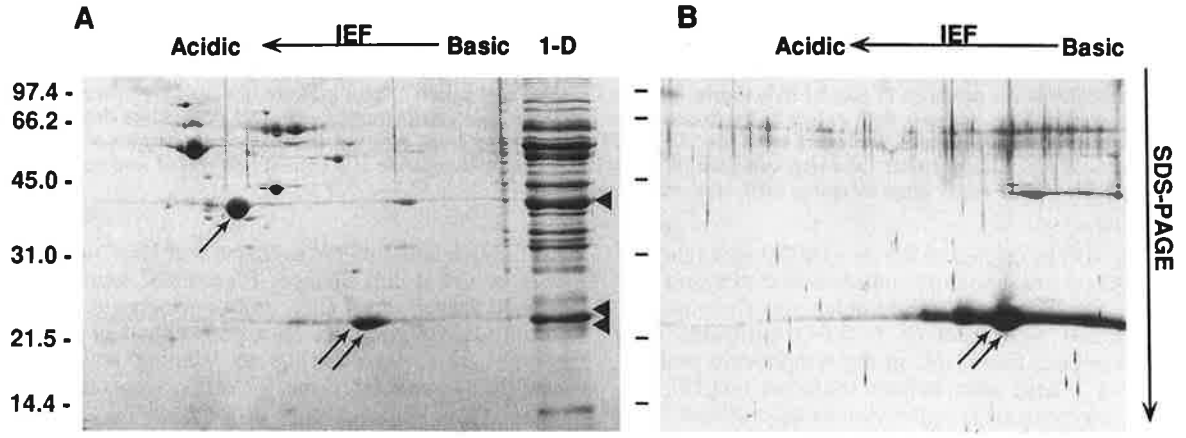

FIG. 1. Analysis of *B. burgdorferi* antigen preparations by 1D and 2D SDS-polyacrylamide gel electrophoresis. (A) The single and double arrows indicate the relative positions of flagellin and OspC, respectively, following 2D gel electrophoresis. Note the alignment of the flagellin and OspC 2D spots with the corresponding (single and double arrowheads, respectively) 1D bands, stained with Coomassie brilliant blue. (B) Immunoblot of an antigen preparation (transferred from a 2D polyacrylamide gel) and reacted with rabbit anti-OspC antiserum. The double arrows indicate the position of the reactive 24-kDa OspC. Positions of molecular size standards are indicated on the left (in kDa).

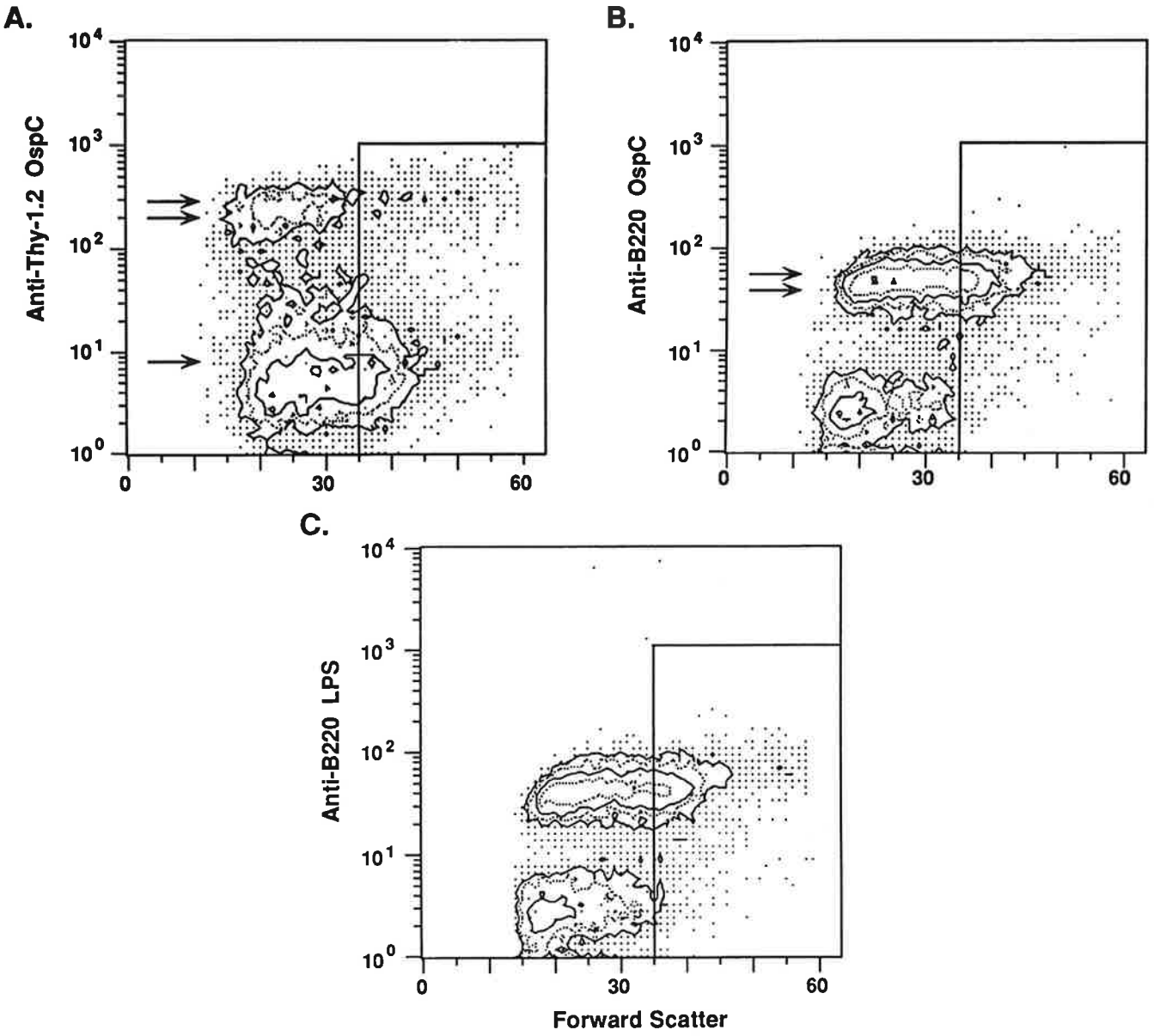

FIG. 2. Fluorescence-activated cytometric analysis of OspC-stimulated murine spleen lymphocytes labeled with monoclonal antibodies directed against Thy-1.2 (A) and B220 (B), representing murine pan-T-cell and murine B-cell markers, respectively. The response of bacterial LPS-stimulated lymphocytes in the presence of anti-B220 is shown in panel C. Single and paired arrows indicate low- and high-fluorescing (labeled) cell populations, respectively, whereas data points in the boxed areas represent blasting (increased cell size) cells. Note that T cells present in OspC-stimulated cultures (A) are predominately small (i.e., lie to left of the boxed area), whereas the fluorescent pattern of OspC- and LPS-stimulated cultures (B and C) are similar following exposure to anti-B220. Lipopolysaccharide is a known mitogen of murine B cells and the B220-positive B cells include many large (blasting) cells after stimulation.

