#13 4/28-29/95

VIII Annual LDF International Scientific Conference on Lyme Borreliosis and other Spirochetal and Tick-Borne diseases

with an emphasis on: Mechanisms of Lyme Borreliosis Persistency

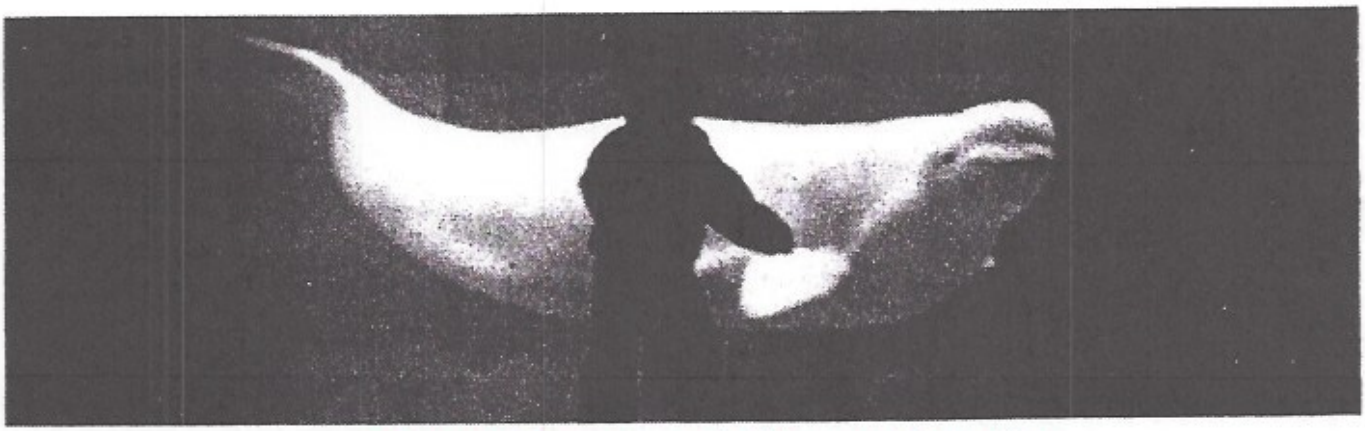
Hyatt Regency Hotel, Vancouver, British Columbia, Canada
April 28 & 29, 1995

Co-Chairs:

S. N. Banerjee, Ph.D., British Columbia Center for Disease Control, Canada
Willy Burgdorfer, Ph.D., M.D. (hon), NIH, Rocky Mountain Laboratories, U.S.
Bettina Wilske, M.D., Max von Pettenkofer Institute, Germany

Poster Session Chair:

Craig Cleveland, M.D., East Hyde Park Internal Medicine, U.S.



... with a special reception at the Vancouver Acquarium

Sponsor: Lyme Disease Foundation

Host: British Columbia Lyme Borreliosis Society

This program has been reviewed and is approved for 15 hours of category 1 study credits by the College of Family Physicians of Canada.

Lyme Disease Foundation, 1 Financial Plaza, Hartford, CT, 06103 USA
203-525-2000 Fax 203-525-TICK

CONFERENCE AGENDA

AM **Friday April 28, 1995**

8:30 **WELCOME**
John Millar, M.D., Provincial Health Officer, B.C.
Diane Kindree, B.S.N., Lyme Borreliosis Society of Canada

GENERAL SESSION

8:45 **Lyme borreliosis clinical overview**
J. Katzel, M.D., *Ukiah Valley Medical Center*

EPIZOOTIOLOGY, EPIDEMIOLOGY SESSION
Chairs: S. N. Banerjee, Ph.D., J. Oliver, Ph.D.

9:00 **Update on the epizootiology of *Borrelia burgdorferi* in southeastern U.S.**
J. Oliver, Ph.D., *Callaway Professor of Biology, Georgia Southern University*

9:20 **Ecology of Lyme disease in Northeastern United States**
J. F. Anderson, Ph.D., *Director, The CT Agricultural Experimental Station*

9:35 **Isolation of *B. burgdorferi* in British Columbia during 1988-1994**
S. N. Banerjee, Ph.D., *Head, B.C.. Center for Disease Control*

9:50 **Lyme disease in British Columbia**
P. Daly, M.D., *Associate Director, B.C. Center for Disease Control*

10:05 **Lyme disease cases acquired in British Columbia 1992-1994**
R. Gill, BSc., *Health Science Officer, B.C. Centre for Disease Control*

10:20 **Surveillance for Tick-borne Relapsing Fever in Texas caves**
J. Rawlings, M.P.H., *IDEAS, Texas Department of Health*

10:35 **Discussion**

VACCINE PROGRESS SESSION
Chair: M. Philipp, Ph.D.

10:45 **Induction of Lyme arthritis and role for *B. burgdorferi* specific T- lymphocytes**
R. Schell, Ph.D., *Professor and Chief Bacteriologist, Wisconsin State Laboratory of Hygiene*

11:05 **Efficacy of recombinant OspA formulations in Rhesus monkey**
M. Philipp, Ph.D., *Chairman, Department of Parasitology*

11:25 **Update on recombinant Lyme disease vaccine development**
A. Barbour, M.D., *Department of Medicine and Microbiology, University of Texas Health Science Center*

11:45 **Discussion**

12:00- 1:00 LUNCH

PATHOGENESIS OF DISSEMINATION SESSION
Chairs: B. Wilske, M.D., D. Dorward, Ph.D.

1:00 **Antigenic variation of *Borrelia burgdorferi sensu lato*: implications for pathogenesis, diagnosis and prophylaxis**
B. Wilske, M.D., *Max von Pettenkofer Institute*

1:25 **Virulent *B. burgdorferi* specifically attach to, activate, and kill TIB-215 Human B-lymphocytes**
D. Dorward, Ph.D., *Senior Staff Fellow, Rocky Mountain Laboratories*

1:50 **The *Borrelia turicatea*-mouse model of Lyme disease**
A. Barbour, M.D., *Department of Medicine and Microbiology, University of Texas HealthScience Center*

2:15 **Discussion**

2:30 COFFEE BREAK

CONFERENCE AGENDA

PM **Friday, April 28, 1995**

INNOVATIVE BIOLOGY SESSION
Chair: W. Burgdorfer, Ph.D., M.D. (Hon.)

2:45 **DNA sequencing of 16S RNA of *Borrelia burgdorferi* isolates from Canada and USA**
M. Altamirano, Ph.D., *Clinical assistant professor, University of British Columbia*

3:05 **Further evidence for a spirochetal etiology of Alzheimer's disease**
J. Miklossy, M.D., *University Institute of Pathology, Division of Neuropathology*

3:25 **Is "Coil/Ag-30" the answer to Lyme borreliosis and other infectious diseases ?**
W. Burgdorfer, Ph.D., M.D. (Hon.), *Rocky Mountain Laboratories*

3:45 **Discussion**

NEW THERAPEUTIC APPROACHES SESSION
Chairs: S. Donta, M.D., C. Garon, Ph.D.

4:05 ***Borrelia burgdorferi* surface DNA network represents a possible target for a new category of antimicrobial agents**
C. Garon, Ph.D., *Chief, RML, National Institute of Allergy and Infectious Diseases*

4:25 **Cellular infection of human fibroblasts, Langerhans cells and leukocytes by isolates of *Borrelia* with emphasis to antibiotic and antibody treatment**
D. Hulinska, Ph.D., *National Institute of Public Health, WHO Collaborating Center for Lyme Disease*

4:45 **Evidence for the intracellular borreliae localization**
S. Donta, M.D., *Professor of Medicine, Boston University Medical Center*

5:05 **Discussion**

5:20 Adjourn

7:30 **RECEPTION** - Specifics on transportation posted at the conference registration desk

AM **Saturday, April 29, 1995**

DIAGNOSTIC ADVANCEMENTS SESSION
Chairs: M. Manak, M.D., B. Schmidt, Ph.D.

8:30 **Use of recombinant, chimeric proteins for the diagnosis of Lyme disease**
B. Luft, M.D., *Chief of the Department of Medicine, SUNY at Stony Brook*

8:50 **Detection of *Borrelia burgdorferi* sequences in clinical specimens by a PCR capture assay**
M. Manak, M.D., *Senior Vice President, Biotech Research Laboratories*

9:10 **Detection of *B. burgdorferi*-DNA in urine from patients with Lyme borreliosis**
B. Schmidt, Ph.D., *Laboratory for dermato-venerological serodiagnosis*

9:30 Discussion

9:40 **A solid-phase ELISA-antigen detection system for Lyme disease**
R. C. Tilton, Ph.D., *Senior Vice President, North American Laboratory Group*

10:00 **Surrogate markers of active infection in Lyme disease**
S. Schutzer, M.D., *Department of Medicine, University of Medicine and Dentistry of New Jersey*

10:20 **New tools in the diagnosis of neurologic Lyme disease**
P. K. Coyle, M.D., *Professor of Neurology, School of Medicine, SUNY at Stony Brook*

10:40 **Morphological heterogeneity of *Borrelia burgdorferi***
A. MacDonald, M.D., *St. Elizabeth's Hospital*

11:00 **Discussion**

11:15 **POSTER SESSION**
Chair: C. Cleveland, M.D. *East Hyde Park Internal Medicine, U.S.*

CONFERENCE AGENDA

PM Saturday, April 29, 1995

12:00-1:00 Lunch

CLINICAL MANIFESTATIONS OF LYME DISEASE SESSION
Chairs: E. Masters, M.D., B. McManus, M.D., Ph.D.

1:00 **Clinical presentations of Lyme borreliosis in Lower Midwest**
E. Masters, M.D., *Family Physicians Group*

1:15 **Lyme arthritis in British Columbia**
G. Price, M.D., *Clinical Associate Professor, University of British Columbia*

1:30 **Cardiovascular manifestations of Lyme disease**
B. McManus, M.D., Ph.D., *Chairman, Pathology and Laboratory Medicine, St. Paul's Hospital*

1:45 **Discussion**

1:50 **Lyme disease case in the Lower Mainland**
E. Murakami, M.D., *Clinical Associate Professor, University of British Columbia Medical School*

2:05 **Acrodermatitis chronica atrophicans**
R. Scrimenti, M.D., *Medical College of Wisconsin*

2.20 **Symptoms and characteristics of chronic Lyme disease in patients**
R. Smithson, Ph.D., *Senior Member of the Research Laboratory, Lockheed Palo Alto Research Laboratory*
I. Vanderhoof, Ph.D., *NY University , Stern School of Business*

2:35 **Discussion**

2:40 Coffee break

CHRONIC/PERSISTING LYME BORRELIOSIS SESSION
Chairs: J. Davies, Ph.D., J. Katzel, M.D.

