Conf #12. Stamford, Ct

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

National Institutes of Health
Rocky Mountain Laboratories
Hamilton, Montana 59840
(406) 363-3211

April 25, 1994

Mr. and Mrs. Thomas Forschner
Lyme Disease Foundation, Inc.
1 Financial Plaza
Hartford, CT 06103

Dear Karen and Tom:

You are to be congratulated for THE BEST LYME CONFERENCE EVER!

Both the tenor and the content of the recent Stamford meeting was excellent. Since we all realize that a quality meeting does not take place in the absence of an extraordinary amount of work and coordination, we were all delighted to have been asked to participate in such a well thought out and executed conference.

Although, as I mentioned in our discussions, all biomedical research is currently being squeezed by personnel and budgetary cutbacks, we will do our very best for your patients, your contributors and the support group members we met at the conference.

Thank you, again, for an enlightening and well spent weekend.

Sincerely,

Claude

Claude F. Garon, Ph.D.
Chief
Laboratory of Vectors and Pathogens

CFG:bk

Conf #12 . Stamford, Ct

88 Harriman Road
Irvington-on-Hudson
New York 10533

April 24, 1994

Thomas and Karen Forschner
Lyme Disease Foundation
1 Financial Plaza
Hartford, Connecticut 06103

Dear Tom and Karen,

A resounding, enthusiastic thank you must be expressed to you both for the fine result you achieved with the conference you sponsored this weekend in Stamford.

The persistent feeling of excitement and the glow of pleasure that still infuse my mood are caused by knowing how wise I was to have attended! The quality -- and content -- of the presenters you chose to comprise this year's event simply reflect the increasing sensitivity you have developed to recognize the timeliness of various issues in Lyme that demand attention, and to then select the very best people to deal with those aspects. Please see this as a vigorous vote of approval for all elements of that gathering.

The newest participants in this conference were a welcome addition to the reliable, well-loved "regulars" in your ranks. Continuously expanding the disparate but not-often-visited corners of the multiple pieces that comprise the picture of Lyme in its entirety sparks the creativity and determination of your audience's members to go back to their own lives and reinvigorate the direction of their own investigations.

It boils down to: you keep inspiring your "following" to higher and higher levels of work toward the goal of solving the mystery of Lyme disease.

See you at your next excellent production!

Very warmest regards,

Betty Gross

#12 4/22-23/94

LYME DISEASE: STATE OF THE ART

WITH AND EMPHASIS ON

NEUROLOGIC MANIFESTATIONS

1994 Conference Compendium

Sheraton Hotel, Stamford, Connecticut
April 22 & 23, 1994

Chair:

Martina Ziska, MD, Lyme Disease Foundation

Co-Chairs:

Andrew D. McBride, MD, MPH	Stamford Director of Health
Julie Rawlings, MPH	Texas Department of Health
Steven Schutzer, MD	UMDNJ - Department of Medicine

Poster Session Chair:

James Katzel, MD	Ukiah Valley Medical Center

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Stamford Department of Health

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16 CME CREDITS

CONFERENCE AGENDA

Friday, April 22, 1994

AM

7:00-9:00 Registration, CME sign-in, & coffee

9:00 **WELCOME**
Martina Ziska, MD — Conference Chair
Andrew D. McBride, MD — Conference Host

9:10 **KEYNOTE**
Karen Vanderhoof- Forschner, MBA, BS

GENERAL SESSION Session chair - Martina Ziska, MD

9:25 **Lyme borreliosis: addressing the issues**
Martina Ziska, MD — Medical Director, Lyme Disease Foundation

9:45 **SCIENTIFIC KEYNOTE**
Historical perspective on Lyme neuroborreliosis
Rudolf Ackerman, MD — Cologne, Germany

10:10 **Lyme borreliosis in the south central United States**
Edwin Masters, MD — St. Francis Hospital

10:40 **Overview of Lyme borreliosis classical clinical manifestations**
Rudolf Scrimenti, MD — University of Wisconsin
Robert Lesser, MD — Yale University

11:20 **Questions**

11:30 **POSTER SESSION** Session chair: James Katzel, MD

Noon-1:00 Lunch

NEUROBORRELIOSIS SESSION Session chair: Patricia Coyle, MD

1:00 **Neuroborreliosis: Diagnosis and differential diagnosis**
Patricia Coyle, MD — SUNY Stony Brook

1:30 **Neurotropism of Borrelia species: the ghost in the closet**
Willy Burgdorfer, PhD, MD (hon) — National Institutes of Health, Rocky Mountain Laboratories

1:45 **Pathogenesis of neuroborreliosis**
Juan Garcia-Monco, MD — Galdakaoko Hospital, Spain

2:10 **Metabolism of the putative neurotoxin quinolinic acid in Lyme borreliosis**
Melvyn P. Heyes, PhD — National Institute of Mental Health

2:30 **Questions**

2:40 Break

NEUROPSYCHIATRY SESSION Session chair: Brian Fallon, MD

3:00 **Lyme borreliosis: Review of neuropsychiatric features**
Brian Fallon, MD — Columbia University

3:30 **Neuropsychiatric Lyme borreliosis and syphilis: is there a parallel?**
Martin M. Schinedling, PhD — St. Mary's Medical Center

CONFERENCE AGENDA

Friday, April 22, 1994

3:45 **Depression and psychopharmacology of Lyme borreliosis**
Francisco Fernandez, MD Bayor College of Medicine

4:10 **Characteristics of cognitive deficit in Lyme borreliosis**
Marian Rissenberg, PhD Neuropsychologist

4:25 **Cognitive/linquistic deficit in Lyme borreliosis**
Nancy Long, PhD Speech/language pathologist

4:40 **Questions**

5:00 **A. Mini-symposium on scientific writing** - For health care professionals only!

B. Community education - For all participants!

5:45 Conclusion of the day & CME sign-out

7:30-8:30 **RECEPTION** - for ticker holders only! Purchase your ticket today!

Saturday, April 23, 1994

AM

7:30-8:30 Registration, CME sign-in, &Coffee

TRACK A - EPIDEMIOLOGY, PUBLIC HEALTH ISSUES Session chair: Julie Rawlings, MPH

9:00 **Tick-borne diseases in North America**
Julie Rawlings, MPH Texas Department of Health

9:30 **Relapsing fever in Canadians visiting USA and India**
S. N. Banerjee, PhD University of British Columbia

9:45 **The epidemiology of Lyme disease in the Lyme Connecticut area**
Matthew L. Cartter, MD State Department of Public Health

10:05 **The epidemiology of Lyme disease in Delaware**
David E. Wolfe, MPH Delaware Devision of Public Health

10:20-10:30 **Questions**

TRACK B - MICROBIOLOGY & TREATMENT IMPLICATIONS Session chair: Claude F. Garon, PhD

9:00 **DNA replication in *Borrelia burgdorferi*: Precision control mechanisms - a potentially attractive target for new antimi- crobial agents**
Claude F. Garon, PhD National Institutes of Health, Rocky Mountain Laboratories

9:30 **Kinetics of killing by and frequency of resistance to an anti-microbial agent**
Scott Samuels, PhD National Institutes of Health, Rocky Mountain Laboratories

9:50 **Disruption of cell surface binding by human transferrin to *Borrelia burgdorferi* by anti bodies targeted at an iron stress-induced outer sheath protein**
David Dorward, PhD National Institutes of Health, Rocky Mountain Laboratories

10:10 **Questions**

TRACK C - DIAGNOSTIC IMAGING Session chair: Richard C. Tilton, PhD

10:30 **Diagnosis of Lyme borreliosis in dairy cattle**
Sandra Bushmich, DVM University of Connecticut

10:45 **Detection of *Borrelia burgdorferi* in mouse urine**
Louis Magnarelli, PhD CT Agricultural Experimental Station