clonal antibody H9724 reacted to the 41 kDa 2D spot (single arrow; Fig. 1A) on immunoblots and identified this spot as flagellin (data not shown). While lymphocytes from naive mice demonstrated significant ($p \leq 0.01$) mitogenic responses to 2D gel-purified OspC in the lymphocyte proliferation assay at 2 days after culture initiation (40,532 ± 4,835 DPM), exposure of lymphocytes to gel-purified flagellin resulted in a low mitogenic response (5195 ± 757 DPM) at equivalent concentrations (15 μG total protein/mL) as OspC. Time course experiments using purified blebs had previously revealed that blastogenic responses after 2 days were similar to or greater than blastogenic responses at 4 or 6 days (data not shown). Analysis of OspC-stimulated spleen cell cultures indicated that OspC-stimulated cells were of the B cell lineage. Blast cells, identified by high forward scatter signal (i.e., cell population within the boxed area) in FACS analysis, were phenotyped as predominately B cells rather than T cells by labeling with the lineage-specific markers B220 and Thy-1.2, respectively (Fig. 2).

DISCUSSION

Two-dimensional gel electrophoresis was used for the isolation of OspC from the Sh-2-82 strain of *B. burgdorferi* because 2D electrophoresis separates proteins by charge as

well as by apparent size and can resolve proteins that differ by a single amino acid (12). This greatly reduces the possibility of two or more proteins comigrating as a single spot during electrophoresis (12). The failure of the 2D gel system to resolve OspA and OspB may have been the result of the basicity (>pH 7.0) of these two proteins that prevented them from entering IEF tube gels (pH 6.9 to 5.2) in the first dimension (12, 14). However, immunoblots with rabbit anti-OspC antiserum indicated that the 2D-resolved 24-kDa protein was OspC, since the rabbit antiserum was previously shown to bind to a 24-kDa protein of strain B31 that also reacted with an anti-OspC monoclonal antibody (6, 9). Antiserum reactivity to the higher molecular weight antigen (approximately 40 Kda) may indicate that this antigen shares epitopes with OspC or that this antigen was present within the OspC immunogen used for production of the antiserum. Similar reactivity has also been observed by other investigators on immunoblots of low-passage spirochetes that were exposed to this same rabbit antiserum (6, 9).

Although both time course experiments and dose response curves were described earlier in demonstrating a mitogenic response to purified bleb preparations (5), similar experiments were not possible here given the extremely limited quantities of 2D gel-purified material available. Limited quantities also hampered attempts to obtain an *N*-terminal protein sequence. However, a comparison of equal quantities of 2D gel-purified flagellin and OspC did verify the relatively high mitogenic potential of the 24-Kda protein preparation. Time course and dose response comparisons must await the development of protein-expressing clones before sufficient quantities of highly purified material become available.

The ability of gel-purified OspC to induce mitogenesis of B cells from naive mice, however, was clearly demonstrated by lymphocyte proliferation and fluorescent cytometric assays. The fact that gel-purified flagellin caused little mitogenesis indicates that sample preparation made no significant contribution to the mitogenic effect of OspC. It is not known whether OspC, or other lipoproteins such as OspA and OspB, is involved in the pathogenesis of Lyme disease. However, peripheral blood lymphocytes from Lyme disease patients and healthy controls have been shown to mount similar proliferative responses to the spirochete (15). Increased B-cell activation in Lyme disease patients has been shown to correlate with the severity of disease as well (16). It is possible that outer surface lipoproteins are responsible for these effects. Such lipoproteins might stimulate autoreactive B cells that are otherwise anergic and initiate tissue injury in certain individuals. This type of autoimmune disease is usually associated with systemic rather than organ-specific complications, correlating with the manifestations of Lyme disease, which is a multisystemic disorder (17, 18). For this reason, the mitogenic capabilities of OspA, OspB, and OspC should be assessed in man, especially if these lipoproteins are components of a candidate vaccine.

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Do General Practitioners Recognize Cases of Lyme Borreliosis in the Netherlands?

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To assess knowledge and recognition of Lyme borreliosis, two photographs of erythema migrans and two written cases of Lyme borreliosis were presented to 51 local general practitioners. The photographs and cases were tested, among 10 other dermatological and rheumatic cases, using 2 different formats: open-ended questions, prompting for the most likely diagnosis, and multiple-probability estimate questions. Each case served as its own "golden" standard, but cases were also presented to a panel of "experts," 13 dermatologists and 23 rheumatologists. In the open-ended questions, the two photographs of erythema migrans were recognized by 16 and 45% of the general practitioners and by 92 and 54% of the dermatologists. In the multiple-probability estimates, 14 and 14% of the general practitioners and 77% and 46% of the dermatologists rated these two photographs as highly probable for erythema migrans. The first case of Lyme borreliosis was correctly diagnosed by 55% of the general practitioners and 96% of the rheumatologists in the open-ended questions. In the multiple-probability estimates, 61% of the general practitioners and 87% of the rheumatologists rated this case as highly probable for Lyme borreliosis. The second case of Lyme borreliosis was never recognized in the open-ended questions, whereas only two general practitioners rated Lyme borreliosis as highly probable in the multiple-probability estimates. General practitioners, as well as dermatologists and rheumatologists, had difficulties recognizing Lyme borreliosis. Better instruction and education in recognizing the manifestations of Lyme borreliosis seems indicated.