3:00 **Antibiotic resistance in spirochetes**
J. Davies, Ph.D., *Professor and Head, Dept. of Microbiology & Immunology, University of British Columbia*

3:15 **Spectrum of antibiotic-responsive menigoencephalomyelitides**
K. B. Liegner, M.D., *New York Medical College*

3:30 **Management of disseminated and chronic Lyme borreliosis**
J. Burrascano, Jr., M.D., *Southampton Hospital*
J. Katzel, M.D., *Ukiah Valley Medical Center*

3:50 **Chronic encephalopathy: to treat or not to treat?**
B. Fallon, M.D., *Columbia University & New York State Psychiatric Institute*

4:05 **Discussion**

4:15 **PUBLIC FORUM** Moderator: J. J. Burrascano, Jr., M.D.
Overview of Lyme disease - M. Ziska, M.D.
Questions and answers - Panel from the day

6:00 Adjourn

ABSTRACTS OF ORAL PRESENTATIONS

Oliver, Jr., James H., MD
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UPDATE ON THE ENZOOTIOLOGY OF B. BURGDORFERI IN THE SOUTHERN U.S.

Dr. James H. Oliver, Jr., Institute of Arthropodology & Parasitology
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We have isolated B. burgdorferi from cotton mice (Peromyscus gossypinus), cotton rats (Sigmodon hispidus), woodrats (Neotoma floridana) and the ticks Ixodes scapularis and Ixodes dentatus. Locations of isolates extend along the coast from Cape Canaveral , FL northward to the northern coasts of South Carolina and inland in central Georgia and southeast Missouri. Prevalence of B. burgdorferi in rodents may be quite high in some foci (75%). Currently it is unclear whether there are several separate parallel enzootic cycles operating or if there are weblike overlap among them. B. burgdorferi strains are more genetically heterogenous in the southern U.S. than those reported from the northern states. Infectivity varies among the isolates and one wonders if there are resulting differences in clinical symptoms and pathology produced in humans.

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ECOLOGY OF LYME DISEASE IN NORTHEASTERN UNITED STATES

John F. Anderson, Ph.D.; The CT Agricultural Experiment Station,
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Some of the highest incidence rates of Lyme disease (>200,000 cases/100,000 population/county) occur in Northeastern United States. The primary tick vector is *Ixodes scapularis* which is extremely abundant in many wooded and suburban areas where white-tailed deer are common. This tick has been recorded feeding on 120 different species of animals (birds, mammals, lizards). The causative-agent, *Borrelia burgdorferi sensu stricto*, has frequently been recovered from humans, *Ixodes scapularis*, and white-footed

mice. Variants of the spirochete have been recovered from *Ixodes scapularis*, the rabbit associated tick *Ixodes dentantus*, and cottontail rabbit. Variants have not been recovered from humans, and therefore are not known to cause human disease. The interaction of the spirochete with its tick vector and wild host reservoirs will be discussed.

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ISOLATION OF BORRELIA BURGDORFERI IN BRITISH COLUMBIA DURING 1992-1994.

S.N. Banerjee, M. Banerjee, K. Fernando, M. Y. Dong, M. Altamirano and J.A. Smith. Provincial Laboratory, B.C.C.D.C., Vancouver, B.C. Canada, V5Z 1L8

The survey of ticks in B.C. during 1988 to 1991 was limited to adult ticks. In 1992 we began trapping rodents in 20 selected sites and juvenile ticks were retrieved from them. Tick guts as well as six organs from rodents, viz., ear, bladder, kidney, spleen, liver and heart were cultured in BSK II medium with antibiotics. All isolates were immunostained with Mab for P31, P34, P39 and P41 ag. SDS-PAGE profiles, PCR for OSPA gene and DNA sequence of 16SrRNA gene were analysed for all axenic cultures. Up to 1991 when we had only adult ticks for culture, no spirochete was isolated 0/1360 (0%). In 1992 B. burgdorferi (Bb) was isolated from 1/539 (0.2%) ticks and 0/28 (0%) mice. In 1993 20/2086 (1%) ticks and 11/243 (4.5%) mice had Bb. In 1994, 7/691 (1%) ticks and 9/211 (4.1%) mice had Bb. SDS-PAGE protein profiles of isolates were comparable to that of Bb. All Bb were positive by Mab tests and by PCR for OSPA. DNA sequences of 16SrRNA gene were similar to Bb-B31. Bb was isolated from I. angustus and I. pacificus ticks. The presence of spirochetes in juvenile and adult ticks as well as in rodents suggests establishment of Bb in B.C. Our results show that culture of host organs and culture of juvenile ticks retrieved from hosts may be more successful than culture of adult ticks only.

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LYME DISEASE IN BRITISH COLUMBIA

Patricia Daly(1), Satyen Banerjee(2), Craig Stephen(1)
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In June, 1994, Borreliosis was made a reportable disease under the Communicable Disease Regulations of the Health Act in the province of British Columbia. This action was taken because of several pieces of evidence. Since 1988, there have been several cases of clinically suspected Lyme disease with positive serology for *Borrelia burgdorferi* who appear to have acquired Lyme disease from within the province. At the same time, studies have identified the presence of tick species in British Columbia that are known to transmit *B. burgdorferi.* Further studies, which are ongoing, have revealed the presence of *B. burgdorferi* in several tick species form the southwest coastal regions of the province, as well as in Deer mice hosts. The case definition to be used for reporting purposes is that developed by the Canadian consensus conference on Lyme disease in 1991 (CDWR 1991; 17(13):66). Based on the Canadian consensus conference guidelines, British Columbia is considered an endemic area for Lyme disease, although the geographical boundaries for endemicity have not been defined and will require further study.

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LYME DISEASE CASES ACQUIRED IN BRITISH COLUMBIA 1992-1994.

Raj Gill, Satyendra Banerjee and Maya Banerjee

Research on Lyme Borreliosis in British Combo has been on going since 1986. Our first positive case was a 2 year old female from Burns Lake in 1988. Two patients were found to be positive in 1989; a 44 year old female from Salt Spring Island and a 71 year old female form Galiano Island. Only on 46 year old male was positive in 1992 from Kootenay Lake. In 1993, three cases were reported; one 58 year old female from Oliver, a 74 year old female from Nanaimo and a 66 year old male from Cortes Island. As of Oct 1994 4 cases have been confirmed; two males from Agassiz and Lumby and 2 females form Port Coquitlam and Port Moody. This makes a total of 11 cases of Lyme disease acquired in B.C. during 1988-1994 out of a total of 43 cases seen at the Provincial Laboratory, B.C.C.D.C. Only 2 cases showed the classical EM rash, most patients were febrile with headache, fatigue and muscle pain. Elderly

Raj Gill → LD cases acquired in BC 1992-1994

patients presented with arthralgia and myalgia. All patients were positive by the ELISA method and confirmed by Western Blot sets and clinical diagnosis by physicians. It is pertinent to note that during 1993-94 Dr. S. Banerjee's Vector Borne Disease Lab isolated *B. burgdorferi* the etiologic agent from the deer mice *Peromyscus maniculatus* and two species of ticks viz. *Ixodes pacificus* and *I. angustus* collected from the places where cases were identified. On the basis of our findings and the Lyme Disease consensus report on case definitions, B.C. should be considered an endemic area for Lyme Disease.

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SURVEILLANCE FOR TICK-BORNE RELAPSING FEVER IN TEXAS CAVERNS

Julie Rawlings, Texas Department of Health

Tick-borne relapsing fever (TBRF) is caused by *Borrelia* spirochetes and is transmitted by *Ornithodoros* ticks. In Texas, the vector is *O. turicata*, found in caves frequented by sheep and goats or rodent and snake burrows; the agent is *B. turicatae*. Spelunkers may be at risk of acquiring TBRF as they explore Texas caves. In an attempt to establish the prevalence of exposure to *B. turicatae* in caverns, a questionnaire was administered to and sera was collected from 112 persons attending a Texas Speliological Society meeting in October, 1994. Data collected by questionnaire were analyzed to identify risk factors for disease were analyzed to identify risk factors for disease and evaluate histories of illness in these caverns. Each specimen was tested by indirect immunofluorescent antibody test (IFA) for antibody to three species of *Borrelia*. Eighteen were reactive.

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INTRODUCTION OF LYME ARTHRITIS AND ROLE FOR *BORRELIA BURGDORFERI* SPECIFIC T-LYMPHOCYTES

Lony C.L. Lim and Ronald F. Schell. Wisconsin State Laboratory of Hygiene and Departments of Bacteriology and Medical Microbiology and Immunology, University of Wisconsin, Madison, Wisconsin 53706. Research for this abstract was completed November 23, 1994.

Row Schell: cont'd

We showed that severe destructive Lyme arthritis developed in vaccinated hamsters after challenge with isolates of *Borrelia burgdorferi* sensu lato and suggested a role for cell-mediated immunity. Specifically, severe destructive arthritis was readily evoked in immunocompetent inbred LSH hamsters vaccinated with a whole-cell preparation of Formalin-inactivated *B. burgdorferi* organisms in adjuvant when challenged with the homologous (vaccine) isolate before high levels of protective borreliacidal antibody developed. Once high levels of protective borreliacidal antibody developed, vaccinated hamsters were protected from homologous challenge and development of arthritis. Vaccinated hamsters, however, still developed severe destructive arthritis when challenged with other isolates of *B. burgdorferi* sensu lato. We now show that *B. burgdorferi* specific T-lymphocytes were responsible for the development of severe destructive arthritis. *B. burgdorferi* specific T-lymphocytes obtained from immunocompetent hamsters vaccinated with a whole-cell vaccine conferred on naive recipient hamsters the ability to develop severe destructive arthritis when challenged with *B. burgdorferi* sensu stricto isolates. The successful demonstration that *B. burgdorferi* T-lymphocytes were responsible for the adoptive transfer of severe destructive arthritis was confirmed by immunological enrichment for T-lymphocytes and characterization of the transferred cells. These studies are important for the development of a safe vaccine (whole or sub-unit) and for determining immunologic modulators to prevent the development of the signs and symptoms of Lyme arthritis.