CONFERENCE AGENDA

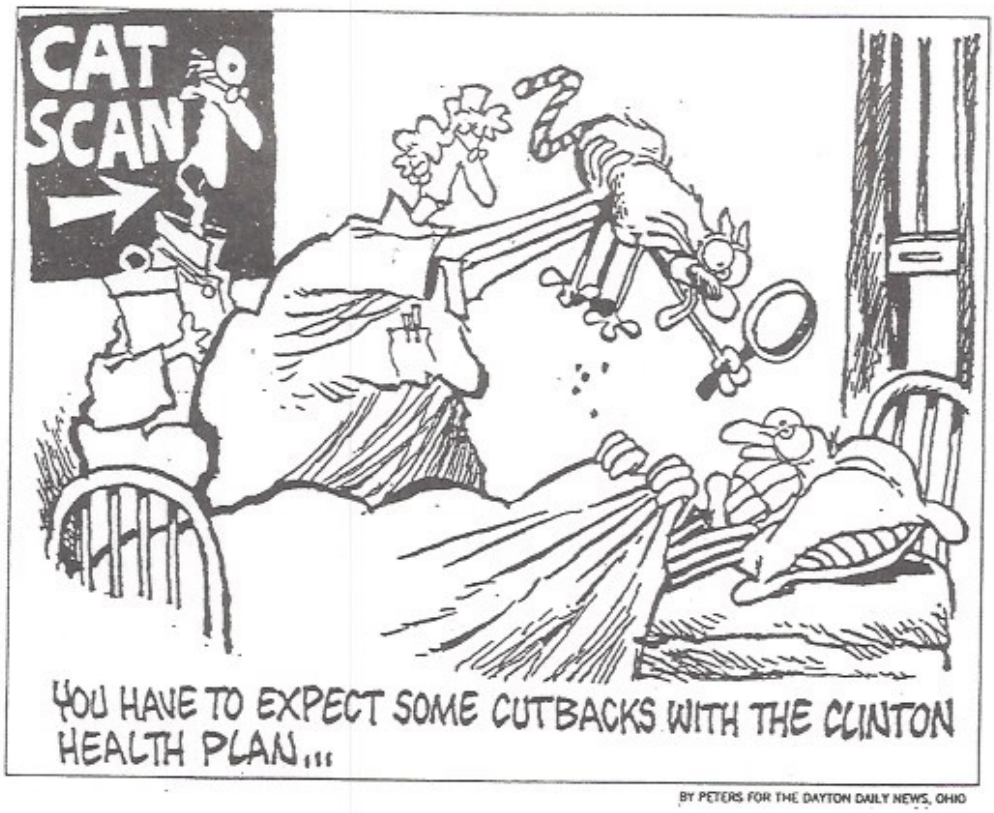
Saturday, April 23, 1994

11:00	**Urine PCR from patients with erythema migrans** Elisabeth Aberer, MD	University of Vienna, Austria
11:15	**Comparison of serological and molecular diagnostic tests for Lyme disease on a non-selected patient population** Richard C. Tilton, PhD	NALG
11:35	**Reference and research of Lyme borreliosis in the Czech republic** Dagmar Hulinska, PhD	WHO Reference Lab, Prague, Czech republic
11:50	**Questions**	
	TRACK D - PROTECTIVE IMMUNITY Session chair: Charles Pavia, PhD	
10:20	**A spectrum of protective humoral immune responses against *Borrelia burgdorferi*** Charles Pavia, PhD	New York Medical Centre
10:40	**Immune response of laboratory mice to *Borrelia burgdorferi* OspA and its relationship to protective immunity** Stephen Barthold, PhD	Yale University
10:55	**Protective immunization againts *Borrelia burgdorferi* in immunocompetent mice characterized by T cell response** Alan B. Frey, PhD	New York University
11:10	**Vaccination induced destructive Lyme arthritis in dog** Ron Shell, PhD	University of Wisconsin
11:25	**Human vaccine efficacy trials** Debra Adler-Klein, MD Michael F. Parry, MD	Infectious Disease Associates, Stamford, CT Infectious Disease Associates, Stamford, CT
11:50	**Questions**	
Noon -1:00	Lunch	
	IMMUNOPATHOGENESIS SESSION Session chair: Steven Schutzer, MD	
1:00	**A rabbit model for erythema migrans and early disseminationof *Borrelia burgdorferi*** James Miller, MD, PhD	University of California, Los Angeles
1:20	**Late infection in rhesus monkey model** Mario Philipp, PhD	Tulane Primate Research Institute
1:40	**Immune modulating activity mediated by *Borrelia burgdorferi*** J. W. Chiao, MD	New York Medical College
1:55	**The role of cerebrospinal fluid research tests in neuroborreliosis** Patricia Coyle, MD	SUNY Stony Brook
2:15	**Questions**	
2:25	Break	
	CLINICAL SESSION Session chair: Martina Ziska, MD	
2:45	**Clinical diagnostic crossroads: locating the bug** Kornelia Keszler, MD	Yale University

CONFERENCE AGENDA

Saturday, April 23, 1994

3:00	**Borrelial etiology of scleroderma**	
	Elisabeth Aberer, MD	University of Vienna, Austria
3:15	**Overview of gestational Lyme borreliosis**	
	Alan B. MacDonald, MD	St. Elizabeth's Hospital
3:30	**Lactation and Lyme borreliosis**	
	Derrick DeSilva, MD	Raritan Bay Medical Center
3:45	**Similarities of Lyme borreliosis in the elderly**	
	Daniel Cameron, MD	Nothern Weschester Hospital Centre
4:00	**Geriatric Lyme borreliosis: is there a difference?**	
	Philip Paparone, DO	Atlantic City Medical Center
4:15	**Overview of Lyme borreliosis treatment**	
	Sam Donta, MD	Boston Universtiy Hospital
4:45	**Questions**	
5:00	Conclusion of the day & CME sign-out	
5:15-7:30	**PUBLIC FORUM** Moderator: James Katzel, MD Ukiah Valley Medical Center	
	Lyme disease: What You Should Know. Questions & Answers - All presenters are expected to participate. All are welcome	



NeuroReport 4, 841–848 (1993)

Alzheimer's disease—a spirochetosis?

Judit Miklossy

University Institute of Pathology, Division of Neuropathology, University of Lausanne, Rue du Bugnon 27, 1005 Lausanne, Switzerland

THE aetiology of Alzheimer's disease (AD), which affects a large proportion of the aged population is unknown and the treatment unresolved. The role of beta amyloid protein (βA4), derived from a larger amyloid precursor protein (APP) in AD is the subject of intense research. Here I report observations that in 14 autopsy cases, with histopathologically confirmed AD, spirochetes were found in blood and cerebrospinal fluid and, moreover, could be isolated from brain tissue. Thirteen age-matched control cases were without spirochetes. Reference strains of spirochetes and those isolated from brains of AD patients, showed positive immunoreaction with monoclonal antibody against the β amyloid precursor protein. These observations suggest that spirochetes may be one of the causes of AD and that they may be the source of the β amyloid deposited in the AD brain.

Key words: Alzheimer's disease; Amyloid; Amyloid precursor protein; β amyloid protein; Spirochetal disease

Introduction

Alzheimer's disease (AD)[1] is characterized by a slow, progressive decline of cortical functions, particularly cognition and memory. The most consistent histological changes are localized in the cerebral cortex: atrophy due to neuronal loss, senile plaques (named also argyrophylic or neuritic plaques), neurofibrillary tangles and astrocytic and microglial proliferation. Fibrillar amyloid proteins are deposited in the leptomeningeal and cortical vessel walls and in senile plaques.[2,3]

The major protein subunit of the amyloid fibril is the beta amyloid protein (βA4), a small, self-aggregating polypeptide of about 4 kDa[2] which is derived from a larger, transmembrane amyloid precursor protein (APP).[4] Molecular and biochemical studies have shown that an excess of βA4 is the primary event in AD, but its source until now has not been established (for a review see reference 5).

Dementia associated with cortical atrophy and microgliosis has also been observed in the late stages of two spirochetal diseases: Lyme disease—a late stage of neuroborreliosis—caused by *Borrelia burgdorferi*[6,7] and general paresis—tertiary stage of neurosyphilis—caused by *Treponema pallidum*. Two cases of concurrent neocortical borreliosis and AD have been reported by MacDonald and Miranda.[8,9] The authors suggest a possible association between *Borrelia burgdorferi* and AD. A careful study, using several methodological approaches, of 18 AD cases failed to support an association between *Borrelia burgdorferi* and AD, but the authors did not rule out the possibility that another spirochete, not detectable by their methods, may be responsible for AD.[10]

Before the era of serological tests the examination of infected material by dark field microscopy was the laboratory procedure of choice for the diagnosis of spirochetal infection. Therefore an attempt to demonstrate spirochetes in AD using this method was the aim of this study. But if, indeed, spirochetes play a role in the pathogenesis of AD, how may this be brought in harmony with the excess of βA4 in the brain reported to cause the manifestations of AD? If spirochetes are found to be immunoreactive with antibodies against APP, these microorganisms may be the source of the βA4.

Materials and Methods

Twenty-seven randomly chosen autopsy cases with various clinical diagnoses were investigated. Autopsy cases with the clinical diagnosis of Alzheimer's dementia, were always taken in the series. The only criterion in selecting cases without Alzheimer's dementia, was that of the age of the patients in the aim to have age-matched control cases for the AD patients (see Table 1). Blood was taken by arterial or intracardiac puncture, and CSF by cisternal puncture. Both procedures were performed under sterile conditions. Blocks (ca 3 × 3 × 1 cm) from frontal, temporal and inferior parietal cortical regions were removed and immediately frozen at −80°C. The timespan between death and autopsy varied from 6 to 16 h. After removing these samples, the brains were fixed in 10% formalin for about one month and processed for routine neuropathological examination and for the systematic investigation of histological signs of AD. The neuropathological diagnosis of AD was based not only on observations made in sections that were silver-stained for AD,[11] but also on the immunohistochemical detection of βA4 using specific antibody (DAKO, M872). The immunohistochemical biotin-avidin peroxidase complex technique was used. A silver method designed for the detection of spirochetes[12] was applied to paraffin or to frozen sections.