KEY WORDS: Lyme borreliosis, Erythema migrans, Lyme arthritis, Education, General practitioners

INTRODUCTION

Lyme borreliosis, a tick-borne spirochetal infection, frequently begins with a characteristic skin lesion, erythema migrans, and is often followed by systemic manifestations involving the heart, nervous system, skin, or the joints (1–6).

Recognition and knowledge of the early dermatologic (especially erythema migrans) cardiac, neurologic, and rheumatic manifestations of the disease and adequate antibiotic treatment of these symptoms are important to prevent later stages of the disease such as chronic arthritis, chronic neurological disorders as encephalopathy and polyneuropathy, and acrodermatitis chronica atrophicans (7–10). One should be aware of the fact that erythema migrans lesions may fade within 3 to 4 weeks, even in untreated patients, and other manifestations of the disease, like arthritis, may occur only months later. One may be unaware of the relationship between subsequent signs and symptoms. Lyme borreliosis may also present itself without preceding skin lesions, for instance, as arthritis of the knee.

In the Netherlands, general practitioners are the first port of call for virtually all patients. As a result, they are probably the first ones to see patients with the early signs and symptoms of Lyme borreliosis. Due to the low incidence of this disease in the Netherlands, they will not frequently encounter patients with Lyme borreliosis (11). However, knowledge of Lyme borreliosis, clinical suspicion and recognition of manifestations, and appropriate and timely antibiotic treatment will decrease the burden of illness. At early stages of the disease (for example erythema migrans), clinical history and physical examination are the most important tools to detect Lyme borreliosis for general practitioners, since antibodies to *Borrelia burgdorferi* only become positive after 6 to 8 weeks (12).

Data of the whole spectrum of Lyme borreliosis have only become available since the first publication by Steere et al. in 1977 (13). The first publications of the early manifestations of the disease in Dutch literature date from 1987 and 1988 (14–18). The general practitioner may have gained his or her knowledge of Lyme borreliosis from medical journals rather than from medical textbooks. The chance that general practitioners have gained experience with Lyme borreliosis from patients is estimated as being low not only due to the low incidence of the disease but probably also due to low suspicion of the occurrence of Lyme borreliosis in the Netherlands.

In this study, we have presented two photographs of erythema migrans and two written case histories of Lyme borreliosis to general practitioners, to assess their knowledge and suspicion of Lyme borreliosis. We also assessed whether the general practitioners had access at all to the articles published about Lyme borreliosis in the Dutch and English literature.

METHODS

This study was undertaken as a part of a study to assess knowledge of general practitioners about rheumatic diseases and was carried out before discussions on a rheumatic post-graduate training program were started. Ten colored photographs of skin lesions that may occur in relation to rheumatic diseases and 10 written case descriptions of pa-

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tients with rheumatic diseases were presented to the general practitioners. The ultimate purpose of the study was to install post-graduate training, which fits the specific needs of the participants. Participation in the study was supported by the board of the Post-Graduate Education Committee of the local organization of general practitioners. The entire evaluation program lasted 45 minutes for each participant.

Participants

In November 1991, a random sample of 58 (73%) of all 79 local general practitioners were invited to participate in the study. A total of 51 general practitioners agreed to cooperate (a response rate of 88%). Seven refused to cooperate, mostly due to lack of time. All participating practitioners were visited in their office by one investigator (AB).

The two photographs of erythema migrans and the two case histories of Lyme borreliosis were also presented to a panel of experts in dermatology and rheumatology, to compare the answers of the general practitioners with their answers. The two photographs of erythema migrans were, therefore, also presented to 13 dermatologists (three from private practice and 10 from an academic setting) during a meeting of the Dutch Society of Dermatology. The two cases of Lyme borreliosis were also presented to 23 rheumatologists (14 from private practice and nine from an academic setting) attending a scientific meeting of the Dutch Society of Rheumatology.

Questions

The photographs of erythema migrans and the cases of Lyme disease were presented in two different formats. The first was an open-ended question, prompting for the most likely diagnosis. The second was a so-called multiple-probability estimate consisting of several (eight) possible diagnoses, for each of which the participant had to rate the probability on a seven points scale (range: highly improbable to highly probable) (20). The questions were presented in these two different formats to assess a possible discrepancy in the answers. It is possible that a participant does not consider Lyme borreliosis spontaneously in the open-ended question. However, when a list of differential diagnoses is provided, including Lyme borreliosis, he or she may implicate this diagnosis into his considerations. This way of presentation does not, of course, reflect the situation as it is in real practice. However, it might give us an idea whether the illness Lyme borreliosis is known by the participants at all.

Photographs of erythema migrans

Two colored photographs (13 × 18 cm) from erythema migrans were placed in plastic binders and presented to the general practitioners (pictures 1 and 2). The two photographs were from patients diagnosed as having erythema migrans by two dermatologists. Both patients developed erythema migrans within 2 weeks of a tick bite. A differential diagnosis was composed for both cases by two dermatologists from the Department of Dermatology of the University Hospital Maastricht. Each dermatologist composed a differential diagnosis for both photographs. The eight diagnoses mentioned the most were used for the definite differential diagnosis used in the study.

Cases of Lyme borreliosis

The clinical cases of Lyme borreliosis were derived from real patients and compiled by one of the investigators (AB). Two different cases were presented. The first case of Lyme borreliosis was a so-called classical case with a bite, skin lesion, atria-ventricular block, and arthritis. The second case was a patient with recurrent arthritis of the knee without any other symptoms of Lyme borreliosis (see Appendix 1 for detailed case descriptions). We felt that Lyme borreliosis should be part of the differential diagnosis for the second case, because arthritis of the knee is the most frequent symptom of Lyme disease and because most patients do not remember any tick bites or even erythema migrans. The patient we described had a recurrent attack of arthritis of the knee. The first attack lasted 8 weeks; the second attack also lasted several weeks. Attacks of arthritis due to Lyme borreliosis are usually short with a median duration of 1 week. However, longer attacks have been described, especially during the 2d and 3d year of the illness (13,19). Episodes of arthritis are often separated by months or even years of complete remission (4).

A differential diagnosis was composed by one of the investigators (AB) and three rheumatologists from the Department of Rheumatology of the University Hospital Maastricht. Each of these four rheumatologists composed a differential diagnosis for each case description in the same way as described for the dermatologists.