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EFFICACY OF RECOMBINANT OspA VACCINE FORMULATIONS IN THE RHESUS MONKEY

Philipp M (1), Lobet Y (2), Robert D (1), Dennis V (1), Desmons P (2), Gu Y. (1), Hauser P (2), Lowrie Jr. R (1).
Tulane University Primate Res. Center, Louisiana, USA (1); SmithKline Beecham Biologicals, Rixensart, Belgium (2).

Three different vaccine formulations, NS1-OspA/aluminium hydroxide (Alum), NS1-OspA/Alum/MPL (an immunostimulant), lipidated OspA/Alum, and one placebo (Alum) were used in a vaccination trial involving 16 male 2-3-year-old Chinese Macaca mulatta (rhesus). Four animals were randomly assigned to each of 4 groups, and each group received one of the vaccine formulations. Three 10 µg doses of each vaccine were given to each animal intramuscularly into the cranial thigh, at 4-week intervals. All animals were exposed to the bite of Ixodes scapularis nymphs infected with the B31 strain of *Borrelia burgdorferi* (*Bb*), 5 weeks after the last injection. Of the 50 ticks that fed upon the animals in

Philipp continued:

1994

the placebo group, 48 contained spirochetes detectable by a direct fluorescence assay (DFA) with an anti-*Bb* polyclonal antibody, whereas only one of 130 ticks on the vaccinated animals had detectable *Bb* by DFA. On Western blots of whole *Bb* antigens, serum antibody from each of the control animals reacted with 10-16 antigens 4 weeks post-challenge (PC), and 22-36 by week 40. Vaccinated animals failed to develop any detectable anti-*Bb* antibodies during the same study period, except for the anti-OspA response induced by the vaccine. Skin biopsy and blood samples obtained weekly during the first 4 weeks PC from both control and vaccinated animals yielded no spirochetes upon in vitro culture. In contrast, skin samples from all of the control but none of the vaccinated animals contained Bb DNA during the first 4 weeks as revealed by PCR using primers that hybridize to a chromosomal DNA fragment of *Bb*. In the same time period, 2/4 controls and 2/16 vaccinated animals had deep perivascular lymphocytic infiltrates in the skin adjacent to the infection site, with cells that stained positive with MAbs reacting with OspA and with a 7.5 kDa lipoprotein of *Bb*. Organs and tissues from several organ systems of the 4 controls and the 4 animals vaccinated with lipidated OspA and one of the animals vaccinated with NS1-OspA/MPL and with NS1-OspA were analyzed postmortem. No gross pathology was observed in any animals. In the control, unvaccinated animals, inflammation often accompanied by positive immunostaining with anti-7.5 kDa protein and anti-OspA MAbs, and by positive PCR was observed in the kidney (in 2 out of 4 animals), urether (2/4), heart (3/4), joints (1/4), nerves (4/4). In the vaccinated monkeys, no inflammation was observed in the kidneys, urether and joints, while an inflammation milder than that seen in control animals was detected in the heart (2/6) and nerves (2/6). The lungs of 4/4 control animals and 5/6 vaccinated animals showed lymphocytic hyperplasia. Some macrophages within these cell clusters stained positive with the anti-7.5 and anti-OspA MAbs. PCR of lung tissue was positive for 2/4 control and 3/4 vaccinated animals. The difference in the tick infection rate between ticks that fed on vaccinated and control monkeys, the lack of seroconversion in the vaccinated animals and the absence of spirochetal DNA in the skin of the vaccinated animals in the weeks following the challenge, indicate that the vaccinated monkeys were protected against the tick challenge. The post mortem analysis, however, suggests that wither a very low spirochete burden (non-immunogenic) or a transient infection may have occured in some of the vaccinated animals. These last observation warrant further analyses of the remaining animals.

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UPDATE ON RECOMBINANT LYME DISEASE VACCINE DEVELOPMENT - Nothing important nor new.

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LYME DISEASE CASE IN THE LOWER MAINLAND, AND A NEW WOOD TICK REMOVAL TECHNIQUE.

Ernie Murakami, M.D., Department of Family practice, UBC; Nima Shojamia, Medical Student, 2nd year, UBC; Satyen N. Banerjee, Ph.D., Provincial Laboratory, BCCDC

A 57 year old man presented to the medical clinic at an Agassiz Correctional Institute in B.C. on April 18, 1994. Complaining of a circular rash on his right leg, neck stiffness and right shoulder chronicum migrans was the diagnosis and serologically Lyme disease was confirmed. Since this patient was incarcerated for three years the local animal vector had to be present at the Agassiz Institution, (rats, mice, cats and raccoons). Erythromycin was given to this patient and he improved clinically. Wood ticks carry disease (second only to mosquitoes) to man and animals: i.e. Rocky Mountain spotted fever, Colorado tick fever, tularemia, babesiosis and Lyme disease. Pediatric abstract summarized five popular methods of removal: jelly, nail polish, alcohol, hot match, forceps and fingers (Pediatric, volume 74, No., June 1985). All these above method may cause increased intraabdominal pressure thus host infection possibilities are greatly increased. The method proposed for the removal involves the injection of Xylocaine intradermally and the hydrostatic pressure causes the tick to fall off the host voluntarily. In some cases a sharp needle or scalpel is required for extraction. This method avoids and prevents any regurgitation of insect abdominal contents. The wood tick is complete and alive for speciation and culturing for infective agents.
This technique has been practiced in Hope, B.C. for the past five years by myself and my associates. All research for this project was done in Hope, B.C.

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ACRODERMATITIS CHRONICA ATROPHICANS

ACA, the first manifestation of LB to be described, is an outstanding example of prolonged latency and chronic infection. It is rarely reported in America. Our knowledge is drawn from a rich European experience. It consists of an early inflammatory stage and a late atrophic stage. A poorly demarcated acral erythema or edema usually ushers in the disease. It disseminates primarily to other acral sites often sparing the torso. Juxta articular modules and fibrotic linear bands may develop over the extensor surfaces of the extremities. Over

Scrimenti, MD - ACA

many months to many years; atrophy may develop. Eventually, lymphadenopathy, weight loss, fatigue, neuropathies, phalangeal joint luxations may appear. Sclerotic and atrophic lesions, indistinguishable clinically and histopathologically from Morphea and Lichen Sclerosus Atrophicans may be seen. Histopathology reveals a predominant CD4 lymphocytic infiltrate, lymphatic telengiectasis and lymphedema. With progression, atrophy of the skin and elastic fibers develops. A rich admixture of plasma cells, if present may distinguish sclerotic and atrophic ACA from Morphea and LSA et A. in the absence of the spirochete. Many lymphocytes and keratinocytes express HLA-DR and HLA-DQ antigens which disappear with therapy. Bb specific IgG titers are usually high. Doxycycline is the usual therapy for uncomplicated ACA. Benzyl penicillin IV and oral Doxycycline is the preferred therapy if neurologic and/or arthritic complications are present.

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SYMPTOMS AND CHARACTERISTICS OF CHRONIC LYME DISEASE PATIENTS

Over the years the Lyme Disease Foundation and the Society of Actuaries have compiled a data base of symptoms, characteristics, and treatment of over 1,000 Lyme disease patients. The data is based upon a questionnaire filled out by the members of the studied group. Since the participants were self selected it is not surprising that the group is primarily composed of patients with long standing illness - the chronic cases.

The analysis is of two types. The first is the calculation of the descriptive statistics of the group. These include symptoms, test results, geographic locations, and cure or continuing impairment. Cost of treatment information is also studied and an attempt is made to correlate this with the other dimensions of the data.

The second type of analysis is the use of a probabilistic neural network technique developed at Lockheed to try to find a pattern for those patients that have continuing illness as opposed to those who are eventually symptom free.

Prevention of Lyme disease through vaccination is now seen as a reasonable and perhaps necessary strategy for control of infection in humans and domestic animals. The focus of vaccine development has been on OspA, an outer membrane protein of the species of *Borrelia* that causes Lyme disease in North America, Europe, and Asia. Studies in animals showed the effectiveness of recombinant OspA in protecting against syringe- and tick- delivered challenges. In the last two years human trials of OspA as a vaccine have begun. The progress in one set of these human trials of safety, immunogenicity, and efficacy will be presented. An additional emphasis will be on vaccine candidates in earlier stages of investigation.

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ANTIGENIC VARIATION OF *BORRELIA BURGDORFERI* SENSU LATO: IMPLICATIONS FOR PATHOGENESIS, DIAGNOSIS AND PROPHYLAXIS OF LYME DISEASE

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Wild type strains of *Borrelia burgdorferi* sensu lato express OspA or OspC (or both) in abundant amounts. Expression of the two proteins is negatively correlated and appears to vary with the environment. Osp A is regularly expressed in *Ixodes ricinus* whereas OspC is rarely detected. Analysis of the human antibody response by Western blot suggest the inverse situation in the human host (strong expression of OspC, in contrast to low expression of OspA). Using a panel of OspA-specific monoclonal antibodies (MAbs) we previously classified European strains into seven OspA-serotypes (confirmed by sequence analysis) (1). Phenotypic analysis with OspC MAbs revealed at least 13 different types (2). OspC sequence analysis confirmed the immunological heterogeneity at a molecular level. In some strains (including also American strains) OspC was considerably heterogenous whereas OspA was highly conserved. Analysis of a large number of European isolates from patients (n=102) revealed a significant association of the OspA-serotype with the clinical manifestation of the disease:

source	type 1	type 2	type 3	type 4	type 5	type 6	type 7	total
CSF	8	5	3	12	3	10	2	43
skin	4	57	0	2	1	4	0	68

1994

Bettina Wilske cont'd.

The high diversity of strains isolated from patients with neuroborreliosis (all seven serotypes were found) has important implications for microbiological diagnosis (serodiagnosis, PCR) as well as for vaccine development.