Examination of the blood and CSF was carried out in all cases using dark field microscopy. 10 µl of blood diluted with 20 µl of sterile distilled water was put on a slide, coverslipped and examined by dark-field microscopy using a ×100 immersion objective. The same procedure was carried out with 30 µl (undiluted) CSF. In each case, several samples were carefully and repeatedly examined. For the isolation of the spirochetes[13] small fragments of the cerebral cortex, with a volume of about 1 cm³, were taken from sterile *post mortem* brain biopsy material. They were finely sliced with a sterile surgical blade and put into a 10 ml sterile tube to which 3 ml of sterile physiological NaCl or PBS was added. After continuous shaking for about 30 min 30 µl of the supernatant was put on a slide, coverslipped and examined in dark field illumination. The modified Noguchi medium[14] used for the cultivation of spirochetes consisted of a mixture of 6 ml of sterile PBS, 2 ml of sterile distilled water and 1 ml foetal calf serum. After inoculation of 1 ml blood taken at autopsy, it was covered with sterile paraffin oil and incubated at room temperature.

For ultrastructural analysis small fragments of cortical sections that were silver-stained for AD[11] as well as fragments of immunostained unfixed frozen sections using a monoclonal antibody against APP (Boehringer, 1285262, dil. 1:5) were embedded in epon. The ultrathin sections from the silver-stained sections which were not contrasted, and the immunostained sections contrasted with uranyl nitrate and lead citrate, were examined in a Philips CM-10 electron microscope.

To study whether spirochetes may be the source of the excess of βA4 deposited in the brain, smears of the Nichols strain of *Treponema pallidum*, the B31 strain of *Borrelia burgdorferi* and those isolated from the cortex of the AD cases were immunostained with an APP monoclonal antibody (Boehringer, 1285262) specific to the *N*-terminal region of all APP isoforms.[15] One may argue that the silver impregnation technique designed for the demonstration of spirochetes is not entirely specific. Therefore an additional case of concurrent AD and serologically confirmed Lyme disease was used to investigate immunohistochemically whether *Borrelia burgdorferi* was present in brain tissue. Here it was assumed that *Borrelia burgdorferi* may also cause AD. If true, then a specific antibody against *Borrelia burgdorferi* would stain the accumulation of spirochetes in the cortex. The presence of spirochetes would allow the comparison in the same brain of the localizations of spirochetes, of APP and of βA4. For the demonstration of the spirochetes the immunoreactions were carried out on acetone-fixed frozen sections using a monoclonal antibody against *Borrelia burgdorferi* (Biodesign, C63780M). The immunohistochemical reaction for APP (Boehringer, 1285262) was also carried out on acetone-fixed frozen sections. For the demonstration of β amyloid (DAKO, M872) formalin-fixed frozen or paraffin sections were also used. As a negative control all immunostaining procedures were carried out with the omission of the primary antibody. As a positive control for the immunohistochemical demonstration of spirochetes, smears of the reference strain of *Borrelia burgdorferi* were used.

Table 1. Demonstration of spirochetes by dark field microscopy in the blood, CSF and those isolated from the cortex and cultured from the blood in 27 autopsy cases, including 14 cases with Alzheimer's disease (AD)

Neuropathological diagnosis	Age	Blood	CSF	Spirochetes isolated from the cortex	Spirochetes cultured from blood
1. AD	74	+	+	+	+
2. AD	79	+	+	+	+
3. AD	86	+	+	+	+
4. AD	78	+	+	+	+
5. AD	76	+	+	+	−
6. AD	78	+	+	+	0
7. AD	89	+	+	+	0
8. AD	73	+	+	+	0
9. AD	64	+	+	+	0
10. AD	72	+	+	+	0
11. AD	97	+	+	+	0
12. AD	88	+	+	+	0
13. AD	81	+	+	+	0
14. AD	74	+	+	+	0
15. Glioblastoma	64	−	−	−	−
16. Subependymoma	78	−	−	−	−
17. Hypertensive encephalopathy	86	−	−	−	−
18. Hypertensive encephalopathy	83	−	−	−	−
19. Hypertensive encephalopathy	84	−	−	−	−
20. Wernicke's encephalopathy	72	−	−	−	0
21. Cerebral hypoxia	91	−	−	−	0
22. Cerebral contusion	72	−	−	−	0
23. Cerebral infarct	82	−	−	−	0
24. Cerebral infarcts	79	−	−	−	0
25. Atheromatosis	64	−	−	−	0
26. Atheromatosis	82	−	−	−	0
27. No cerebral lesion	62	−	−	−	0

+: presence of spirochetes; −: no spirochetes; 0: not investigated.

Results

In the blood and the CSF from 14 out of the 27 cases, motile, coiled spirochetes were observed (Table 1; Fig. 1A, B). Their diameter was approximately 0.2 to 0.3 µm and their length varied between 8 and 30 µm. A rough estimate of the number of spirochetes per cc of blood was 100 to 400; and per cc of CSF, 50 to 200. In the remaining 13 cases spirochetes were observed neither in the blood nor in the CSF (Table 1). Severe histological and immunohistochemical changes typical of AD were found in all 14 cases whose blood and CSF contained spirochetes (Fig. 3). An astrocytic and a microglial proliferation particularly in association with senile plaques were also observed (Fig. 3E, F).

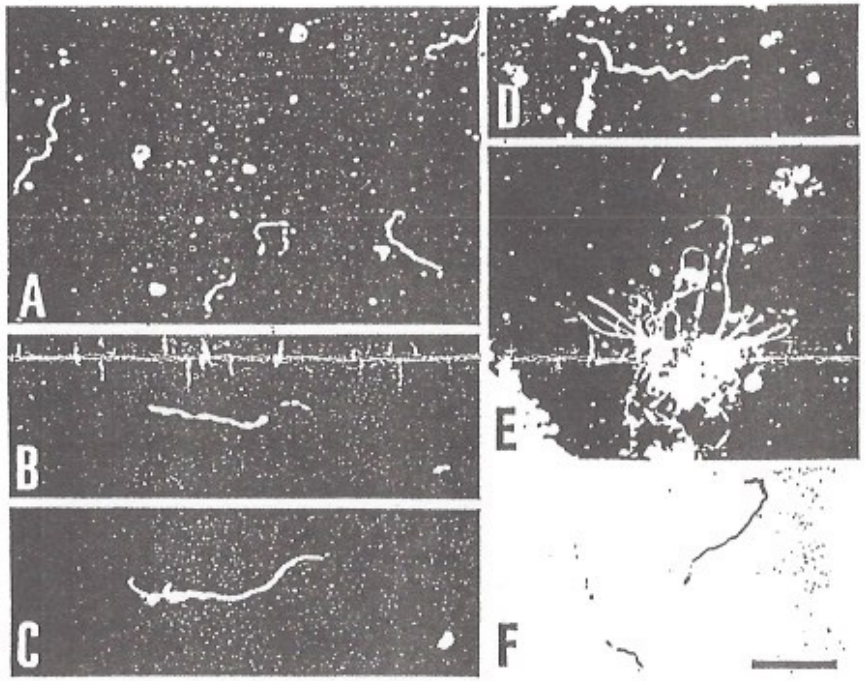


FIG. 1. A and B: Dark field photomicrographs showing spirochetes in the CSF in two autopsy cases of Alzheimer's disease (AD). C: Smear of *Borrelia burgdorferi* (B31 strain) immunostained with a fluorescein conjugated rabbit polyclonal antibody against this spirochete (Biodesign, B65303F, dil.1:30). D and E: Dark field photomicrographs showing the morphology of spirochetes isolated from the cortex of two AD cases. F: Spirochetes from the same strain as shown in C which were immunoreactive with an antibody against APP (Boehringer, 1285262 dil.1:5), biotin-avidin peroxidase complex technique. As a negative control the reaction was carried out with omission of the primary antibody (not shown). Bar for A–F is shown in F and corresponds to 10 μm.

When using a silver method for the demonstration of spirochetes,[11] the accumulations of the silver-stained spirochetes are, both in architecture and in pattern of distribution, the perfect resemblance of senile plaques (Fig. 3B). There were no perivascular lymphocytic cuffs, microglial nodules, or signs of meningitis in these 14 AD cases. Leptomeningeal fibrosis and granular ependymitis were often observed.

In the remaining 13 cases, those without spirochetes in the blood and CSF, the neuropathological examination revealed no signs of AD, but several other pathologies, including hypertensive, hypoxic or traumatic brain damage and Wernicke's encephalopathy due to chronic alcoholism. In two cases a brain tumour (subependymoma and glioblastoma) was found (see Table 1). In the silver- and immunostained sections for AD there were no senile plaques or neurofibrillary tangles in the cortex of these 13 control cases.

Spirochetes were isolated from the unfixed cortical tissue from all 14 AD cases (Table 1; Fig. 1D, E). The rough estimate of the number of spirochetes per cc of cortex was 1000 to 2000. The number of microorganisms in the cortex is probably underestimated because not all spirochetes were detached from the cortical fragments and therefore were not clearly visible in the isolation fluid. From the cortex of three out of four AD cases tested, spirochetes were cultured with success in a synthetic (BSK) medium. No spirochetes were found in the cortex from the 13 cases without AD (Table 1). In addition, spirochetes were cultured from the blood, in four out of five arbitrarily chosen AD cases, in a modified Noguchi medium (Table 1). The spirochetes not only survived but also proliferated markedly after 2 weeks of incubation. The rough estimate of the number of spirochetes per 1 cc of culture medium was between 2000 and 8000, i.e. an increase of about ×20 with respect to the initial samples taken from the blood. No spirochetes could be cultured from the blood in five cases tested without AD (Table 1).