Presentation

The photographs were presented to the general practitioners and dermatologists by two of the investigators (AB and MR) without any specific comment. First, the photographs were presented with the open-ended question. The diagnoses for each photograph were written on separate sheets. Second, the same photographs were presented with the multiple-probability estimates. The differential diagnoses for the multiple-probability estimate questions were presented on separate sheets. The participants were not allowed to read back or to correct answers already given to the open-ended questions after they had seen the differential diagnoses in the multiple-probability questions.

The two cases were presented to the participating general practitioners and rheumatologists using a computer interface specially developed for testing (21). First, the two cases were presented linked to the open-ended question. Second, the same cases were presented but were then linked to the multiple-probability estimate. It was not possible to return to a previous screen or correct answers already given to the open-ended questions after a participant had seen the differential diagnosis given in the multiple-probability estimate questions. The answers to all questions were immediately filed in the computer.

Scoring

Since all cases and photographs were derived from real patients, each photograph or case served as its own "golden" standard. Answers to the open-ended questions were considered correct when the participants mentioned either erythema migrans or Lyme borreliosis within the first three answers. In the multiple-probability ratings, rates 6 and 7 were considered highly probable, rates 3, 4, and 5 were probable, and rates 1 and 2 were highly improbable.

Medical journals

At the end of the test, a list of 17 Dutch and 10 English medical journals of supposedly easy access to general practitioners was presented. The instruction for this list was to rate how frequently these journals were read by the general

TABLE 1
Presence of Correct Answers Given to Two Photographs of Erythema Migrans and the Two-Cases of Lyme Borreliosis in the Open-Ended Questions by the General Practitioners, Dermatologists, and Rheumatologists

	Dermatologists		General Practitioners		Rheumatologists	
	$N = 13$		$N = 51$		$N = 23$	
	N	%	*N*	%	*N*	%
Photo 1	12	92.3	8	15.6		
Photo 2	7	53.8	23	45		
Case 1			28	54.9	22	95.7
Case 2			0	0	1	4.3

practitioners on a scale from 1 (never) to 5 (every issue). The number of published articles about Lyme borreliosis (period 1983 to 1991) was known for each journal.

Statistics

Counts of correct answers were calculated for each group; variance and means were compared to each other. For categorical data, an X^2 test was used to test for significant differences between groups. A probability value $p < 0.05$ was considered statistically significant.

RESULTS

In Table 1, the results of the correct answers to the open-ended questions linked to the photographs of erythema migrans and the two cases of Lyme borreliosis are presented.

Only eight general practitioners (15.6%) recognized the first photograph as erythema migrans compared to 12 of the dermatologists (92.3%) ($p < 0.001$). There was no significant difference between the general practitioners and the dermatologists when recognizing the second photograph as erythema migrans. Forty five percent of the general practitioners gave the correct answer compared to 53.8% of the dermatologists.

A variety of diagnoses was suggested by both general practitioners and dermatologists for the two photographs. Diagnoses mentioned the most were contact allergy (6 times), mycosis (4 times), discoid lupus erythematosus (4 times), erythema annulare centrifugum (2 times), and epizoonosis (27 times). All other diagnosis were mentioned only once.

Twenty eight general practitioners (54.9%) recognized the first case as Lyme borreliosis. None of the general practitioners mentioned Lyme borreliosis as a possible diagnosis for the second case. Strikingly, only one rheumatologist mentioned Lyme borreliosis.

Again, a variety of diagnoses was mentioned for the two cases by the general practitioners and the rheumatologists. The diagnoses mentioned for the first case by the general practitioners were rheumatic fever (8 times), reactive arthritis (6 times), septic arthritis (3 times), endocarditis (3 times), gout (3 times), postinfectious arthritis (3 times), viral infection (1 time), erysipelas (1 time), and arthritis of unknown etiology (2 times). In the second case, gout was considered the most likely diagnosis by 24 general practitioners (47.1%) and by 10 rheumatologists (43.5%). Fourteen general practitioners (27.5%) mentioned arthritis of unknown etiology as a diagnosis. Other diagnoses made by the rheumatologists in this case were reactive arthritis (26.1%) and ankylosing spondylitis (17.4%).

In Tables 2 and 3, the results of the probabilities awarded to the multiple-probability estimate questions are presented. It is remarkable that only seven general practitioners (13.7%) rated the second photograph of erythema migrans as highly probable, whereas 23 of them (45%) mentioned erythema migrans in their answers to the open-ended questions. Listing of erythema migrans in the differential diagnosis probably confused the general practitioners; 35 of them even rated erythema migrans as highly improbable. There were no remarkable differences between the open-ended questions and the multiple-probability estimate questions regarding the two cases of Lyme borreliosis. Only two general practitioners rated Lyme borreliosis as highly probable in the second case. So, concerning the second case of Lyme borreliosis, listing Lyme borreliosis in the differential diagnosis could not even lure the rheumatologists to consider Lyme borreliosis!

Two general practitioners correctly diagnosed both photographs of erythema migrans; however, they did not recognize the first case. Two general practitioners recognized photograph 1 but did not recognize case 1. Seven general practitioners recognized photograph 2 but did not recognize case 1 as Lyme borreliosis. Twenty-eight general practitioners (54.9%) suggested Lyme borreliosis in their differential diagnosis to case 1. Only three of these 28 correctly diagnosed both photographs of erythema migrans. Six diagnosed photograph 1, and 13 diagnosed photograph 2. As stated before, none of the general practitioners recognized case 2 as Lyme borreliosis.

Eight Dutch medical journals were read regularly by more than 50% of the general practitioners. Articles about Lyme borreliosis appeared in five of these eight journals in 1987 to 1991 (14–18, 22–36). One journal published an article about Lyme borreliosis, which included the first photograph of erythema migrans just 1 month before the study was started (11). The other journals were read by less than 12% of the general practitioners.

DISCUSSION

As part of a study assessing knowledge of rheumatic diseases of general practitioners, we evaluated the recognition and knowledge of Lyme borreliosis.