(1) Wilske, B. et al., J. Clin. Microbiol.: 31 (1993) 340-350 (2) Wilske, B. et al., J. Clin. Microbiol. (1995), in press

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VIRULENT *BORRELIA BURGDORFERI* SPECIFICALLY ATTACH TO, ACTIVATE, AND KILL TIB-215 HUMAN B-LYMPHOCYTES

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In order to examine the effects of Lyme disease spirochetes on immune effector cells, experimental co-incubations of *B. burgdorferi* Sh-2-82 and cultured human and B- and T-lymphocytes, TIB-215 and H9 cells respectively, were begun in the fall of 1994. Comparisons were made in the structure and viability of B- and T-cells incubated with varying concentrations of virulent or attenuated *B. burgdorferi*, or virulent *B. hermsii* after 1, 24, 48 and 72 hr. The levels of IgM antibody secretion of B-cells exposed to spirochetes and /or the known B-cell activators IL-5 and IL-6 were also compared. Examination by light and electron microscopy showed that at relative concentrations of 100:1, low passage *B. burgdorferi* attach rapidly to 90=% of B-cells. Video microscopy revealed that initial attachment involved spirochetal apices. Within one hour, affected B-cells began to aggregate in culture wells. After 24 hours, numerous spirochetes covered B-cell surfaces, and up to 50% of B-cells were lysed. Such effects were dependent on infectious dose. No significant attachment and killing was noted in co-incubations involving high passage *B. burgdorferi*, virulent *B. hermsii*, or T-cells. Furthermore, heat killed or sonicated spirochetes, or spirochetal culture supernatants had no apparent effects on B-cells, suggesting that attachment and cytolysis require inducible factors. Since there was no evidence of B-cell invasion, and cytolysis was serum complement-independent, B-cell killing may involve a previously undocumented toxigenesis. Electrophoretic and western blot analysis, using convalescent human Lyme disease serum, showed that at least four proteins and human immunogens were produced by virulent spirochetes in response to co-incubation with this B-cell line. Whether any or all of these factors are involved in B-cell killing remains to be determined. Quantitative comparisons of IgM secretion by B-cells, incubated with spirochetes and/or interleukins 5 and 6, showed that all three spirochetal populations effectively activated the B-cells and stimulated IgM secretion. Such activation is consistent with previous studies with normal human B-cells. These studies document that *B. burgdorferi* exhibits specific

Area of Research that needs to START! *

[24 year old idea!]

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cytopathic effects on cultured B-cells, and provide a model fro examining the possibility that such interactions between spirochetes and B-cells may play a role in colonization and persistence of *B. burgdorferi* in mammals.

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THE *BORRELIA TURICATAE*-MOUSE MODEL OF LYME DISEASE

Mice infected with *Borrelia burgdorferi* develop arthritis and carditis, in common with humans with Lyme disease. However, mice and other non-primate animal models of *B. burgdorferi* infection only transiently or inconsistently have involvement of the central nervous system. We have found that a strain of *B. turicatae*, an agent in relapsing fever in southwestern North America, produces infection and involvement of both peripheral and central nervous system of mice. In addition, the neurotropism of this species appears to be limited to certain serotypes. Serotype A notably infects the brain, but serotype B does. In contrast serotype B infections are characterized by a severe polyarticular arthritis; serotype A produces only mild arthritis. The only discernable difference between serotype A and B of *B. turicatae* is in an outer membrane protein that is homologous to OspC proteins of *B. burgdorferi*. These findings suggest that disease manifestations of Lyme disease and other borrelial infections are in part determined by features of an infecting organism.

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DNA SEQUENCES OF 16S RNA OF *B. burgdorferi* ISOLATES FROM CANADA AND U.S.A.

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The phylogenetic relationship between some of the Borrelia isolates form Canada and U.S.A. has been investigated by amplification of a 276 base pairs segment of the 16S RNA gene. The DNA sequences were compared with those analyzed for *B. burgdorferi* B31 and other groups such as *B. hermsii*, *B. anserina* and *B. garinii*. Primers for the Polymerase Chain Reaction (PCR) were selected from conserved regions of the gene that codes for the 16S RNA

Bb isolates from Canada & U.S.A.
Altamirano, Manuel

in Borrelia strains. DNA extracted from isolates was amplified by PCR in a 50 ul reaction for 35 cycles, using 48° C for annealing temperature. The PCR products were purified by HPLC and both strands were sequenced using fluorescein labeled primers in cycling reactions in the presence of *Thermus thermophylus* DNA Polymerase. The sequencing reaction were analyzed in the automatic laser DNA sequencer. The 16S RNA sequences from the Canadian isolates were clustered and similar to *B. burgdorferi* B31 strain. Differences in sequences were seen in some isolates previously identified as *B. burgdorferi*. The phylogenetic relationships of those isolates along with further molecular biology studies shall be discussed.

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FURTHER EVIDENCES FOR A SPIROCHETAL ETIOLOGY OF ALZHEIMER'S DISEASE.

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Recently we reported that spirochetes were found by dark field microscopy in the blood, cerebrospinal fluid and were isolated form the brain tissue of Alzheimer's disease (AD) cases, including two familial AD cases. Moreover the spirochetes were cultured from the cortex tissue in three out of four AD cases investigated. Reference strains of spirochetes showed a positive immunoreaction with a monoclonal antibody against the ß amyloid precursor protein (APP) of AD, indicating that spirochetes may contain APP or at least an APP-like (APLP) protein. Using scanning and atomic force microscopy we observed that the isolated and cultured microorganisms from the AD brain possess axial filament. Using a monoclonal antibody against Borrelia burgdorferi we found a positive immunoreaction in senile plaques and in neurons in the brain of a patient with concurrent AD and Lyme disease. Individual spirochetes free in the neuropil were also observed.
The 4',6-diamidine-2-phenylindole dihydrochloride (DAPI) Which binds selectively to DNA is frequently used for the visualization of the bacterial DNA of Mycoplasma in cell cultures. We expected that DAPI would also bind to the DNA of spirochetes. If AD is caused by spirochetes, consequently in addition to resident cell nuclei we would find DNA also in senile plaques, neurofibrillary tangles and in the neuropil threads. Indeed when stained with DAPI reference spirochetes may be visualized, but also senile plaques, neurofibrillary tangles and neuropil threads in AD exhibit fluorescence which can be abolished be DNAase pretreatment. Further investigations demonstrated some similar histochemical properties of reference spirochetes and those of the AD type histological changes. These observations seem to reinforce the hypothesis that AD may correspond to the tertiary stage of neurospirochetosis.

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Development of Destructive Arthritis in Vaccinated Hamsters Challenged with *Borrelia burgdorferi*

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We present the first direct evidence that adverse effects, particularly severe destructive arthritis, can develop in vaccinated hamsters after challenge with *Borrelia burgdorferi* sensu lato isolates. Hamsters were vaccinated with a whole-cell preparation of Formalin-inactivated *B. burgdorferi* sensu stricto isolate C-1-11 in adjuvant. A severe destructive arthritis was readily evoked in vaccinated hamsters challenged with the homologous *B. burgdorferi* sensu stricto isolate C-1-11 before high levels of protective borreliacidal antibody developed. Once high levels of C-1-11 borreliacidal antibody developed, hamsters were protected from homologous challenge and development of arthritis. Vaccinated hamsters, however, still developed severe destructive arthritis when challenged with other isolates of the three genomic groups of *B. burgdorferi* sensu lato (*B. burgdorferi* sensu stricto isolate 297, *Borrelia garinii* isolate LV4, and *Borrelia afzelii* isolate BV1) despite high levels of C-1-11 specific borreliacidal antibody. Vaccines that contained whole spirochetes in adjuvant induced destructive arthritis, but this effect was not dependent on the isolate of *B. burgdorferi* sensu lato or the type of adjuvant. These studies demonstrate that caution is necessary when employing whole spirochetes in adjuvant for vaccination to prevent Lyme borreliosis. Additional studies are needed to identify the antigen(s) responsible for the induction and activation of arthritis and to define the immune mechanisms involved.

Lyme borreliosis is caused by the spirochete *Borrelia burgdorferi* sensu lato (3, 5, 19) and is transmitted primarily to human hosts by *Ixodes* ticks (6, 49, 50). Its most characteristic clinical feature is an expanding skin lesion, erythema migrans, yet patients with the illness may present with arthritic, cardiac, or neurological symptoms without a skin lesion (46, 47, 53). The signs and symptoms of the disease change frequently and are often intermittent. If the infection is left untreated, chronic arthritis may develop in weeks or months. Today, Lyme borreliosis has become the most frequently reported tick-associated illness in the United States (8) since it was first consistently reported in the 1970s (48, 51, 52).

The high level of interest in Lyme borreliosis has facilitated a worldwide effort to develop an effective vaccine against *B. burgdorferi* sensu lato. The feasibility of vaccination has been demonstrated in dogs (9, 26), gerbils (33), hamsters (17, 18, 20), and mice (12, 13, 43, 44, 54, 55). Viable (21, 23, 30, 32, 36, 39, 40) and nonviable (9, 17, 18, 20, 22, 26) Lyme borreliosis spirochetes and several of their outer surface proteins, including OspA (12, 13, 34, 35, 37, 38, 43, 44, 54, 55), OspB (14, 34, 35, 55), OspC (33), and the 39-kDa protein (41), have been shown to induce protective antibodies capable of killing isolates of *B. burgdorferi* sensu lato in vitro or preventing infection in animals. Other spirochetal components may also be involved. Recently, a whole-cell vaccine for dogs has become available commercially (9, 18, 26), and field trials involving humans with OspA, as a vaccine are being conducted. Although vaccination studies are progressing, there are important concerns about the heterogenicity (4, 15, 24, 31, 56–59) and immunogenicity (10, 54) of nonviable whole spirochetes and subunit components. In addition, the possibility of adverse effects developing from vaccination with or without adjuvants must be considered.

In this study, we present the first direct evidence that an adverse effect, especially severe destructive arthritis, can develop after vaccination against the Lyme borreliosis spirochete. We describe the development of severe destructive arthritis in mature immunocompetent hamsters vaccinated with a whole-cell preparation of Formalin-inactivated *B. burgdorferi* sensu lato in adjuvant after challenge with isolates of the three genomic groups of *B. burgdorferi* sensu lato.