The ultrastructural study of silver-stained and anti-APP-immunostained sections of the cerebral cortex in three AD cases tested also revealed coiled elements

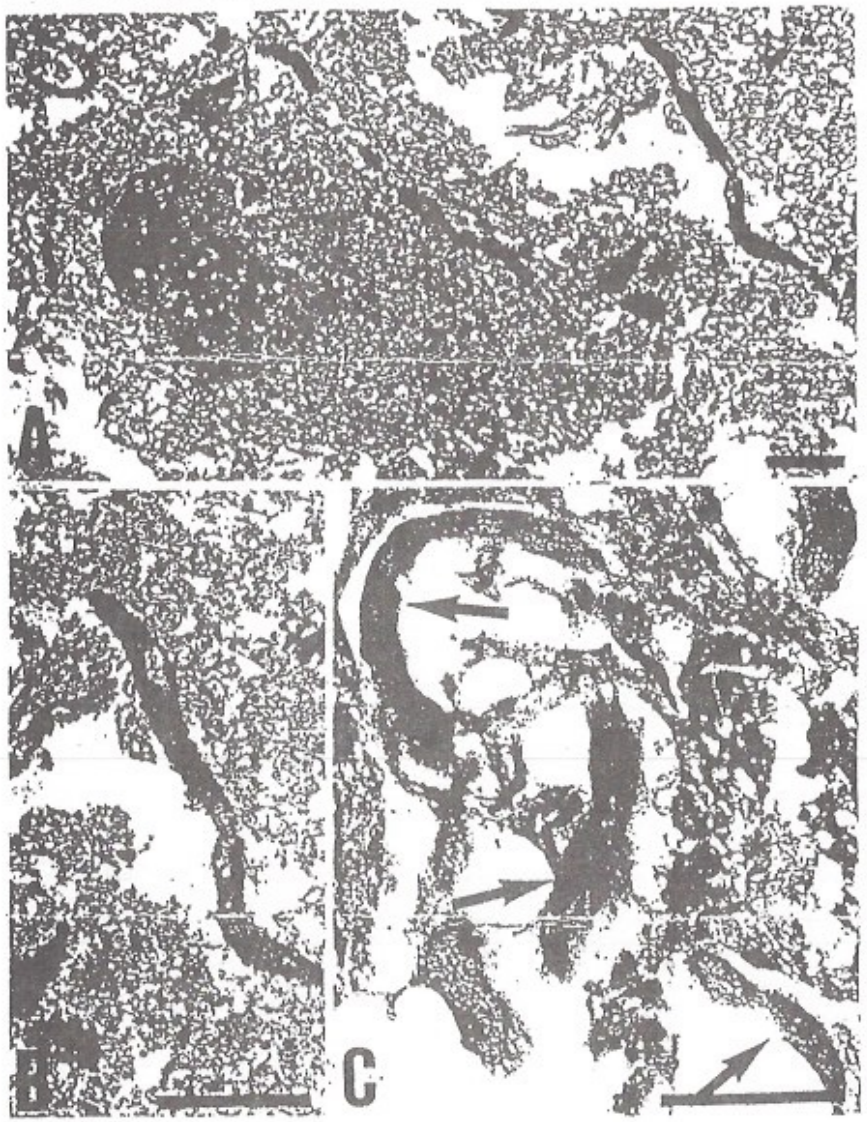


FIG. 2. A: Electron micrograph from an ultrathin section prepared from a frozen section, which had been silver-stained for senile plaques[11] in an AD case. Illustration shows silver deposits within elongated elements morphologically compatible with spirochetes. B: The silver-impregnated element lying at the right hand side of A shown at higher magnification. C. Electron micrograph from an immunostained frozen section in an AD case. Immunostaining was carried out using a monoclonal antibody against APP. Arrows point to elongated elements compatible with fragments of spirochetes localized in a senile plaque. The material shown in A–C derives from parietal cortex. Bars in A–C represent 1 μm.

whose morphology was compatible with that of spirochetes (Fig. 2A–C). Smears of reference strains of *Treponema pallidum* (Nichols strain), of *Borrelia burgdorferi* (B 31 strain) and smears of spirochetes isolated

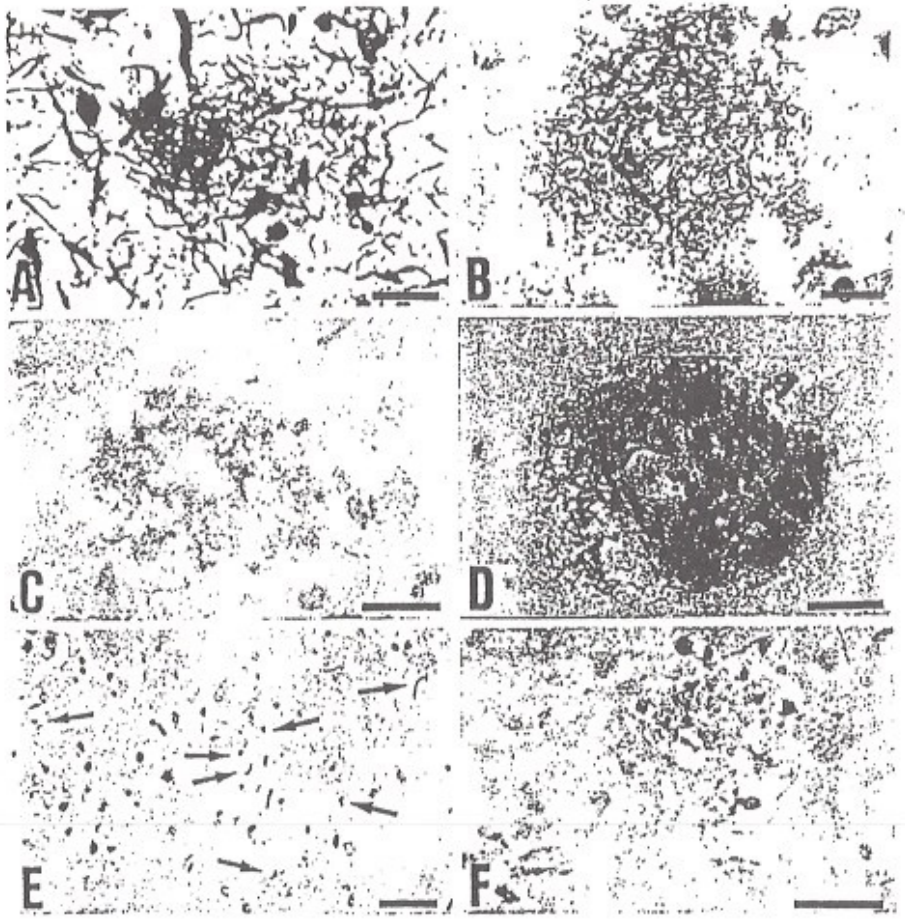


FIG. 3. Photomicrographs taken from the inferior parietal cortex of the brain of a patient from the 14 AD cases investigated in this study. A: Frozen section stained with a silver-technique for AD[11] showing senile plaque. For B a technique designed for the visualization of spirochetes[19] was used. The accumulation of silver-stained spirochetes follows the pattern of the senile plaque. C and D: Cryostat sections fixed in acetone and immunostained with a monoclonal antibody against APP (Boehringer, 1285262, dil.1:5) and β amyloid (DAKO, M872), respectively, showing senile plaques. E: Paraffin section stained with haematoxylin and eosin. Arrows point to putative reactive microglia. F: Cryostat section stained with a monoclonal antibody against human monocytes and macrophages (DAKO, CD68 PGM1: M718) showing microgliosis, particularly in association with senile plaques. Bars in A–D correspond to 20 μm, and in E and F to 50 μm.

from the AD cases, showed a positive immunoreaction, using a monoclonal antibody against APP (Fig. 1F). The negative controls which were carried out with the omission of the primary antibody were always negative.

In an additional case with concurrent AD and Lyme disease, using a specific antibody against *Borrelia burgdorferi*, spirochetes were found in senile plaques (Fig. 4B, D), in the leptomeningeal and cortical vessel walls, in neurones (Fig. 4E, F), and in microglial cells. They were also found as solitary elements in the neuropil (Fig. 4G). The location of spirochetes was similar to

that of the immunostained APP and βA4 accumulations (Fig. 4A–D), and follows the pattern of the senile plaques. The neurofibrillary tangles were also immunoreactive with the antiserum against *Borrelia burgdorferi*. Sections in which the immunoreaction was carried out with the omission of the primary antibody did not show spirochetes.