Most of the general practitioners did not recognize the photographs of erythema migrans. On the other hand, the second photograph of erythema migrans was only recognized by 54% of the dermatologists. When patients with erythema migrans are not recognized as having Lyme borreliosis, they will probably not be treated with appropriate antibiotics. This will then increase the risk of the development of later stages of Lyme borreliosis. Despite the fact that several papers about Lyme borreliosis have been published in the Dutch literature and also in journals commonly read by general practitioners, it is clear that more information about the different manifestations of erythema migrans should be given to both general practitioners and dermatologists. Most of these articles focus on the entire spectrum of the disease.

A typical case of Lyme borreliosis with almost all clinical features of the disease was easily recognized by more than 50% of the general practitioners and most rheumatologists. However, a case of Lyme borreliosis with recurrent arthritis of the knee, without other preceding signs and symptoms, was not diagnosed either by rheumatologists or by general practitioners. Recurrent arthritis attacks, especially of the knee, are a common feature of Lyme borreliosis and should be recognized by rheumatologists at all events (4, 37).

TABLE 2

Probability Ratings in the Multiple-Probability Estimate Questions to Two Photographs of Erythema Migrans Presented to General Practitioners and Dermatologists

	General Practitioners (n = 51)						Dermatologists (n = 13)					
	Highly Probable		Probable		Highly Improbable		Highly Probable		Probable		Highly Improbable	
	N	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%
Photo 1	7	13.7	10	19.6	34	66.7	10	76.9	3	23.1	0	0
Photo 2	7	13.7	9	17.6	35	68.6	6	46.2	6	46.2	1	7.6

TABLE 3

Probability Ratings in the Multiple-Probability Estimate Questions to Two Cases of Lyme Borreliosis Presented to General Practitioners and Rheumatologists

	General Practitioners (n = 51)						Rheumatologists (n = 23)					
	Highly Probable		Probable		Highly Improbable		Highly Probable		Probable		Highly Improbable	
	N	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%
Case 1	31	60.7	7	13.7	13	25.5	20	86.9	0	0	3	13
Case 2	2	3.9	4	7.8	45	88.2	0	0	4	17.4	19	82.6

We presented the photographs of erythema migrans and the two cases of lyme borreliosis also with a differential diagnosis to assess whether Lyme borreliosis is a diagnosis considered by the general practitioners at all. Presentation of the multiple-probability estimate questions had a negative influence on the answers of the general practitioners concerning the two photographs of erythema migrans. Rheumatologists did not even rate Lyme arthritis as probable or highly probable in their differential diagnosis for the second case. One might carefully conclude, relying on the results of this study, that a diagnosis of Lyme borreliosis will be overlooked not only by the general practitioners but also by the dermatologists and the rheumatologists.

Although physicians consistently report, for the purpose of gathering knowledge for later use, that reading, primarily of medical journals, is their predominant source of information; publication of several articles about Lyme borreliosis in the Dutch literature apparently did not influence the results of this study (38). This is probably due to lack of direct knowledge and low suspicion of Lyme borreliosis, but it is probably also due to the low chance of encountering a patient with Lyme borreliosis. It is not said that the results of this study will be applicable to all general practitioners (or dermatologists and rheumatologists) or to general practitioners in parts of the country where the chance of encountering patients with Lyme disease will be higher. Although ticks, infected with *Borrelia burgdorferi*, have been found in all parts of the country and people who have been bitten by ticks are at risk everywhere, one might assume that in certain areas with a high infection rate of ticks, general practitioners more often encounter patients with Lyme disease, which will increase their suspicion (31).

The use of standardized (simulated) patients with different stages of Lyme borreliosis would have been the best way to evaluate the real performance of the general practitioners but appeared impracticable (39). Regarding the photographs of erythema migrans, the procedure of observing a patient's cutaneous problem directly and providing a questionnaire to gather accompanying symptoms and to palpate the skin lesion is of course a better assessment procedure. We presented the photographs of erythema migrans without any comment, because about half the patients with erythema migrans do not remember a tick bite and sometimes only notice their skin lesion by accident. Frequently, there are no additional supportive data from the clinical history. The cases of Lyme borreliosis were presented as written cases. A possible criticism of this method is that hypothetical case scenarios may include selected aspects of clinical reality while neglecting others and that physicians may not respond in the same way to hypothetical scenarios as they do to real ones. We tried to avoid this criticism by deriving our cases from real patients. A study by Rethans and van Boven suggested no significant difference in the overall score for written case simulations and the use of standardized real patients (39). Written performance testing may still supply valid information and may well be used in assessment situations (40).

Is it necessary for general practitioners to have knowledge of Lyme borreliosis at all regarding the low incidence of the disease in the Netherlands? There are several reasons to suppose so. As stated before, the disease, especially erythema migrans, is easily treated with antibiotics by general practitioners. Timely treatment with antibiotics will prevent the later stages of the disease and reduce the burden of illness substantially. There is also increasing awareness of Lyme borreliosis not only in the medical press but also in the lay press. Overdiagnosis of Lyme disease has been described recently (41–43). Knowledge of the natural history and suspicion of the signs and symptoms of Lyme borreliosis will not only provide for adequate treatment of the patients that do have Lyme disease but will also prevent that the patients without the symptoms described in the reports about the natural history of untreated Lyme patients are unnecessarily exposed to prolonged treatment with antibiotics. Too often, patients with only vague symptoms of nonspecific fatigue and arthralgia or myalgia are unnecessarily treated with antibiotics, even with antibiotics intravenously (41–44). We stress that we do not want to suggest that general practitioners consider erythema migrans in their differential diagnosis of every "red" skin lesion or consider Lyme arthritis for every patient with arthritis and start antibiotic treatment without a considered judgement. The possibility of a tick bite, visits to tick-infested areas, the oc-

currence of an expanding skin lesion after a possible bite, populations at risk as hunters, and the clinical history of a patient are all important tools for the diagnosis of Lyme borreliosis (45).

In summary, based on this study, better education aimed at recognition of the (early) manifestations of Lyme borreliosis and especially erythema migrans is necessary for general practitioners as well as for rheumatologists and dermatologists in the Netherlands.

APPENDIX 1

Case 1

A 35-year-old male nurse from a wooded area visits your practice complaining about a warm, swollen right knee he has had for a couple of days. In fact, he already complained about his knee during a short stay in the coronary care unit. He was admitted because of acute dizziness. The diagnosis was second-degree atria-ventricular block, which came into remission spontaneously. You prescribed him some acetaminophen some weeks before this admission because of flulike complaints with slight fever, chills, and lymphadenopathy. He remembered some sort of bite with redness on his right leg, which had been visible during a couple of weeks after this bite.