MATERIALS AND METHODS

Hamsters. Six- to 8-week-old inbred LSH/Ss WSLH hamsters were obtained from our breeding colony located at the Wisconsin State Laboratory of Hygiene. Hamsters weighing 60 to 120 g were housed three per cage at an ambient temperature of 21°C. Food and water were available ad libitum.

Organisms. Low-passage (<10) virulent isolates of the three genomic groups (28, 29) of *B. burgdorferi* sensu lato (*B. burgdorferi* sensu stricto isolates C-1-11 and 297, *Borrelia garinii* isolate LV4, and *Borrelia afzelii* isolate BV1) and *Borrelia hermsii* were cultured once in modified Barbour-Stoenner-Kelly (BSK) medium (7) at 32°C to a concentration of 5×10^7 spirochetes per ml. Five-hundred-microliter samples were then dispensed into 1.5-ml screw-cap tubes (Sarstedt, Newton, N.C.) containing 500 μl of BSK supplemented with 30% glycerol (Sigma, St. Louis, Mo.), sealed, and stored in liquid nitrogen. When needed, a frozen suspension of spirochetes was thawed, and an aliquot was used to inoculate fresh BSK. The culture was incubated at 32°C for 72 h and diluted with fresh BSK to yield 5×10^6 spirochetes per ml. Spirochetes were enumerated by dark-field microscopy and with a Petroff-Hausser counting chamber. Isolates were obtained from S. M. Callister, R. C. Johnson, and G. Stanek.

Preparation of vaccines. *B. burgdorferi* sensu stricto isolates C-1-11 and 297 were grown in 4 liters of BSK to 5×10^8 spirochetes per ml. Spirochetes were harvested by centrifugation (10,000 × *g*, 4°C, 30 min) after three washes with phosphate-buffered saline (PBS, pH 7.4). The pellet was suspended in 1% Formalin and incubated at 32°C for 30 min. The Formalin-inactivated spirochetes were then washed three times by centrifugation and suspended in PBS. Five-hundred-microliter samples containing 5×10^9 spirochetes were dispensed into 1.5-ml screw-cap tubes (Sarstedt) and stored at −70°C. Subsequently, frozen samples of Formalin-inactivated *B. burgdorferi* C-1-11 and 297 were thawed and suspended in 10 ml of aluminum hydroxide gel (HPA-3; Rehels, Inc., Berkeley Heights, N.J.), aluminum hydroxide (Imject alum; Pierce, Rockford, Ill.), Freund's incomplete adjuvant (Sigma), or PBS.

Vaccination of hamsters. Hamsters were mildly anesthetized with ether contained in a nose-and-mouth cup and vaccinated intramuscularly in each hind leg with a single dose containing 0.2 ml of 10^8 Formalin-inactivated organisms of *B. burgdorferi* sensu stricto isolate C-1-11 or 297 in various adjuvants. The protein concentration was 50 to 100 μg per inoculum (Bio-Rad Laboratories, Hercules, Calif.). Controls consisted of nonvaccinated hamsters and hamsters inoculated with 0.2 ml of the adjuvants alone or Formalin-inactivated *B. burgdorferi* sensu stricto isolate C-1-11 or 297 in PBS. Similar or higher concentrations of spirochetes have been used to vaccinate hamsters (20, 22).

Hamster sera. Sera were obtained from hamsters vaccinated with Formalin-inactivated *B. burgdorferi* sensu stricto isolate C-1-11 or 297 with and without adjuvant at various intervals after vaccination. Concomitantly, sera were obtained from noninfected normal hamsters. Hamsters were anesthetized with ether and bled by intracardiac puncture. The blood was allowed to clot, and serum was separated by centrifugation at 500 × *g*, pooled, divided into 1-ml aliquots, dispensed into 1.5-ml screw-cap tubes (Sarstedt), and frozen at −20°C until used.

Borreliacidal assay. A previously described procedure (30) was modified and used to determine borreliacidal activity. Sera from vaccinated and nonvaccinated hamsters were heat inactivated at 56°C for 30 min, diluted 1:10 with fresh BSK, and filter sterilized through a 0.22-μm-pore-size filter apparatus (Acrodisc; Gelman Sciences, Ann Arbor, Mich.). Frozen suspensions of *B. burgdorferi* sensu stricto isolates C-1-11 and 297, *B. garinii* isolate LV4, *B. afzelii* isolate BV1, and *B. hermsii* in BSK were thawed, inoculated into fresh BSK, and incubated at 32°C for 72 h. Spirochetes were enumerated by dark-field microscopy and with a Petroff-Hausser counting chamber, and the suspensions were adjusted to contain 10^6 spirochetes per ml with BSK. One-hundred-microliter samples of the spirochetal suspensions were added to round-bottomed wells of a 96-well microtiter plate (GIBCO Laboratories, Grand Island, N.Y.). Subsequently, 100 μl of sera or twofold dilutions of sera from vaccinated and nonvaccinated hamsters and 20 μl of sterile guinea pig complement (hemolytic titer, 200 CH50 units per ml; Sigma) were added to each well of the microtiter plate. The plate was shaken gently and incubated at 32°C for 16 h. All assays were performed in duplicate.

Flow cytometry data acquisition and analysis. After incubation of assay samples, 100 μl was removed and diluted 1:5 with PBS (pH 7.4), and 50 μl of acridine orange (5.4 nM; Sigma) was added (27). Controls included samples containing normal serum with viable or heat-killed (56°C for 30 min) spirochetes in BSK and complement. The samples were then analyzed with a FACScan flow cytometer (Becton Dickinson Immunocytometry Systems, Mountain View, Calif.) with FACScan LYSIS II software for data acquisition. Initially, viable and heat-killed spirochetes were detected and differentiated from BSK, serum, and complement particles by using forward scatter, side scatter, and acridine orange fluorescence. Live gating was performed only on profiles of spirochetes during data acquisition to exclude all BSK, serum, and complement particles. Data were acquired for 1 min. Assay samples were then analyzed by histogram profiles of acridine orange fluorescence with FACScan LYSIS II software. Gates were established for viable and heat-killed spirochetes based on their incorporation of acridine orange. Three parameters were evaluated: events per minute (number of labeled spirochetes), percent shift in fluorescence (number of dead spirochetes), and mean channel fluorescence (intensity of fluorescently labeled spirochetes). Borreliacidal activity was determined by a decrease in events per minute and increases in percent shift in fluorescence and mean channel fluorescence compared with values obtained with normal serum. Spirochetes were sorted based on the flow cytometric parameters. Sorted spirochetes were incubated in fresh BSK medium and monitored for growth for 5 weeks. No growth of spirochetes was detected (27). The borreliacidal titer was the highest dilution of immune serum that killed spirochetes compared with normal serum.

Infection of hamsters. Vaccinated hamsters were mildly anesthetized with ether and challenged subcutaneously in each hind paw with 0.2 ml of BSK containing 10^6 viable organisms of *B. burgdorferi* sensu stricto isolate C-1-11 or 297, *B. garinii* isolate LV4, *B. afzelii* isolate BV1, or *B. hermsii*. Controls included nonvaccinated hamsters and hamsters vaccinated with various adjuvants alone or Formalin-inactivated spirochetes in PBS and challenged with 10^6 viable spirochetes or BSK. We have infected hamsters with 10^2 to 10^8 *B. burgdorferi* organisms and recovered spirochetes from bladders, spleens, kidneys, and hearts cultured in BSK medium. Recovery rates varied (≤66%) for challenge inocula of between 10^3 and 10^4 spirochetes. When hamsters are challenged with 10^6 spirochetes, the measurement of arthritis by plethysmograph is less variable and spirochetes are recovered from all tissues.

Assessment of arthritis. Swelling of the hind paws of hamsters challenged with isolates of *B. burgdorferi* sensu lato and *B. hermsii* was used to evaluate the inflammatory response. The hind paws were measured every other day for 21 days with a plethysmograph (Buxco Electronics, Sharon, Conn.). Measurements were obtained by mildly anesthetizing hamsters with ether, carefully dipping a hind paw into a column of mercury up to the ankle, and measuring the amount of mercury displaced (in milliliters). The mean plethysmograph value for three hamsters (six hind paws) per group was used as an index of severity of swelling from arthritis. Mercury displacement was standardized with a volume calibrator.

Recovery of spirochetes from tissues. Twenty-one days after challenge, hamsters were killed by CO_2 inhalation. The urinary bladder, spleen, left kidney, and heart were removed aseptically, homogenized through a 5-ml syringe, and inoculated into 5 ml of BSK supplemented with rifampin (100 μg/ml; Sigma) and phosphomycin (100 μg/ml; Sigma). Cultures were incubated at 32°C and examined weekly for 2 weeks by dark-field microscopy for motile spirochetes. If spirochetes were not detected, 0.5 ml of the culture was inoculated into 4 ml of BSK supplemented with antibiotics and examined weekly for another 3 weeks. If no spirochetes were detected, the culture was

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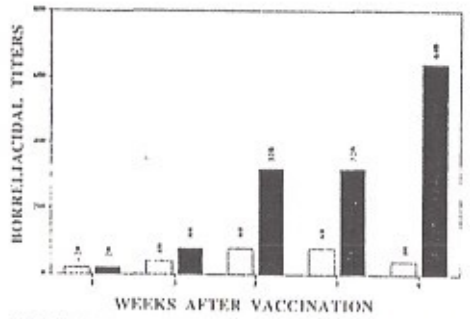

FIG. 1. Development of borreliacidal antibody titers in hamsters after vaccination with Formalin-inactivated *B. burgdorferi* sensu stricto isolate C-1-11 in PBS (stippled bars) or aluminum hydroxide gel (solid bars).

considered negative. For recovery of *B. hermsii*, blood was inoculated into 5 ml of BSK supplemented with antibiotics.

Preparation of tissues for histology. The hind legs of all vaccinated and nonvaccinated hamsters were amputated 21 days after challenge at the midfemur, fixed in 10% neutral buffered Formalin, placed in decalcifying solution (Lerner Laboratories, Pittsburgh, Pa.) for 18 h, and stored in 10% Formalin prior to processing. The knees and hind paws were bisected longitudinally, embedded in paraffin, cut into 6-μm sections, placed on glass slides, and stained with hematoxylin and eosin. Hind legs were randomly selected from each group of hamsters for histopathological examination.