Discussion

There is evidence suggesting an infectious aetiology for AD. Cases of familial AD can be transmitted to the chimpanzee in the form of spongiform encephalopathy, resembling Creutzfeldt-Jakob disease (CJ).[18] In addition, the inoculation of experimental animals by

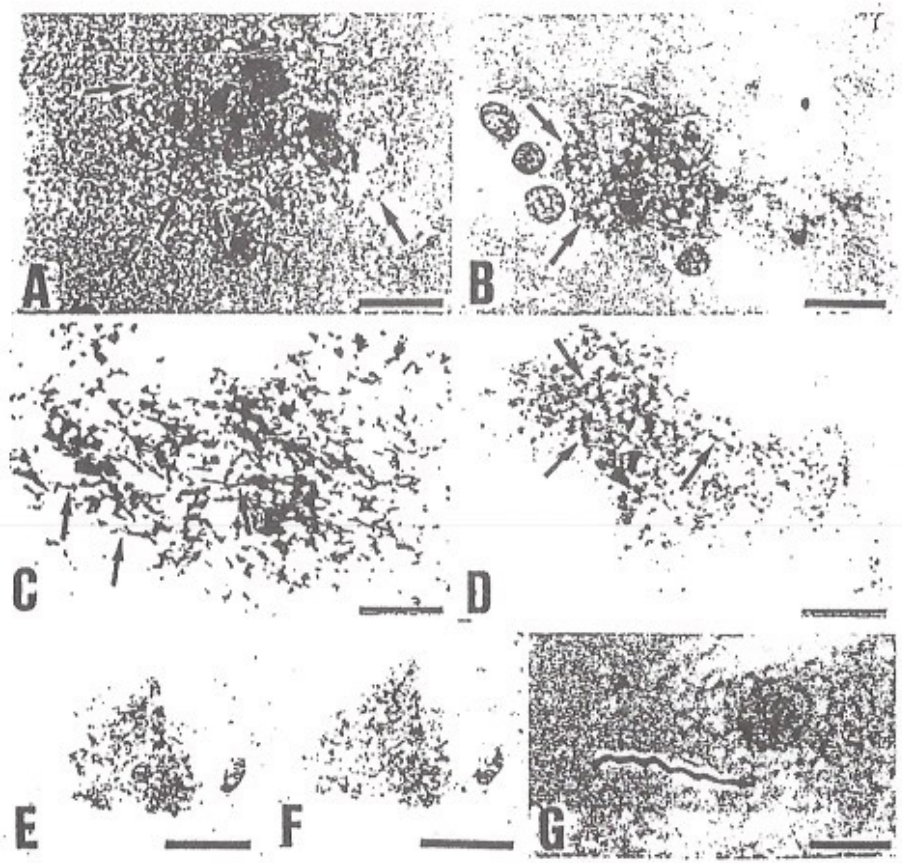


FIG. 4. Photomicrographs documenting the immunohistochemical findings of a case with concurrent AD and Lyme boirreliosis, showing similar accumulation of APP, βA4 and spirochetes in the cerebral cortex. The biotin-avidin peroxidase complex technique was used, preparations were counterstained with haematoxylin. A: Cryostat section fixed in acetone and immunostained with anti-APP monoclonal antibody (Boehringer, 1285262, dil.1:5) showing fine, coiled elements (arrows) within a senile plaque. B: Cryostat section fixed in acetone and immunostained using monoclonal antibody against *Borrelia burgdorferi* (Biodesign, B653078, dil.1:50). C and D show immunostained filaments (arrows) in subpial plaques when using monoclonal antibodies against βA4 (DAKO, M872) in a formalin-fixed paraffin section (C) and against *Borrelia burgdorferi* (D). E and F show the same neurone photographed at different levels. The nucleolus in E helps to identify the cell in question as a neurone, while in F filamentous elements immunoreactive with a monoclonal antibody against *Borrelia burgdorferi* (Biodesign, B653078, dil.1:50) are seen. G: shows a solitary coiled immunostained spirochete in the neuropil. Bars in A–F correspond to 20 μm, and in G to 10 μm.

the scrapie agent may induce the formation of senile plaques, morphologically similar to, but biochemically distinct from, AD plaques.[9] Masters *et al*[10] suggested that there are sufficient similarities between the amyloid filaments and proteins in AD and in scrapie to suspect that they are derived by mechanisms which have in common the generation of self aggregating polypeptides of low molecular weight. The authors' comment that if the scrapie filaments and proteins in CJ are an integral part or a direct effect of the infectious agent, it follows that AD is also an infectious process similar to scrapie. The present finding that reference strains of *Treponema pallidum* and *Borrelia burgdorferi* as well as the spirochetes found in the AD cases showed positive immunoreaction with a monoclonal antibody against APP, suggests that the APP may be an integral part of the infectious agent and thus may be the source of the excess of βA4 deposited in the AD brain. The fact that spirochetes of the *Borrelia burgdorferi* reference strain, cultured in a synthetic medium also exhibit a positive immunoreaction with the APP antibody may suggest that spirochetes synthesize their own APP.

In AD, βA4 is often deposited in the leptomeningeal and cortical vessel walls. In addition βA4 deposits were detected in tissues other than brain[11] suggesting that AD is a systemic disorder. This would be in agreement with spirochetal invasion of the brain via the vascular system. Probably, as in syphilis, spirochetes may invade the CNS during the early stage of the disease. In a small proportion of patients infection takes place but may remain latent for periods of varying length. In general paresis, dementia may appear as long as 43 years after the primary infection.[12] Subsequently, the accumulation of the microorganisms in small clusters in the cortex may cause the formation of 'argyrophylic', 'senile' or 'neuritic' plaques while damage to the neurones may lead to neurofibrillary changes.

The clinical reports made no mention of signs or symptoms of meningitis, encephalitis or polyradiculoneuritis, nor were histological signs of meningitis or encephalitis in the 14 AD cases investigated here. Spirochetes may invade the parenchyma of several organs including the brain without the challenge of an inflammatory reaction.[14] However, we cannot exclude the possibility that the amyloid-bearing plaques may be the sites of a chronic inflammatory process.[15]

There were no Alzheimer-like changes in the 13 age matched control cases investigated in this study. It is well known that in the older population there are cases demonstrating a small number of plaques and neurofibrillary tangles. These cases where the number of plaques and tangles with respect to the age of the patients[12] are not sufficient for the neuropathological diagnosis of AD, are said to reflect 'normal ageing'. In the cortex of these cases one may expect to find spirochetes in a small or moderate number. This was not seen in any of the 13 control cases (with use of the silver techniques for AD, or with the immunohistochemical technique for the demonstration of βA4), probably because of the restricted number of cases investigated. When a larger, particularly an aged, population without AD is tested, one may well find spirochetes in the blood in a number of cases but not in the CSF and in cerebral cortex. In a number of AD cases spirochetes may well be present in the CSF and in the cerebral cortex but not in the blood.

Recently the microorganism, Actinomyces, has been reported to be present in the brain of patients with an incidence that was four times higher than in other pathological or normal conditions.[13] Gram staining or ultrastructural analysis did not reveal the presence of Actinomyces in the AD cases presented here.

It was demonstrated that the messenger RNAs encoding the three major APPs, are present in many tissues besides brain in both control and AD cases, suggesting that APP is a natural constituent of a variety of cells.[14,15] The finding that the APP gene resides also on chromosome 21,[16] suggested the possibility that the APP gene, as the site of mutations, may cause AD. This hypothesis was subsequently challenged, when further studies revealed that the AD locus and the APP gene are not linked and not coinherited in familial cases of AD.[17,18]

Few familial AD cases, representing only a very small proportion of all AD cases (familial and sporadic cases together) are caused by a genetic defect.[19–21] The similar localization and distribution of the βA4 deposited in the brain in both forms—familial and sporadic—are difficult to explain. An alternative reconciliation of the genetic and infectious aetiology of AD lies in the supposition that the genetic defects associated with AD may lead to a predisposition for spirochetal infection, or may favour its progress.

Conclusion

In conclusion, the isolation of spirochetes from all the 14 AD cases investigated suggests that AD may correspond to a tertiary stage of neurospirochetosis. The fact that spirochetes isolated from AD brains, as well as reference strains of *Treponema pallidum* and *Borrelia burgdorferi*, express positive immunoreactivity with a specific antibody against APP would seem to indicate that several types of spirochetes may contribute to the aetiology of AD. An immunohistochemical and ultrastructural analysis that demonstrated a similar localization of APP, of βA4 and of spirochetes in the brain of a patient with concurrent AD and Lyme disease, would appear to support this hypothesis.

The characterization of the spirochetes found in AD is now needed. Knowing the genus and species of these spirochetes would indicate the source, the mode of transmission, and the site of the primary infection. It would enable one to develop serological tests for early detection of the infection. The pathological process is thought to begin long before the diagnosis of 'dementia' is made; and thus, appropriate antibiotic treatment should start early in order to prevent the development of dementia.

The presence of histological signs of AD in the brain, even in small numbers, would signify that the illness is in progress. At all times the late latent stage may turn into tertiary clinical manifestations.[20] In this light, the use of quantitative criteria for the neuropathological diagnosis of AD is unjustified.

ACKNOWLEDGEMENTS: I am grateful to H. Van der Loos for encouragement and critical remarks; to R. C. Janzer for helpful discussions. I thank L. Gem and E. Pradervand for reference strains of spirochetes; M. R. Dorsaz for advice; P. Dorsaz, S. Gros, A. Montoro and S. Trapey for technical assistance; S. Burki and J. Malfardet for photography.

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CALL FOR ABSTRACTS
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IMMUNOBLOTTING FOR THE DETECTION OF LYME BORRELIOSIS IN AUSTRALIA

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An indigenous form of LB, indistinguishable clinically from its northern hemisphere equivalent, is quite common throughout the entire eastern seaboard of continental Australia. The suspected vector is *Ixodes holocyclus* which is known to carry fastidious, slow growing borreliae. Although not yet characterized antigenically, it is likely that these spirochaetes are members of the rapidly expanding and antigenically diverse range of *B. burgdorferi* related genospecies associated with LB.