At physical examination, there is a warm, heavily swollen knee with severe limitation of movements.

Open-ended question: What is (are) your most likely diagnosis (diagnoses)?

Multiple-probability estimate question: How would you rate the probability of each of the following diagnoses on a scale from 1 (highly improbable) to 7 (highly probable)?

Viral arthritis
Rheumatic fever
Reactive arthritis
Gout
Lyme borreliosis
Septic arthritis
Systemic Lupus Erythematosus
Rheumatoid arthritis

Case 2

A 47-year-old pilot visits your practice complaining about a warm, swollen left knee, which has lasted several weeks already. He does not complain about any pain. Flexion is severely limited. He has used some NSAIDs without any result.

Last year he had the same problem. The arthritis lasted at that time for 8 weeks. Due to his job, he travels around the whole world. After long-distance flights, he has some lower back pain. He takes a drink now and then to fall asleep.

Open-ended question: What is (are) your most likely diagnosis (diagnoses)?

Multiple-probability estimate question: How would you rate the probability of each of the following diagnoses on a scale from 1 (highly improbable) to 7 (highly probable)?

Rheumatoid arthritis
Reiter's syndrome
Gout
Spondylitis ankylopoetica
Lyme borreliosis
Osteoarthritis
Meniscal tear
Gonococcal arthritis

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Ticks of California and Their Public Health Significance

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In California, ticks are known to transmit seven microbial disease agents to humans. The etiologic agents include one virus (Colorado tick fever virus), five bacteria (*Borrelia (B.) burgdorferi*, *B. hermsii*, *B. parkeri*, *Francisella tularensis*, and *Rickettsia rickettsii*), and one protozoan (*Babesia* sp.). These agents cause Colorado tick fever, Lyme disease, relapsing fever, tularemia, Rocky Mountain spotted fever, and babesiosis. Virtually all cases of relapsing fever are caused by *B. hermsii* in montane regions; *B. parkeri* is associated rarely with human illness at lower elevations. In 1992, ticks transmitted 98% of the vector-borne pathogens contracted indigenously in California with Lyme disease alone accounting for 94% of cases reported that year. Among the 48 species of ticks established in the state, five species are of actual or potential public health importance because of allergic reactions to their bites (*Ornithodoros coriaceus*), their capacity to transmit one or more microbial disease agents to people (*Dermacentor andersoni, D. occidentalis, Ornithodoros hermsi*), or both (*Ixodes pacificus*). A sixth human-biter (*Dermacentor variabilis*) is not known to transmit microbial disease agents in California, though it is a vector of *F. tularensis* and *R. rickettsii* in the eastern United States.

Key words: Ticks, Diseases, California

INTRODUCTION

In California, ticks are of considerable public health importance because of allergic reactions induced by their bites or their capacity to transmit several microbial disease agents (1, 2). The tick fauna is among the most diverse in the United States; 48 of the 86 species of ticks recognized nationwide occur in California. This total includes three genera and 20 species of soft ticks (Argasidae) and four genera and 28 species of hard ticks (Ixodidae) (3–5). In addition, the lone star tick, *Amblyomma americanum*, and the cattle tick, *Boophilus annulatus*, have been introduced occasionally into California, but as yet, neither tick has become established. In 1980, Lane and Murray (1) reviewed the statewide incidence of tick-borne diseases, summarized relevant ecological studies, and delineated the California state surveillance program for tick-borne diseases. This communication provides a brief update of the medical importance of ticks in California since the last review.

PUBLIC HEALTH SIGNIFICANCE

Ticks are of public health significance because their attachments may cause various kinds of skin disorders (e.g., erythema, pain, swelling); they may rarely invade the auditory canal producing a condition known as otoacariasis; females of certain ixodids may cause a flaccid, ascending, and sometimes fatal paralysis known as tick paralysis; individuals bitten repeatedly by some ticks may develop allergic or even anaphylactic reactions (2); and most importantly, ticks transmit a wide array of bacterial (including rickettsial), viral, and protozoan disease agents (6).

Six species of ticks infest humans with some regularity in California, four of which serve as primary vectors of one or two of five microbial disease agents (Table 1). These include the argasid *Ornithodoros hermsi* and the ixodids *Dermacentor andersoni*, *D. occidentalis*, and *Ixodes pacificus*. Further, *Ornithodoros parkeri* rarely transmits *Borrelia parkeri* to people in this state (see below), and the primary vector of human babesiosis is unknown. *Dermacentor variabilis* and *Ornithodoros coriaceus* also attach to humans, but neither tick has been implicated as a vector in this region. However, *O. coriaceus* is notorious for the persistent dermatoses and, rarely, the systemic reactions it provokes in some individuals. The erythematous, expanding skin lesions that sometimes result from the attachment of *I. pacificus* in persons previously sensitized to its bite, may be confused with erythema migrans, the clinical marker for early Lyme disease in up to 60 to 80% of patients. Moreover, the first case of tick-bite-induced anaphylaxis in the New World occurred in a person who was bitten repeatedly by *I. pacificus* over a period of many years (2).

The vertebrate reservoirs of tick-borne disease agents in California also are presented in Table 1. Insofar as is known, rodents are the principal reservoir hosts of all the viral and bacterial pathogens listed except for lagomorphs (rabbits and hares), which in conjunction with rodents perpetuate *R. rickettsii* in enzootic foci. The reservoir host(s) of the agent of human babesiosis is (are) unknown in this region, but *I. pacificus* is a competent experimental vector of *B. microti*, the agent of babesiosis in the eastern United States (7).

In 1992, 243 cases of vector-borne diseases were reported by California State health authorities (Table 2); 98% resulted from exposure to ticks or animals presumed to have been infected by tick bite (i.e., tularemia). Mosquitoes and fleas were the source of exposure in the remaining 2% of cases. Lyme disease ranked first with 228 cases (of these, 216 cases were contracted or probably contracted in California) or 94% of the total number reported that year, and 496 cases of the disease were reported for 1991 and 1992 combined (8, 9). Similarly, Lyme disease accounted for over 90% of all the vector-borne illnesses reported nationally in 1992 (10).