Statistics. The plethysmograph values obtained from hamster hind paw measurements were tested by analysis of variance. The Fisher least-significant-difference test (45) was used to examine pairs of means when a significant F ratio indicated reliable mean differences. The alpha level was set at 0.05 before the experiments were started.

RESULTS

Development of borreliacidal antibody in vaccinated hamsters. Two groups of 15 hamsters each were vaccinated with a single dose of a whole-cell preparation of Formalin-inactivated *B. burgdorferi* sensu stricto isolate C-1-11 in PBS or an aluminum hydroxide gel. Borreliacidal antibody titers were determined at 1, 3, 5, 7, and 9 weeks after vaccination by flow cytometry. One week after vaccination, low levels of borreliacidal antibody were detected in pooled sera from three hamsters vaccinated with the preparations of Formalin-inactivated spirochetes (Fig. 1). However, hamsters vaccinated with Formalin-inactivated spirochetes in adjuvant had a 2- to 16-fold increase in borreliacidal antibody titer 3 weeks or later after vaccination.

Development of severe destructive arthritis in vaccinated hamsters. Five groups of three hamsters each were vaccinated with Formalin-inactivated *B. burgdorferi* sensu stricto isolate C-1-11 in aluminum hydroxide gel. At 1, 3, 5, 7, and 9 weeks after vaccination, hamsters were challenged subcutaneously in the hind paws with 10^5 viable organisms of *B. burgdorferi* sensu stricto isolate C-1-11. Hamsters challenged 5 weeks or less after vaccination developed severe swelling of the hind paws (Fig. 2 and 3C). Swelling was detected 5 days after challenge and increased rapidly, with peak swelling occurring on days 14 to 16 after challenge before gradually decreasing. Spirochetes were also recovered from the urinary bladder, spleen, kidney, and heart of these hamsters after cultivation in BSK for 10 days

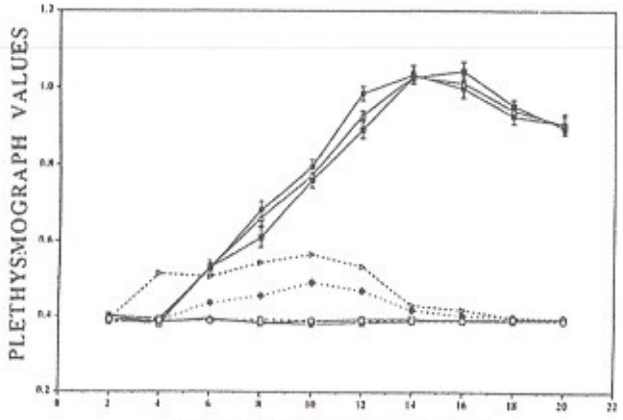

FIG. 2. Development of hind paw swelling in hamsters challenged with *B. burgdorferi* sensu stricto isolate C-1-11 and vaccinated with Formalin-inactivated *B. burgdorferi* sensu stricto isolate C-1-11 in aluminum hydroxide gel for 1 (-■-), 3 (-△-), 5 (-●-), 7 (-□-), and 9 (-▲-) weeks. Controls included nonvaccinated hamsters challenged with BSK (- - ○ - -) or *B. burgdorferi* sensu stricto isolate C-1-11 (- - ◆ - -) and hamsters vaccinated with aluminum hydroxide gel and challenged with *B. burgdorferi* sensu stricto isolate C-1-11 (- - ▷ - -). Nonchallenged vaccinated hamsters showed no adverse clinical manifestations.

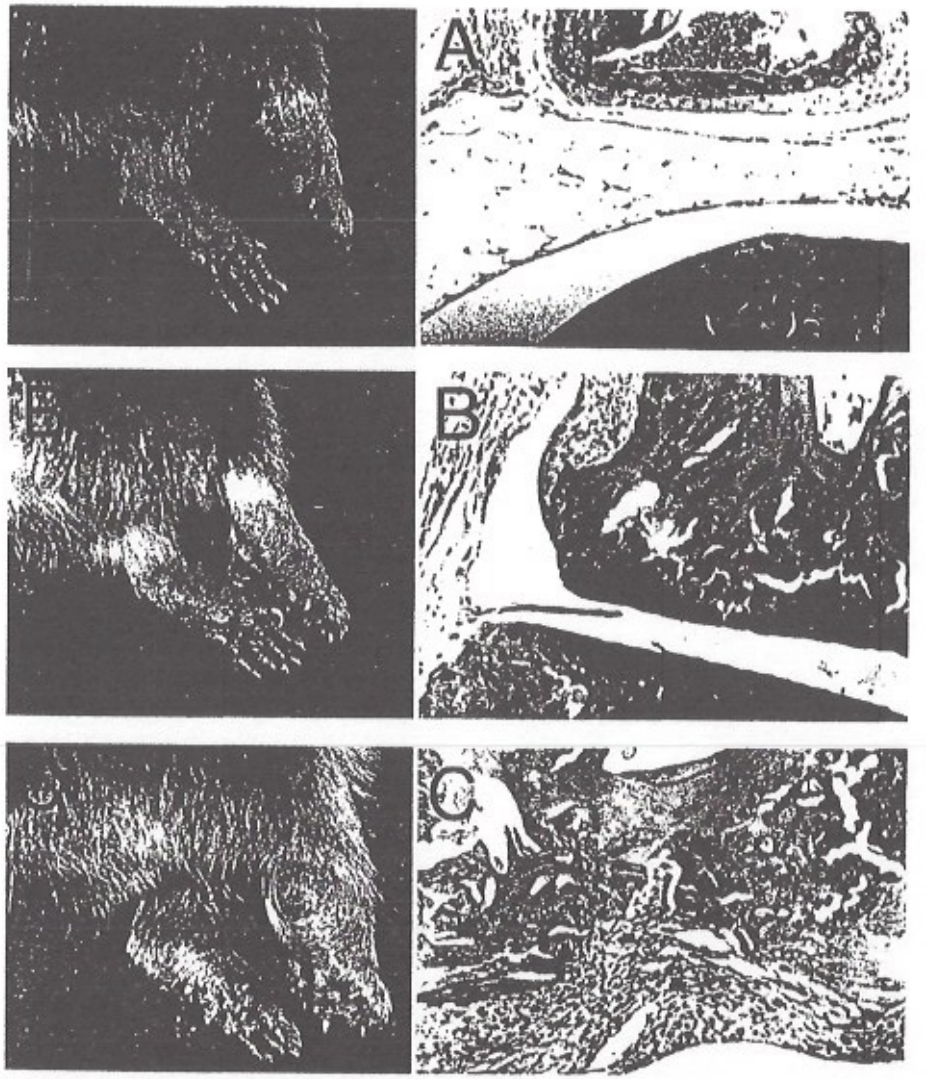

FIG. 3. Appearance (left) and histopathology (right) of hind paws. (A) Nonvaccinated hamster challenged with BSK. (B) Nonvaccinated hamster challenged with *B. burgdorferi* sensu stricto isolate C-1-11. (C) Hamster vaccinated with Formalin-inactivated *B. burgdorferi* sensu stricto isolate C-1-11 in aluminum hydroxide gel and challenged with the same isolate. Results were determined 21 days after challenge. Arrows point to areas of infiltration of inflammatory cells into the synovium. Destructive arthritis was also observed in hamsters vaccinated with *B. burgdorferi* sensu stricto isolate 297 in adjuvant and challenged with *B. burgdorferi* sensu stricto isolate 297. In addition, destructive arthritis was evoked when hamsters vaccinated with *B. burgdorferi* sensu stricto isolate C-1-11 or 297 in adjuvant were challenged with *B. garinii* isolate LV4 and *B. afzelii* isolate BV1. The presence of high-titer isolate-specific borreliacidal antibody prevented the induction of arthritis when vaccinated hamsters were challenged with the homologous isolate. However, arthritis still developed in vaccinated hamsters challenged with nonhomologous isolates of *B. burgdorferi* sensu lato.

TABLE 1. Recovery of *B. burgdorferi* from tissues[a]

Group and time of challenge (wk postvaccination)	No. of animals positive for spirochetes in:			
	Urinary bladder	Spleen	Kidney	Heart
Vaccinates				
1	3	2	3	3
3	3	2	2	2
5	2	3	2	3
7	0	0	0	0
9	0	0	0	0
Controls				
Nonvaccinated	3	3	3	3
Adjuvant alone	3	2	3	2

[a] Hamsters were vaccinated with Formalin-inactivated *B. burgdorferi* isolate C-1-11 in aluminum hydroxide gel and challenged 1, 3, 5, 7, and 9 weeks after vaccination with *B. burgdorferi* isolate C-1-11. Controls included nonvaccinated hamsters and hamsters inoculated with adjuvant alone. There were three hamsters in each group.

(Table 1). By contrast, hamsters challenged 7 and 9 weeks after vaccination failed to develop swelling of the hind paws (Fig. 2), and their tissues did not grow spirochetes when cultured in BSK (Table 1). Slight swelling of the hind paws was detected when nonvaccinated hamsters and hamsters inoculated with adjuvant alone were challenged with *B. burgdorferi* sensu stricto isolate C-1-11 (Fig. 2 and 3B). The swelling, however, was significantly less ($P < 0.01$) and of shorter duration than the swelling in hamsters challenged 1, 3, and 5 weeks after vaccination with Formalin-inactivated spirochetes in adjuvant. The tissues of these hamsters also grew spirochetes when cultured in BSK for 10 days (Table 1). In addition, nonvaccinated hamsters and hamsters vaccinated with BSK failed to develop any swelling of the hind paws (Fig. 2 and 3A). Vaccinated hamsters that were not infected showed no adverse clinical manifestations. Likewise, vaccinated and nonvaccinated hamsters challenged with nonviable spirochetes failed to develop arthritis. In other experiments, arthritis was evoked in vaccinated hamsters challenged intradermally and intraperitoneally. The onset of arthritis was delayed by approximately 20 days and the swelling of the hind paws was less severe after challenge by these routes.