In the absence of direct detection methods for the laboratory confirmation of LB, we investigated the usefulness of immunoblotting (WB) for disease confirmation. Using B31 and two European *B. burgdorferi* strains (ACA-1, NBS-16) as antigen, data are presented for 9 patients representing various forms of LB who, unlike controls, contain antibodies to many, but not all, of the immunodominant antigens. Most notably, patients react with the well-defined 41kDa (flagellin), 31kDa (OspA), often with the 34kDa (OspB) and antigens in the 20-30kDa range (OspC, OspD), thus complying with the criteria established by Dressler *et al* for WB positivity.

Using OspA reactivity as a criterion for positivity, our experience is similar to elsewhere in that a relatively small proportion of LB patients is positive. A survey of 450 likely LB positive sera indicated that only 75 (16.6%) were OspA positive. Of interest, was the finding that only 9 (1.5%) reacted with B31 OspA, while 27 (6%) reacted only to ACA-1 (a European skin isolate) and 40 (9%) were positive to NBS-16 (a Swedish tick isolate). These data suggest that several types of LB borreliae may be co-circulating in Australia.

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MULTISYSTEM INVOLVEMENT WITH LYME BORRELIOSIS IN AUSTRALIA

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Controversy exists in Australia about the presence of an indigenous form of Lyme borreliosis. We will present clinical with supportive immunoblot data on 9 cases (6 female, 3 male) of Lyme-like illnesses acquired in Australia, for which no other cause was found. Average age was 47 years, range 9-67 years. Clinical features were: skin lesions (5 cases); physician diagnosed erythema migrans (2 females); patient reported erythema migrans (2 females); benign lymphocytic infiltration of Jessner-Kanof (1 male); complete heart block in a 52 year old man, requiring permanent pacemaker insertion; pauciarticular arthritis (3 females, 1 nine year old male); clinical signs of neurological abnormalities supported by abnormalities of nerve conduction, nuclear magnetic resonance imaging (3 females), but in all 3 cases who had spinal taps, no abnormalities were detected on cerebrospinal fluid examination. Neurological abnormalities were: bilateral facial nerve palsy (1); retrobulbar neuritis (1); radiculopathy (1). All patients had serum antibodies to outer surface protein A antigen (OspA) on immunoblot testing using European *B.burgdorferi* isolates, but not on testing to B31 strain. We feel, on the basis of these patients, that an indigenous form of Lyme borreliosis exists in Australia and that it produces multisystem involvement as seen in known endemic areas in the northern hemisphere.

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CAN MS SOMETIMES BE A CHRONIC FORM OF LYME BORRELIOSIS ?

Many symptoms considered characteristic of Multiple Sclerosis [MS] are seen in some patients diagnosed with Lyme Borreliosis [LB]. The distinguishing features of an MRI used to confirm an MS diagnosis are also seen in some chronic LB patients. MS is the most common severe neurological disease of man. LB is seen with many different symptoms including many forms of neurological disease. Physicians often find it difficult to differentiate between the two diseases. Many patients are first diagnosed as having MS on the basis of MRI or symptoms and then later rediagnosed as having chronic LB. There are many dangers that can accompany this misdiagnosis. Patients who are misdiagnosed as having MS are often treated with steroids which can promote a much more rapid progression of LB than is seen in untreated patients. If patients are eventually rediagnosed as having LB and treated with antibiotics, the previous steroid treatments can sometimes interfere with the proper treatment of the LB. Fifteen years ago the treatment regimen for LB patients shifted from steroids to antibiotics enormously improving the success of the treatment..

The MRI which is often used to confirm a suspected case of MS is indistinguishable from an MRI of patients with either LB or Optic Neuritis. Optic Neuritis is most commonly seen in young women. A large majority of them will eventually develop MS. Patients with LB can also develop Optic Neuritis. Progression of LB to Optic Neuritis and of Optic Neuritis to MS is commonly seen. Their common MRI and their other inter-relationships provide evidence that LB, Optic Neuritis and MS may very well all have the same etiological agent. The etiological agent of LB is definitely a Borrelia. The etiological agent of Optic Neuritis and MS are considered by some as "unknown", but since most Optic Neuritis patients eventually develop MS it is safe to say that they may well have the same etiological agent. A neuropathologist who conducted postmortems on hundreds of MS patients over 40 years ago was able to demonstrate spirochetes in many of the MS lesions. He published -- 30 years before the same Borrelia were shown to be involved in the newly identified LB.

In Borrelia infections, the earlier in the disease that antibiotic treatments are initiated the more effective they usually are. If Optic Neuritis and MS are also Borrelia infections, they also should be treated as early as possible. Both diseases are now often treated with steroids, but much strong evidence has been published that steroids should not be used in either condition. Because of a possible or probable Borrelia etiology, the use of early antibiotic treatments of MS and Optic Neuritis patients should be considered by physicians. Some practitioners have treated many MS and Optic Neuritis patients with antibiotics with greater success than with any standard steroid treatments. This deserves close attention since the present treatments of Optic Neuritis and MS are under strong criticism because of their dangers and ineffectiveness.

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Introduction and general session.

VII Annual International Scientific Conference on Lyme borreliosis and other spirochetal and tick-borne diseases was held on April 22, 23, 1994 in Stamford, CT. The Conference was organized by Lyme Disease Foundation and Stamford Department of Health and co-sponsored by Stamford Hospital. The Conference was attended by 350 people, health care professionals mostly, but open to public. The emphasis of the Conference was on neurologic manifestations and neuropsychiatric aspects of Lyme borreliosis.

The general session was chaired by who also had the opening presentation. The importance of clinical observation and publishing data were discussed in general and applied then specifically on situation in Lyme disease. Steps, which would replace numerous controversies with knowledge and data were suggested.

Doctor Ackermann, distinguished neurologist from Germany gave a detailed and comprehensive historical perspective on Lyme neuroborreliosis. His presentation surprised not only with the depth of the presented data, but also with the statement, that chronic Lyme disease predominantly manifests as Lyme arthritis and not neurologic disease, as thought before!

Doctor Edwin Masters, family physician from Missouri had presented data, suggesting Lyme disease is prevalent in Missouri. Missouri, as well as the entire area of south central U. S. is known as nonendemic area for Lyme disease. The isolation of borrelias in this area can represent the missing link between the existence of disease in these areas and patients with clinical symptoms. More attention and research is needed to determine the extent and potential of the infection.

The closing presentation of this session reviewed the classical clinical manifestations of Lyme borreliosis. Dr. Scrimenti and Dr. Lesser reviewed dermatologic, arthritic, cardiac and neurologic symptoms in very illustrative and educational manner.

Clinical Session

Clinical session was started with very interesting case report presented by Dr. Kornelia Keszler. She demonstrated the difficulty, primary care physicians are having in attempt to provide full care to patients, whose clinical manifestation of Lyme disease is not well defined and the course of infection is rather complicated. This case offered more questions, than answers, the trend, which was confirmed by following presentations as well.

The clinical spectrum of disease is not fully recognized even in the most researched areas of Lyme disease as is dermatology. Dr. E. Aberer presented interesting data on borrelial etiology of circumscribed scleroderma. In the diagnostic assessment, the lymphocyte stimulation test, dismissed in the U.S. with skepticism, was the most helpful. Treatment antibiotic trial confirmed the etiology by *Borrelia burgdorferi*.

Question of lactation and Lyme disease, remains to be answered, despite presentation of three cases by Dr. D. DeSilva. *B. burgdorferi* PCR-positive breast milk suggests, but not confirms, that this is the possible way of transmission for the baby. In this respect, transplacental transmission of the infection is much more serious consideration, but clearer answer should be given to lactating women.

Dr. Cameron and Paparone had presentation on characteristics of geriatric Lyme disease and its comparison to other age groups. This is rather unresearched area and presented data cannot be compared to larger population. The conclusion suggests, that clinical picture, treatment response and length of treatment in geriatric group is similar to other age group. Results of Dr. Paparone also suggest, that serology probably correlates with severity of symptoms, but this need to be confirmed by further studies.

Last presentation of the session and Conference was brilliant and provocative overview of Lyme borreliosis treatment given by Dr. Sam Donta. He has shown the most insight and innovative approach, which, if adopted by other, could bring some fast and more definitive answers to the most controversial issue of Lyme borreliosis: the treatment. However, as this point, presentation had to be limited to questions and hypothesis, rather than answers and solutions.

SUMMARY

LYME DISEASE: 1994 STATE OF THE ART CONFERENCE
TRACK B
MICROBIOLOGY & TREATMENT IMPLICATIONS

The session was opened by chairman, Dr. Claude F. Garon with a welcome. He commented that while in the past we have often congratulated ourselves on the conquest of infectious diseases, microorganisms causing disease have continued to do what microorganisms have always done - adapt and survive. Although vaccine and antibiotic development have produced wonders, problems still exist including new disease presentations, old pathogens that are now multiply drug resistant, and old pathogens with new virulence determinants. Progress in finding real solutions to these problems is often hindered by a lack of real understanding of exactly when and how microorganisms cause disease. Dr. Garon described the basic research conducted at the federal Rocky Mountain Laboratories and at other laboratories around the world as a molecular dissection of a pathogen, such as Borrelia burgdorferi, with the aim of explaining, in molecular terms, how disease is produced by infection. An important by-product of this approach is the ability to use well characterized bioproducts for the production of new generation vaccines and development of designer drugs aimed at specific targets. Research leading to the identification of three such targets in Borrelia burgdorferi was discussed: 1) a coordinated DNA replication system; 2) a critical circular DNA coiling/uncoiling enzyme; and, 3) a specific iron-acquisition surface receptor.