During the 1970s when Lyme disease was not yet recognized to be a public health problem in California, ticks transmitted 75% of the major vector-borne infections (1). A total of only 223 confirmed cases of arthropod-borne diseases was reported for the entire decade, with Colorado tick fever and relapsing fever ranking first and second with 85 and 73 cases, respectively. The first recognized case of Lyme disease in California was an individual who had been bitten by a tick while hiking in Sonoma County in 1975 (11). Surveillance for the disease was initiated in 1983 by the

TABLE 1
Primary Reservoirs and Vectors of Tick-Borne Microbial Agents Afflicting Humans in California

Disease (Agent)	Primary Vertebrate Reservoir	Primary Tick Vector to Humans
Viruses		
Colorado tick fever (*Coltivirus*)	Rodents	*Dermacentor andersoni*
Bacteria		
Lyme disease (*Borrelia burgdorferi*)	Rodents	*Ixodes pacificus*
Relapsing fever (*Borrelia hermsii*)	Rodents	*Ornithodoros hermsi*
Rocky Mountain spotted fever (*Rickettsia rickettsii*)	Lagomorphs, rodents	*Dermacentor occidentalis*
Tularemia (*Francisella tularensis*)	Rodents	*D. occidentalis*
Protozoa		
Babesiosis (*Babesia* sp.)	Unknown	Unknown

TABLE 2
*Reported Cases of Selected Vector-Borne Diseases in California, 1992**

Disease	Number of Cases	Percent of Total
Tick-borne		
Lyme disease	228	93.8
Relapsing fever	5	2.1
Rocky Mountain spotted fever	3	1.2
Tularemia	2	0.8
Mosquito-borne		
St. Louis encephalitis	2	0.8
Flea-borne		
Murine typhus	2	0.8
Plague	1	0.4

*Source: R.A. Murray, Division of Communicable Disease Control, California Department of Health Services, Berkeley, California.

California Department of Health Services, but Lyme disease was not made officially reportable until March of 1989 (12).

The first isolate of *B. burgdorferi* from western North America was derived from an adult *I. pacificus* collected at the University of California Hopland Field Station (now the Hopland Research and Extension Center, HREC) in southeastern Mendocino County, California, in 1984 (13). Since then, *B. burgdorferi* has been isolated from ixodid ticks, rodents, or both in ~32 of the 58 counties in this state (14, 15). *Ixodes pacificus* is an important vector to humans with spirochetal infection rates in nymphs and adults usually ranging from < 1 to 6% (14). Several other species of ixodid ticks have been found infected naturally with *B. burgdorferi* in California including *Ixodes neotomae*, an efficient enzootic (maintenance) vector (16, 17). This tick feeds predominantly on rodents and lagomorphs and normally does not bite people. Two species of rodents, the dusky-footed woodrat (*Neotoma fuscipes*) and the California kangaroo rat (*Dipodomys californicus*), serve as reservoirs in California (17, 18). These rodents can infect up to nearly 40 to 50% of noninfected *I. pacificus* larvae fed xenodiagnostically on them (R. N. Brown and R. S. Lane, unpublished data). In the Far West, *N. fuscipes* is a more important reservoir host, because it has a much broader geographical range and occupies more habitat types than *D. californicus*. However, *D. californicus* may be important locally as a reservoir host because of its abundance in certain habitats (e.g., grassland and chaparral) and the high prevalence of *B. burgdorferi* infection detected in some populations. In 1994, for example, 29 (83%) of 35 kangaroo rats collected from a localized population inhabiting grassland at the HREC were found to contain spirochetes with the ear-punch biopsy technique (R. S. Lane, unpublished data).

The relationship of *B. burgdorferi* to its tick vectors, vertebrate hosts, and potential risk factors for human infection have been studied intensively in northern California since 1982. This research sought to determine the basic mechanisms by which the Lyme disease spirochete is maintained and distributed in natural foci, including the modes of transmission to humans and other animals. Lane et al. (14) summarized this body of work as well as research pertaining to *Ixodes scapularis (I. dammini)* in eastern North America and *Ixodes ricinus* in Europe through 1990. The reservoir potential of rodents, the vector competence of their associated ticks, and the epidemiology of Lyme disease also have been treated in several more recent reports (17–20).

Among the other borreliae that occur in California, the montane relapsing fever spirochete *B. hermsii* has been responsible for hundreds of cases since the disease was made officially reportable in 1931 (21). The closely related spirochete *B. parkeri* has been associated rarely with relapsing fever. One group of cases originated in sandy caves in Stanislaus County, where *O. parkeri* ticks (and presumably *B. parkeri*) were found to be the source of infection (21). In contrast to *O. hermsi*, which normally transmits *B. hermsii* spirochetes to persons as they sleep in summer cabins or cottages, *O. parkeri* inhabits the burrows of rodents (e.g., California ground squirrels) and, therefore, seldom has an opportunity to feed upon and infect people.

Limited clinical and serologic evidence suggest that another species of *Borrelia* isolated from the soft tick, *Ornithodoros coriaceus*, may sometimes infect people in California (22). This spirochete, named *B. coriaceae* after its tick vector (23), also is suspected of being the etiologic agent of epizootic bovine abortion, an important disease of rangeland cattle in the Far West, particularly in California (24).

In addition to the proven human pathogens listed in Table 1, several characterized and uncharacterized spotted fever group (SFG) rickettsiae and one typhus group rickettsia, *Rickettsia canada*, have been isolated from ixodid ticks in California (25–27). Of these, only the unclassified SFG rickettsia, 364-D, has been implicated as a cause of human illness (26, 27). This rickettsia is closely related to, but distinct from *Rickettsia rickettsii*, the etiologic agent of Rocky Mountain spotted fever.

Finally, ticks may occasionally transmit other zoonotic disease agents to people in California. Although no human cases of Powassan encephalitis have been reported from the state, the causative virus was isolated from cell cultures of the kidney of a spotted skunk from Sonoma County (28),

and antibodies to this virus were detected in 13 species of mammals from Kern County (29). Also, several cases of human babesiosis have been recorded in California, which appear to be caused by a species other than *Babesia microti*, the etiologic agent of human babesiosis in the eastern United States (30). The three cases reported through 1991 occurred in patients who had been splenectomized prior to diagnosis (30). Studies are under way to elucidate the etiology, clinical spectrum, ecology, and epidemiology of babesiosis in the far-western United States (31, 32).