Changes in movement and behavior of hamsters. Hamsters that had been vaccinated 5 weeks or less previously with Formalin-inactivated *B. burgdorferi* sensu stricto isolate C-1-11 in aluminum hydroxide gel were challenged subcutaneously in the hind paws with 10^6 viable organisms of *B. burgdorferi* sensu stricto isolate C-1-11. These hamsters displayed unusual behavior patterns and had difficulty moving about their cages. They became unusually vicious about 10 days after challenge and refused to eat and drink despite placement of food and water directly on the floors of the cages. Simply touching the cages caused the hamsters to squeal. Hamster movement was greatly restricted because they could not move their legs. Hamsters maneuvered about the cages extremely slowly on their abdomens, dragging their hind legs. These responses gradually waned 20 days after challenge and correlated with the onset and decrease in the swelling of the hind paws. These clinical findings may be the result of severe pain rather than inflammation of the central nervous system. No unusual changes were observed in nonvaccinated hamsters or hamsters inoculated with adjuvant alone and then challenged with *B. burgdorferi* sensu stricto isolate C-1-11 or with BSK.

Histopathology of hind paws. Twenty-one days after challenge with *B. burgdorferi* sensu stricto isolate C-1-11, an erosive and destructive arthritis was detected in the hind paws of hamsters vaccinated 5 weeks earlier with Formalin-inactivated *B. burgdorferi* sensu stricto isolate C-1-11 in adjuvant (Fig. 3C). The synovia of the tibiotarsal and intertarsal joints displayed chronic hypertrophy and hyperplasia characterized by bridging of villi mixed with fibrin (pannuslike), erosion of the articular cartilage, and focal destruction of underlying bone (Fig. 3C). A

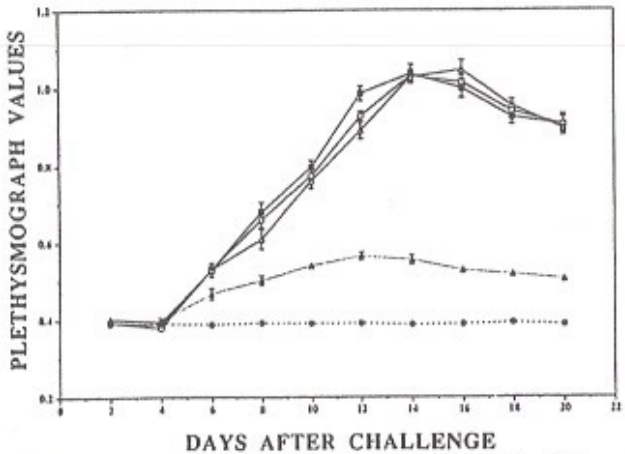

FIG. 4. Development of hind paw swelling in hamsters vaccinated with Formalin-inactivated *B. burgdorferi* sensu stricto isolate C-1-11 in aluminum hydroxide gel (-■-), aluminum hydroxide (-○-), and Freund's incomplete adjuvant (-△-) after challenge with *B. burgdorferi* sensu stricto isolate C-1-11. Controls included nonvaccinated hamsters challenged with *B. burgdorferi* sensu stricto isolate C-1-11 (- - ▲ - -) or BSK (- - ● - -).

TABLE 2. Recovery of *B. burgdorferi* from tissues of hamsters vaccinated with different preparations[a]

Group and prepn	No. of animals positive for spirochetes in:			
	Urinary bladder	Spleen	Kidney	Heart
Nonvaccinated	3	2	3	3
Vaccinated				
Aluminum hydroxide gel	3	3	3	3
Aluminum hydroxide	2	2	3	3
Freund's incomplete adjuvant	2	3	2	3

[a] Hamsters were vaccinated with the indicated preparation of Formalin-inactivated *B. burgdorferi* sensu stricto isolate C-1-11 and then challenged 5 weeks later with the same isolate. Nonvaccinated hamsters were used as controls and were also challenged. There were three hamsters in each group.

cellular infiltrate of neutrophils, macrophages, mast cells, lymphocytes, and plasma cells was also present in the subsynovial and periarticular tissues. Chronic arthritis was pronounced and characterized by fibromyxoid changes with residual granulation tissue and occasional cartilaginous metaplasia. In contrast, the hind paws of nonvaccinated hamsters challenged with *B. burgdorferi* sensu stricto isolate C-1-11 displayed an acute synovitis (Fig. 3B). A cellular inflammatory infiltrate was present, but the joint spaces were free of pannus tissue formation and the development of erosive and destructive arthritis. (Fig. 3B). Similar histopathological findings were also seen in the hind paws of hamsters infected with *B. burgdorferi* sensu stricto isolate C-1-11 after vaccination with Formalin-inactivated *B. burgdorferi* sensu stricto isolate C-1-11 in PBS or with adjuvant alone (data not shown). Nonvaccinated hamsters inoculated with BSK also failed to develop any significant histological changes (Fig. 3A).

Effects of various adjuvants on development of severe destructive arthritis. Three groups of three hamsters each were challenged subcutaneously in the hind paws with 10^6 viable organisms of *B. burgdorferi* sensu stricto isolate C-1-11 5 weeks after vaccination with Formalin-inactivated *B. burgdorferi* sensu stricto isolate C-1-11 in aluminum hydroxide gel, aluminum hydroxide (Imject alum), or Freund's incomplete adjuvant. All vaccinated hamsters developed severe swelling of the hind paws 5 days after infection (Fig. 4). The swelling peaked on days 14 to 16 after challenge and gradually decreased. Spirochetes were also recovered from their tissues after cultivation in BSK for 10 days (Table 2). Although nonvaccinated hamsters challenged with *B. burgdorferi* sensu stricto isolate C-1-11 developed slight swelling, the severity of swelling was significantly less ($P < 0.01$) than that measured in vaccinated hamsters and was of shorter duration (Fig. 4). Nonvaccinated hamsters inoculated with BSK failed to develop any swelling of the hind paws. When these experiments were repeated with Formalin-inactivated or other preparations of whole *B. burgdorferi* sensu lato isolates in various adjuvants, similar results were obtained.

Induction of severe destructive arthritis in vaccinated hamsters challenged with other isolates of the three genomic groups. Five groups of three hamsters each were vaccinated with formalin-inactivated *B. burgdorferi* sensu stricto isolate C-1-11 or 297 in aluminum hydroxide gel and challenged 7 and 3 weeks later, respectively, with 10^6 viable organisms of *B. burgdorferi* sensu stricto isolate C-1-11 or 297, *B. garinii* isolate LV4, *B. afzelii* isolate BV1, or *B. hermsii*. Hamsters vaccinated with *B. burgdorferi* sensu stricto isolate C-1-11 or 297 in adjuvant had borreliacidal antibody titers of 1:320 and 1:1,280, respectively, at the time of challenge (Table 3). No swelling of the hind paws was detected in vaccinated hamsters challenged with the homologous isolate, nor were spirochetes isolated from the tissues of these animals. When vaccinated hamsters were challenged with the nonhomologous isolate of *B. burgdorferi* sensu stricto, *B. garinii*, or *B. afzelii*, severe swelling of the hind paws developed and spirochetes were isolated from the tissues of these hamsters even though high levels of isolate-specific borreliacidal antibody were present (Table 3). Hamsters vaccinated with Formalin-inactivated *B. burgdorferi* sensu stricto isolate C-1-11 or 297 in adjuvant failed to develop any swelling of the hind paws when challenged with *B. hermsii*, although spirochetes were isolated from the blood.

DISCUSSION

This is the first documentation that severe destructive arthritis can develop after vaccination against infection with *B. burgdorferi* sensu lato. Arthritis was readily evoked in vaccinated hamsters challenged with isolates of *B. burgdorferi* sensu lato before high levels of protective borreliacidal antibody had developed. Once high levels of isolate (vaccine)-specific borreliacidal antibody developed, hamsters were protected from homologous challenge and development of arthritis. However, vaccinated hamsters still developed severe destructive arthritis when challenged with other isolates of the three genomic groups of *B. burgdorferi* sensu lato. Our results demonstrate

TABLE 3. Induction of severe destructive arthritis in vaccinated hamsters[a]

Challenge isolate	No vaccine			Isolate C-1-11 vaccine			Isolate 297 vaccine		
	Borreliacidal antibody titer	No. with arthritis	No. positive by culture	Borreliacidal antibody titer	No. with arthritis	No. positive by culture	Borreliacidal antibody titer	No. with arthritis	No. positive by culture
B. burgdorferi sensu stricto isolates									
C-1-11	<20	0	3	320	0	0	<20	3	3
297	<20	0	3	20	3	3	1,280	0	0
B. garinii LV4	<20	0	3	<20	3	3	<20	3	3
B. afzelii BV1	<20	0	3	20	3	3	<20	3	3
B. hermsii	<20	0	3	<20	0	3	<20	0	3

[a] Groups of three hamsters were vaccinated with whole-cell preparations of Formalin-inactivated *B. burgdorferi* sensu stricto isolate C-1-11 or 297 in aluminum hydroxide gel and then challenged with the same or a different isolate. Nonvaccinated hamsters were used as controls. The borreliacidal antibody titer of nonvaccinated and vaccinated hamsters was determined prior to challenge. The number of hamsters with severe destructive arthritis and the number of hamsters whose urinary bladder, spleen, kidney, or heart tissues were positive for growth of spirochetes in culture were also determined. Blood samples were cultured in BSK for recovery of *B. hermsii*.

that vaccination induces protection against homologous challenge, but vaccination also primes hamsters for development of arthritis. If vaccinated hamsters are challenged with isolates of *B. burgdorferi* sensu lato that are resistant to the vaccine-induced specific antibody, they will develop severe destructive arthritis. This is an important finding and suggests that vaccines must be composed of several isolates of *B. burgdorferi* sensu lato to induce a comprehensive borreliacidal antibody response to prevent development of arthritis.