Dr. Garon introduced his own presentation by showing a high resolution transmission electron micrograph of the causative agent of Lyme disease - Borrelia burgdorferi and a schematic drawing showing the total genetic capacity of the microorganism. Although made up of both unique linear and circular molecules, the relative copy number of these molecules appears to be tightly controlled. In contrast to some autonomously replicated, microbial extrachromosomal elements which are represented hundreds, if not thousands, of times in each cell, the number of individual DNA molecules seen in Borrelia burgdorferi, whether linear or circular, is low and seems not to vary significantly over many generations of culture. Structures which appeared to show the intimate association of DNA molecules into networks within spirochetes were presented. Since important genes have been mapped to the linear chromosome, and to both linear and circular plasmids, this linkage control mechanism seems to be important and may be exploited using new biochemical agents which target DNA replication. Of the replication inhibitors tested, agents which cross-link DNA strands appear to be the most effective at inhibiting the growth of Borrelia burgdorferi in the laboratory in culture.

Dr. Scott Samuels presented data on the kinetics of killing by the antimicrobial agent coumermycin. Coumermycin is a non-clinically useful antibiotic, but one which is valuable in the laboratory to examine the role of DNA gyrase in the growth of Borrelia burgdorferi. DNA gyrase is a critically important enzyme which controls the coiling and uncoiling of DNA molecules within a cell. The effect of various concentrations of coumermycin over time on the survival and growth were established during laboratory culture. Concentrations of coumermycin

down to 0.003 μg/ml showed some effect, although 1000-fold more drug was required to completely inhibit growth. Cell killing required at least 2 μg/ml coumermycin. One hundred per cent killing was achieved with either 50 μg/ml coumermycin for at least 12 days or 2 μg/ml for 30 days. Resistance to the drug, which was shown to require but a single point mutation in the DNA gyrase B gene, spontaneously occurred in several static populations exposed to less than 50 μg/ml coumermycin.

Dr. David Dorward presented data on the disruption of cell surface binding by human transferrin to Borrelia burgdorferi by antibodies targeted at an iron stress-induced outer sheath protein. Acquisition of ferric iron is required for persistent systemic infection by Borrelia burgdorferi. In order to understand the molecular mechanism of iron acquisition by these spirochetes, studies were undertaken to monitor the effects of iron deprivation on spirochetal growth and metabolism, and to examine in detail the interaction between spirochetes and physiological iron sources. Initial experiments demonstrated that Borrelia burgdorferi could utilize human holotransferrin to support growth in iron restricted medium. Furthermore, 41 and 45 kilodalton proteins were preferentially expressed under iron stress. Murine antibodies raised against the 41 kilodalton protein bind to cell surfaces and appear to block binding of holotransferrin. By western blot analysis, the antibodies recognized a protein which was affinity purified from spirochetal cultures using holotransferrin coated magnetic beads. This membrane protein was also recognized by anti-Borrelia burgdorferi rabbit serum. These results show that iron acquisition from holotransferrin involves a surface-exposed transferrin receptor. Since antibodies to an iron stress-inducible protein appear to block binding of holotransferrin to its receptor, specific antibodies may also inhibit critical iron acquisition by the spirochetes.

An open question and answer session followed involving the session presenters and the audience.

NORTH AMERICAN LABORATORY GROUP
A Boston Biomedica Company

DATE: April 29, 1994

TO:

Lyme Disease Foundation, Inc.

FAX NO: 203/525-8425

FROM: Dr. Richard C. Tilton

COMPANY: NALG FAX NO: (203) 223-6279

OF PAGES (including this sheet): 3

Re: Track C-Diagnostic Imaging
Dr. Richard C. Tilton

Dr. Tilton convened the session which was devoted primarily to the diagnostic aspects of Lyme Disease.

Dr. Buschmich described her very interesting work on dairy cattle. She tested cattle from both endemic and non-endemic areas and found both populations to have antibodies against the OspA and OspB antigen of B. burgdorferi. These findings suggest either infection with another Borrelia or cross reactivity due to a rumen spirochete.

Both Dr's. Magnarelli and Aberer discussed diagnostic tests using urine as a sample source. Dr. Magnarelli described a mouse model in which he was able to detect B. burgdorferi antigen in mouse urine using an inhibition ELISA. Of the 87 urine samples tested, approximately two-thirds had antigen, over 60% were antibody positive, and over half were culture positive.

Dr. Aberer studied the shedding of B. burgdorferi in human urine as detected by PCR. The majority of patients with ECM were PCR positive prior to antibiotic therapy. She also observed excretion of DNA up to 10 months after the initiation of disease. There was speculation that excretion of B. burgdorferi DNA can serve as an important indicator of disease activity.

Dr. Tilton reported on over 500 patients from practices in New York, New Jersey, and Connecticut. PCR on plasma samples from all patients were negative, even in those patients who were seropositive and demonstrated classical symptoms of Lyme disease. The conclusion of this study was that blood is not a satisfactory specimen for detection of B. burgdorferi by PCR unless methods can be improved to detect as few as 1-5 spirochetes per ml. of blood.

Dr. Hulinska described the work done on Lyme borreliosis in Prague, Czech Republic. During 1993, over 33,000 cases were seen and the best clinical marker was ECM and neurologic symptoms. She showed some definitive electron micrographs of spirochetes in a variety of tissue samples such as skin, synovium, and muscle. It was postulated that Borrelia survive in their intracellular locale and may be protected from antibodies.

In summary, there were several caveats:

- Serologic tests for Lyme disease performed on cattle from both endemic and non-endemic areas reflect either infection with another Borrelia or cross reactions due to rumen treponemes.
- Urine appears to be a desirable specimen for detection of B. burgdorferi antigens and B. burgdorferi DNA, particularly in acute disease.
- With the present sensitivity of PCR, plasma does not appear to be an adequate specimen in late disseminated Lyme disease.
- Borrelia survive intracellularly and are protected from some antibiotics and maybe host factors.

Sincerely,

Richard C. Tilton, Ph.D.
Sr. Vice President and
Chief Scientific Officer

RCT/hlm

J. RAWLINGS

Four papers were presented in the Epidemiology and Public Health Issues section of the program. The first was an overview of various tick-borne diseases by Julie Rawlings of the Texas Department of Health. It is important to remember that although Lyme borreliosis is the most prevalent tick-borne disease, there are others such as Rocky Mountain spotted fever, Colorado tick fever, tularemia, human ehrlichiosis, babesiosis and tick-borne relapsing fever to be aware of. Dr. S.N. Banerjee presented four cases of tick-borne relapsing fever diagnosed at the University of British Columbia in Vancouver, Canada in 1993. Three patients had vacationed in Idaho prior . onset of their illness; the other had visited India. Laboratory diagnoses were based on clinical manifestations, the observation of spirochetes in blood and/or serologic test results. Dr. Matthew Cartter, with the State Department of Public Health and Addiction Services, discussed the epidemiology of Lyme disease in the 12-town area around Lyme, Ct, an area which continues to have one of the highest rates of Lyme disease in the world. The incidence of reported cases has increased nearly 9-fold since 1977. In addition, David Wolfe summarized the epidemiology of Lyme disease in Delaware, where the number of reported cases increased 246% between 1989 and 1992. Further, he found that in 1992, 94% of Delaware physicians treated patients with acute Lyme disease for at least three weeks; whereas only 52% did so in 1991.

The New York State Psychiatric Institute
722 West 168th Street #13
New York, New York 10032

5/8/94

Lyme Disease Foundation
1 Financial Plaza
Hartford, CT. 06103

Att: Steven Schutzer, MD
Martina Ziska, MD

Dear Steve and Martina:

I've asked each of the speakers to summarize their talks for me. Unfortunately, I haven't yet received anything. I know you are under a deadline pressure. So, I'll summarize the talks myself.

Neuropsychiatry Section

1. Lyme Disease: A Review of its Neuropsychiatric Features

Dr. Brian Fallon reviewed the literature regarding the psychiatric features associated with Lyme borreliosis. The literature consists primarily of case reports, although a few controlled series have been reported. Case reports have linked Lyme borreliosis with paranoia, thought disorders, delusions, auditory and/or olfactory hallucinations, stereotypies, anorexia nervosa, obsessive compulsive disorder, major depression, disorientation, confusion, violent outbursts, mood lability, panic attacks, mania, personality changes, catatonia, and dementia. Nine reports of larger series of patients with Lyme disease have been reported. Irritability, mood lability, and'or depression were reported in 7 of the 9 studies with a frequency ranging from 26% to 66% of the sample. One study of children with neurologic Lyme disease found that behavioral or mood disturbances were the second most common symptom. Although each of the reports had methodologic limitations, the presence of mood disturbance was a consistent finding. The psychiatric aspects of this disorder need further study and greater recognition.