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Disseminated Lyme Disease after Short-Duration Tick Bite

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Lyme disease, an Ixodes tick-borne spirochetal infection, has been the subject of much controversy. One problematic area has been the prophylactic treatment of deer-tick bites in endemic areas. Some have argued against routine antimicrobial prophylaxis based upon the belief that transmission of *Borrelia burgdorferi* is unlikely before 24–48 hours of tick attachment. Others have suggested that it is cost effective to administer prophylactic antibiotics against Lyme disease when embedded deer-tick bites occur in endemic areas. Herein, a case of disseminated Lyme disease after only 6 hours of tick attachment is presented. The current recommendation against treatment of short-duration tick bites may need reconsideration, particularly in hyperendemic areas.

Key words: Lyme disease, Tick-bite, Prophylaxis

CASE REPORT

A healthy 9-year-old caucasian female acquired an embedded deer-tick bite on her lower left abdomen while playing outdoors on April 6, 1993, in Ocean County, New Jersey, a known endemic area for Lyme disease. Prior to going outdoors, the child was dressed and examined by her mother, who is a microbiology technician. There was no tick bite noted. After 6 hours of outdoor play, the mother noted a slightly engorged, embedded deer tick on the child's abdomen in the left lower quadrant. After removal using tweezers, the tick was identified as a deer tick but was not viable and could not be tested for infection with *Borrelia burgdorferi*. Only viable ticks are tested for infection by the Ocean County Health Department. An infectious disease consultant recommended observation only. There was no initial rash and the child remained well for 2 months.

Approximately 6–8 weeks later, in early June 1993, the child insidiously developed headache, stiff neck, mood changes, and withdrawal from usual interests. On June 29, the child began to manifest intermittent nausea, anorexia, fever, chills, headache, fatigue, and irritability. On July 7, there appeared three small erythematous circular skin lesions on the upper abdomen remote from the original bite location. On July 12, a larger circular erythematous rash appeared surrounding the site of the earlier deer tick bite. On July 13, numerous other lesions consistent with disseminated erythema migrans appeared on the abdomen, back, chest, legs, and arms (Fig. 1). Eighteen lesions were noted altogether, and the child was admitted to the hospital by her pediatrician because she appeared acutely ill.

The laboratory evaluation revealed the white blood cell count to be 11,400 mm^3. There was a mild anemia with a hemoglobin of 11.1 gm/dL and a hematocrit of 33%. The platelet count was elevated at 512,000/mm^3, and the erythrocyte sedimentation rate was increased at 98 mm/h. The Lyme titer by ELISA was markedly elevated at 100.3 (negative <20). The Lyme Western blot was markedly positive with IgG bands at 66, 60, 41, 39, 34, 17, and 15 KDA. The 34 KDA antibody is widely accepted to be species specific for *Borrelia burgdorferi*. The ELISA assay was performed at Community Medical Center, Toms River, New Jersey. The Western blot was performed by Smith-Kline Laboratories.

The child was treated with 28 days of intravenous ceftriaxone 1-g daily and had gradual resolution of all signs and symptoms of Lyme disease and remains well at this time, some 10 months after infection.

DISCUSSION

The current recommendations regarding the treatment of deer-tick bites include reassurance and observation (1). It has been widely publicized that short-duration deer-tick bites are unlikely to transmit Lyme disease (2). Some have suggested, however, that in Lyme endemic areas, prophylactic antibiotic treatment of embedded deer-tick bites would be cost effective (3), while others disagree (4). The degree of tick engorgement, however, may be a better indicator of the risk of transmission (5). Matuschka and Spielman have suggested that in endemic areas where the deer tick carriage rate for *Borrelia burgdorferi* is high, presumptive antibiotic treatment is indicated in the event that a nearly replete nymphal Ixodes tick is found on a person and the branches of the tick's gut cannot be readily distinguished with a hand lens (6). Ocean County, New Jersey is an intensely endemic area for Lyme disease where the carriage rate among deer ticks is about 50%.

Another factor that may strongly affect risk of transmission is the specific removal method. Most presumptions about the duration of tick attachment required for infection are based upon experimental models in which researchers using a careful technique and fine forceps remove ticks after various intervals, noting rates of transmission (7). Realistically, most individuals do not have fine forceps and may not use a careful technique when removing ticks. Unfortunately, many ticks are crushed upon removal because blunt forceps, tweezers as in this case, and even pliers are used in haste by individuals anxious to remove ticks quickly. In this case a partly replete deer tick was crushed during removal, and this may have facilitated transmission of *Borrelia burgdorferi*. Since the actual transmission mechanism is poorly defined and regurgitation of gut contents or saliva may be possible routes of transmission (8), the removal technique may be as important a factor as duration of attachment or degree of deer-tick repletion.

This case provides suggestive epidemiologic, serologic,

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and photographic evidence that disseminated Lyme disease can occur after deer-tick bites of as little as 6 hours duration. This child has only had one known deer-tick bite. Within 6–8 weeks, she developed appropriate signs and symptoms of disseminated Lyme disease in the appropriate time span and 18 erythema migrans lesions including one at the site of the deer tick bite. Although another unrecognized deer-tick bite is theoretically possible, there is no history of any other tick bite or rash. It is suspected that despite the short duration of attachment, the partly engorged deer tick's gut was crushed during removal, which may have facilitated transmission of *Borrelia burgdorferi*. The authors believe this to be the most plausible explanation for her disseminated Lyme disease. The child's markedly positive serology and Western blot are supportive. Although antibody to 34 KDA is often seen in later-stage Lyme disease, there is wide variability in serologic results among commercial laboratories. Reconsideration of the current recommendations regarding prophylaxis of embedded deer-tick bites in intensely endemic areas is suggested, particularly if the deer tick was crushed during removal.

Finally, the risk and cost of antibiotic treatment is substantial compared to the oral antibiotics used for prophylaxis. Liegner (9–11) has drawn attention to the "toll of human suffering" associated with disseminated Lyme disease. It may be not only cost effective but would serve the greater good if embedded deer-tick bites in intensely endemic areas, particularly if crushed upon removal, were treated with prophylactic oral antibiotics, as is offered to those with substantial exposure to syphilis.

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