Adjuvants are frequently used in vaccines to augment immune responses without major side effects (2). We showed that vaccination with Formalin-inactivated spirochetes in the absence of adjuvant induced only a weak borreliacidal antibody response. Borreliacidal antibody accurately reflects the level of protective antibody (40). When Formalin-inactivated spirochetes were incorporated in adjuvant, high levels of borreliacidal antibody developed 7 weeks or more after vaccination. Vaccinated hamsters were protected from homologous challenge. Surprisingly, hamsters vaccinated with spirochetes in adjuvant developed severe destructive arthritis when challenged with the homologous isolate before high levels of borreliacidal antibody were detected. Furthermore, vaccinated hamsters developed severe destructive arthritis when challenged with other isolates of the three genomic groups of *B. burgdorferi* sensu lato, even when high levels of isolate-specific borreliacidal antibody were present. Our results also showed that development of arthritis was caused by the spirochetes and not by the adjuvants. These findings suggest that vaccines prepared from whole spirochetes should be tested for their ability to induce arthritis or other clinical manifestations of Lyme borreliosis by challenging vaccinated animals with multiple isolates of *B. burgdorferi* sensu lato before and after the establishment of high levels of protective borreliacidal antibody.

Our results suggest that alternative approaches, besides whole spirochetes, need to be considered for the development of Lyme borreliosis vaccines. One approach would be to use selected proteins or other antigens of *B. burgdorferi* sensu lato that are known to induce protective borreliacidal antibody. Several *B. burgdorferi* sensu lato proteins, including OspA (12, 13, 43, 44, 54, 55), OspB (14, 34, 35, 55), OspC (33), and the 39-kDa protein (40) have been shown to induce killing antibody. Although OspA is currently the leading vaccine candidate, it has considerable immunologic (4, 15, 38) and molecular (24, 31, 59) heterogeneity. This may require several OspA proteins for development of a comprehensive vaccine. We have shown that isolates of *B. burgdorferi* sensu lato can be separated into at least five distinct seroprotective groups (28, 29). This suggests that combinations of *B. burgdorferi* sensu lato protective proteins may be required to provide a comprehensive vaccine for humans and other animals. The ability of these proteins alone or in combination to induce or elicit adverse clinical manifestations is unknown and must be evaluated before their inclusion in a vaccine. Likewise, the immunogenicity of these purified proteins must be determined. An adjuvant may be required for the induction of an adequate protective antibody response and may enhance the potential for adverse effects. Another approach for vaccination would be to use whole spirochetes and eliminate or remove the antigen(s) responsible for the induction of arthritis or other possible autoreactive pathologic responses (1, 16, 25, 42). Although this approach is not popular, it should be evaluated because of the immunogenic and molecular heterogeneity of vaccine protein candidates and the number of antigens that can induce borreliacidal activity.

Why has severe destructive arthritis not been documented in other vaccinated animals? Vaccination of dogs (9, 26) and hamsters (17, 18, 20) has been done previously with whole spirochetes in adjuvant. One explanation is that vaccinated animals are commonly challenged with the homologous infectious *B. burgdorferi* sensu lato isolate during periods when levels of borreliacidal antibody are high. Generally, several weeks elapse between vaccination and challenge, allowing sufficient time for development of specific borreliacidal antibody. In this study, the protective antibody response to vaccination with *B. burgdorferi* sensu stricto isolate C-1-11 developed slowly. It took 7 weeks for the development of sufficiently high levels of borreliacidal antibody to prevent infection and the development of arthritis. By contrast, hamsters vaccinated with *B. burgdorferi* sensu stricto isolate 297 developed high levels of borreliacidal antibody as early as 1 week after vaccination and were protected from homologous challenge and development of arthritis. Another explanation is that investigators failed to challenge vaccinated animals with isolates of *B. burgdorferi* sensu lato belonging to distinct seroprotective groups. Lovrich et al. (28, 29) identified five seroprotective groups among North American and European isolates of *B. burgdorferi* sensu stricto, *B. garinii*, and *B. afzelii* by using the borreliacidal assay. We showed that hamsters vaccinated with Formalin-inactivated *B. burgdorferi* sensu stricto isolates C-1-11 and 297 developed severe destructive arthritis when challenged with distinct seroprotective group isolates in the presence or absence of homologous borreliacidal antibody. A third explanation may be that the route of challenge influenced the development of arthritis. We have also evoked arthritis in vaccinated hamsters challenged intradermally and intraperitoneally. However, the onset of arthritis was delayed by approximately 20 days and the swelling of the hind paws was less severe in these animals. The late development of arthritis may not have been observed by other investigators. A fourth explanation is that the hamster is unique. Hamsters may process whole spirochetes in adjuvant differently than other animals. It is important to note that serious adverse reactions in dogs to a commercially available whole-cell vaccine containing an adjuvant have not been reported to date (9, 26). Likewise, other animals have not developed arthritis despite vaccination with whole spirochetes in adjuvant (17, 18, 20). Even if other animal models do not develop arthritis after vaccination, the hamster's propensity for development of arthritis may lead to a better understanding of the immune mechanisms responsible for the induction and development of arthritis.

Our results also suggest that humorally mediated responses are not responsible for the development of severe destructive arthritis in hamsters. Arthritis was evoked in the presence and absence of borreliacidal antibody and of antibody used for the serodiagnosis of Lyme disease. Passive transfer of serum from hamsters vaccinated with Formalin-inactivated *B. burgdorferi* sensu stricto isolate C-1-11 did not induce arthritis when naive syngenic recipient hamsters were challenged with the homologous isolate or other isolates of *B. burgdorferi* sensu lato, even after daily administration of serum for 7 days (data not shown). Likewise, Fikrig et al. (11) showed that active immunization with OspA did not enhance arthritis but hastened its resolution. These results and those obtained by histopathology suggest that cell-mediated responses are involved. Additional studies are needed to determine whether severe destructive arthritis can be passively transferred with cells or abrogated in vaccinated hamsters by treatment with specific T-lymphocyte reagents.

In summary, severe destructive arthritis developed in vaccinated hamsters. Although vaccinated hamsters failed to develop arthritis when challenged with the homologous isolate during periods when the levels of isolate-specific borreliacidal antibody were high, they developed severe destructive arthritis when challenged before borreliacidal antibody developed or when challenged with different isolates of *B. burgdorferi* sensu lato. The induction and development of arthritis was not dependent on the isolate of *B. burgdorferi* sensu lato or the type of adjuvant used. Additional studies are needed to define the antigen(s) responsible for the induction of arthritis and the mechanism(s) involved. These investigations are needed to understand the immune mechanisms responsible for arthritis and for the development of a safe vaccine for prevention of Lyme borreliosis.

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VIII ANNUAL SCIENTIFIC CONFERENCE ON LYME BORRELIOSIS

Call For Abstracts

you would like to present data, in poster form, please send your abstract/s to the LDF by November 30, 1994. Selected abstracts will published in the Compendium. Abstracts should be typed within the abstract box outline. No additional pages are allowed. Please : capitol letters for the title, underline main author and include the address where research was done & the timeframe. A conference mmittee member will contact you regarding more information, as needed. Selections will be made by the end of February, 1995.

ategory (check one):

- Microbiology
- Pathogenesis
- Diagnosis
- Clinical manifest.
- Treatment
- Veterinary Issues
- Epidemiology
- Other spirochetal, ck-borne diseases
- Other (list)

LYME DISEASE SURVEILLANCE IN ATLANTIC CANADA. H.Artsob, M. Garvie, D. Dick, B. Horney, R. Maloney and H. Whitney. Laboratory Centre for Disease Control, Health Canada, Ottawa; Atlantic Veterinary College, Charlottetown; Department of Forestry and Agriculture, St. John's. Timeframe: 1989-1994.

A study was undertaken to monitor for the possible presence of Borrelia burgdorferi in Atlantic Canada. Isolation attempts were undertaken on Ixodes scapularis and I. uriae ticks, a serosurvey initiated on dogs and deer, and diagnostic serology performed on humans and dogs that exhibited symptoms possibly consistent with Lyme disease. Three isolates of B. burgdorferi were obtained - one from an adult Ixodes scapularis taken off a cat in Charlottetown, Prince Edward Island in 1991 and two from adult I. scapularis taken off dogs in Summerside and Brudenell, Prince Edward Island in 1994. Serological studies indicated infection with B. burgdorferi in one dog that had recently travelled outside of Atlantic Canada and three deer from Anticosti Island, Quebec which is situated in the Gulf of St. Lawrence.

Several human Lyme disease infections were diagnosed. The majority of these infections were recognized in individuals who had histories of travel to known Lyme endemic areas. One infection was encountered in a taxidermist in New Brunswick who had mounted the head of a deer shot in a known Lyme endemic area in New York state.

Serological studies do not indicate that B. burgdorferi has a widespread distribution in Atlantic Canada at present. A possible role of birds in the distribution of I. scapularis in this region will be presented.

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ABSTRACTS OF ORAL PRESENTATIONS

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UPDATE ON THE ENZOOTIOLOGY OF B. BURGDORFERI IN THE SOUTHERN U.S.

Dr. James H. Oliver, Jr., Institute of Arthropodology & Parasitology
Georgia Southern University, Statesboro, GA 30460-8056

We have isolated B. burgdorferi from cotton mice (Peromyscus gossypinus), cotton rats (Sigmodon hispidus), woodrats (Neotoma floridana) and the ticks Ixodes scapularis and Ixodes dentatus. Locations of isolates extend along the coast from Cape Canaveral , FL northward to the northern coasts of South Carolina and inland in central Georgia and southeast Missouri. Prevalence of B. burgdorferi in rodents may be quite high in some foci (75%). Currently it is unclear whether there are several separate parallel enzootic cycles operating or if there are weblike overlap among them. B. burgdorferi strains are more genetically heterogenous in the southern U.S. than those reported from the northern states. Infectivity varies among the isolates and one wonders if there are resulting differences in clinical symptoms and pathology produced in humans.

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ECOLOGY OF LYME DISEASE IN NORTHEASTERN UNITED STATES

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Some of the highest incidence rates of Lyme disease (>200,000 cases/100,000 population/county) occur in Northeastern United States. The primary tick vector is *Ixodes scapularis* which is extremely abundant in many wooded and suburban areas where white-tailed deer are common. This tick has been recorded feeding on 120 different species of animals (birds, mammals, lizards). The causative-agent, *Borrelia burgdorferi sensu stricto*, has frequently been recovered from humans, *Ixodes scapularis*, and white-footed