2. Central Nervous System Cognitive & Emotional Effects of Chronic Lyme Disease.

Dr. Martin Schinedling reported his results on a study of 46 patients with Lyme disease who had received a battery of neuropsychological tsts, 16 of whom had been sick less than 4 years and 20 who had been sick greater than nine years. He noted that those sick longer had a higher degree of unemployment, greater difficulty on a test of abstract concept formation, and greater difficulty with shifting cognitive set. The most common symptoms included memory problems, abnormal sensations, fatigue, pain, phono/photophobia, concentration problems and speech/language difficulties. On the MMPI, the Lyme patients scored high on

depression, hysteria, and schizophrenia. Dr. Schinedling noted that these high scores need to be interpreted in the context of patients who have a physical illness, as is done in studies of patients with brain trauma. High scores on the depression subscale seemed to reflect a truly depressed mood, while the high scores on the hysteria and schizophrenia sub-scales were misleading. The high hysteria sub-scale scores resulted from the report of multiple somatic symptoms and the high scores on the schizophrenia sub-scale resulted from the cognitive difficulties and unusual sensory experiences of these patients. Given incorrect conclusions that can easily be drawn from a casual use of the MMPI, Dr. Schinedling urged that the MMPI be interpreted cautiously among patients with Lyme disease, with particular attention being paid to the individual items within the subscales.

3. Depression and Psychopharmacology of Lyme

Dr. Catherine Saunders reviewed the key symptoms of depression which include two weeks of five of the following features: depressed or irritable mood; anhedonia; weight loss or gain; insomnia or hypersomnia; psychomotor agitation or retardation; fatigue or loss of energy; feelings of worthlessness or inappropriate guilt; poor concentration or indecisiveness; or recurrent thoughts of death. Depression due to organic factors, such as a CNS infection, can be effectively treated with standard anti-depressant therapies, as long as the underlying infection has also been sufficiently treated. Because tricyclic antidepressants have significant anti-cholinergic side-effects, many psychiatrists prefer to treat patients with an organic affective disorder with the selective serotonergic reuptake inhibitors, such as fluoxetine (Prozac), sertraline (Zoloft), or paroxetine (Paxil). Failure to respond to these interventions may warrant switching to an anti-depressant of another class or the addition of adjunctive medications, such as Lithium, T3, or the psychostimulants.

4. The Cognitive Profile of Lyme Disease

Dr. Marian Rissenberg reported on 37 patients with putative Lyme Disease (60% seropositive, 27% seronegative, 13% equivocal) who had been given an extensive battery of neuropsychological tests. The average duration of illness was 43 months with more than 90% reporting a fluctuating symptom pattern. Dr. Rissenberg noted that a fluctuation in mental acuity ("better" one day, "worse" the next) seemed characteristic of Lyme disease and caused considerable vocational and family problems. Typical symptoms included memory loss (100%), irritability (89%), confusion (76%), decreased initiative (70%), depression (70%), anxiety (49%), and personality change (43%). The performance on the neuropsychological battery did not reveal a specific typical pattern. Rather, deficits for the group were noted across all measures - short term memory, mental tracking, abstract reasoning, planning and sequencing, expressive and receptive language, and visuospatial processing. These deficits occurred independently of degree of depression. Neuropsychological evaluation can be

helpful in the documentation of objective deficits, in the assessment of treatment efficacy, and in the planning of strategies of cognitive remediation.

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I hope this is what you wanted. If you want something briefer, just let me know.

Brian

Brian Fallon, MD

James H. Katzel, M.D.

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THE POSTER SESSION

The poster session, chaired by Dr. James Katzel, drew much attention from the conference attendants. The poster area became a location of intense discussions sparked by the varied topics presented in poster form. Abstract were accepted from around the world. Dr. Katzel presented an interactive tour of Lyme disease risk behaviors, clinical signs and symptoms, and treatment results. Medical education was the primary focus. On the topic of laboratory diagnosis, Dr. Tilton described the importance of a quality assurance panel for antibody test kit validation.

Clinical manifestations of Lyme disease were represented in a number of interesting displays. Dr. Schwartzberg introduced us to a woman patient hospitalized with a polymyalgia rheumatica like syndrome, and a positive lyme elisa and western blot. Dr. Marshall questioned a possible link between MS and Lyme disease. Dr. Cleveland showed yet another presentation of Lyme disease, that of a chest wall mass which developed months after antibiotic treatment. An intriguing case of frontal type dementia with Lyme disease was discussed by Dr. Wanick. Two beautiful posters came to us from Australia; Drs. Barry and Hudson showed the laboratory detection and the epidemiology of the disease down under. A rheumatologist in training, Dr. Powell, showed the association of borrelia specific immune complexes in patients with chronic fatigue syndrome. Dr. Feaga called for prompt research to study humans who voluntarily exposed themselves to the veterinary Lyme disease vaccine. And finally, a high school student stressed the occurrence of behavioral and cognitive deficiencies in adolescents with Lyme disease.

The complete abstract for each of these poster presentations is available in the conference compendium published by the Lyme Disease Foundation.

James Katzel, M.D.
Poster Session Chair

Jim H. Katzel

(Photo of part of the poster area enclosed)

Acute Lyme Disease in an American Tourist Returning from Germany

SIR—Lyme disease, caused by infection with the tick-transmitted spirochete *Borrelia burgdorferi*, is the most commonly reported arthropod-borne disease in the United States, with ~9,000 cases reported annually [1]. Lyme disease also occurs in Europe, the Commonwealth of Independent States (the former Soviet Union), northern China, and Japan [2]. We report a case of acute Lyme disease in a Wisconsin resident who acquired the infection while visiting Germany.

A 43-year-old woman from Milwaukee discovered a tick attached to her right upper arm on 29 June 1990 while visiting relatives in Bergkirchen, Germany, northeast of Munich. The tick was removed and discarded without identification. Her relatives' home was at the edge of the village, in a heavily wooded area. She had been hiking in the surrounding mountains 2 days before the tick was noticed. On 12 July a circular lesion of 2–3 cm in diameter appeared at the bite site, and the woman experienced nausea and malaise. She returned to Milwaukee without medical evaluation. On 28 July the patient noted extreme fatigue, headache, neck pain, and anorexia. She was examined by her personal physician on 31 July. No definitive clinical diagnosis was made, and no treatment was prescribed. A serum sample was reportedly negative for antibodies to *B. burgdorferi*. The rash then expanded and became more erythematous.

On 14 August a dermatologic consultant noted an elliptical 10 × 8–cm erythematous plaque on the right deltoid region. A reddened margin and irregular areas of partial central clearing were present. Lymph nodes were palpable in the right anterior cervical and axillary regions. The patient's temperature was 99.9°F. A clinical diagnosis of early Lyme disease with erythema migrans was made. No rheumatologic, neurological, or cardiac signs of Lyme disease were present. A 4-mm punch biopsy specimen was obtained from the leading edge of the skin lesion, placed in 7 mL of Barbour-Stoenner-Kelly (BSK) medium [2a], and shipped overnight to the Centers for Disease Control and Prevention (Diagnostic and Reference Laboratory, Bacterial Zoonoses Branch, Fort Collins, Colorado).

Treatment was begun with amoxicillin and probenecid (500 mg of each, administered three times a day by mouth). The patient experienced chills the first evening after beginning therapy with amoxicillin, but there was no other evidence of a Jarisch-Herxheimer reaction. The skin rash resolved within 6 days of the initiation of treatment, but constitutional symptoms resolved more slowly. Eight days after treatment was begun, a maculopapular rash developed, which was suspected to be the result of an allergic reaction to amoxicillin. Treatment was then changed to a regimen of doxycycline (100 mg twice a day by mouth). Doxycycline therapy was continued for a total of 7 weeks, after which the patient was asymptomatic and the rash and adenopathy had disappeared. She has remained asymptomatic.

The culture of the skin biopsy sample obtained on 14 August was positive for *B. burgdorferi*. Results of an enzyme immunoassay for antibodies to *B. burgdorferi* in serum drawn on 17 September 1990 were equivocal; a whole-cell sonicate of an American reference strain (B31; provided by Dr. Alan G. Barbour, University of Texas, San Antonio) was used. A repeated assay on 5 December 1990 was unequivocally negative, and the patient was still seronegative when the test was performed once again in March 1991; these results suggest that antibiotic treatment aborted an antibody response to infection [3].

It is important that physicians consider the diagnosis of Lyme disease in patients who have traveled to Europe or northern Asian countries, including Japan, particularly those who traveled during spring or summer and who participated in activities that may have exposed them to ticks. It is also important to consider that patients who develop erythema migrans usually do not have a tick attached at the time the rash develops and may not have observed the tick that transmitted the infection or its bite [3]. Such patients frequently do not have detectable antibodies against *B. burgdorferi* [3]. As the present case and other studies have shown, culture of a punch biopsy specimen [4–6] or of aspirate [7] from an erythema migrans lesion can be useful to confirm a clinical suspicion of Lyme disease.

Grant L. Campbell, David T. Dennis, Thomas J. Quan, and Rudolph J. Scrimenti

Bacterial Zoonoses Branch, Division of Vector-Borne Infectious Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Fort Collins, Colorado; and Department of Dermatology, Medical College of Wisconsin, Milwaukee, Wisconsin

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