#6

1990 NATIONAL LYME BORRELIOSIS SCIENTIFIC SYMPOSIUM

Orlando, FL

1st compendium

MARCH 29 & 30, 1990

CLINICAL MANAGEMENT & NEW DISCOVERIES

CO-CHAIRMEN:
WILLY BURGDORFER, PhD, MD
JOHN F. ANDERSON, PhD

LYME BORRELIOSIS FOUNDATION
in cooperation with
The University of Miami School of Medicine

CME credits have been applied for.

LYME BORRELIOSIS FOUNDATION, INC.

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LYME BORRELIOSIS SCIENTIFIC SYMPOSIUM

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CLINICAL MANAGEMENT & NEW DISCOVERIES - March 29 & 30, 1990

CONFERENCE CHAIRS: JOHN F. ANDERSON, PhD & WILLY BURGDORFER, PhD, MD

Thursday - 3/29/90

7:00 am — Registration & Continental Breakfast

8:00 am — **WELCOME & HISTORY**
Lyme Borreliosis celebrates its 107th birthday

John F. Anderson, PhD
Director
Connecticut Agricultural Experiment Station

Willy Burgdorfer, PhD, MD
Laboratory of Pathogens
Rocky Mountain Laboratories, NIAID

8:30 am — **THE SPIROCHETE BORRELIA BURGDORFERI**
A genetically and structurally complex bacterium its ability to confound the immune system , and antigenic variation... an important concept for treating patients.

Moderator: Thomas G. Schwan, PhD
Acting Head, Arthropod-Borne Diseases Section
Rocky Mountain Laboratories, NIAID
James N. Miller, PhD
Professor of Microbiology & Immunology
UCLA School of Medicine
Warren J. Simpson, PhD
Visiting Associate, New Zealand
Rocky Mountain Laboratories, NIAID

9:30 am — **PATHOLOGY, PATHOGENESIS AND TESTING**
Damage to all organ system.the pathogenesis, and serologic testing. Human and Veterinary aspects compared.

Moderator: Paul H. Duray, MD
Acting Chairman, Department of Anatomic Pathology
Fox Chase Cancer Center
Louis A. Magnarelli, PhD
Chief Scientist
Connecticut Agricultural Experiment Station

10:20 am — **BREAK**

10:35 am **CLINICAL CASES - PEDIATRICS**

Case Presentations, Decision Making, Treatment Protocols, Follow-up, New Trials, Early, Chronic, Relapsing and Life Threatening Cases .

Moderator: Dorothy M. Pietrucha, MD
Pediatric Neurology
New York Hospital - Cornell Medical Center & Jersey Shore Medical Center
Lawrence S. Zemel, MD
Director of Pediatric Rheumatology
Newington Childrens Hospital
Edwin J. Masters, MD
Family Practice
St. Francis Hospital & S.E. Missouri Hospital

Noon **LUNCH**

1:15 pm **CLINICAL CASES - ADULTS**

Case Presentation, Decision Making, Treatment Protocols, Follow-up, New Trials, Early, Late, Chronic, Relapsing, and Life Threatening Cases

Moderator: Paul E. Lavoie, MD - Rheumatologist
Associate Clinical Professor
University of California San Francisco
Gerold Stanek, MD
Hygiene-Institut der Universitat Wein, Austria
Andrew R. Pachner, MD - Neurologist
Georgetown University
J. Lawton Smith, MD - Neuro Ophthalmologist
Professor of Ophthalmology
University of Miami, School of Medicine
Philip T. Williams, MD - Internist/Gastroenterologist
Ajax & Pickering General Hospitals Ontario, Canada
J. Joseph Burrascano, Jr. , MD - Internal Medicine
Southampton Hospital

3:00 pm **BREAK** (15 minutes) - continuing Adult session.

4:15 pm **OBSTETRICAL/GESTATIONAL LYME - DIAGNOSTIC & THERAPEUTIC DILEMMAS**

Moderator: Paul E. Lavoie, MD
Associate Clinical Professor,
University of California San Francisco
Dorothy M. Pietrucha, MD
Pediatric Neurology
New York Hospital - Cornell Medical Center
Jersey Shore Medical Center
George D. Wendell, MD
Associate Professor of Obstetrics & Gynecology
University of Texas, Southwestern Medical Center

5:15 pm - 6:15 pm **RECEPTION**

7:00 pm - 9:00 pm **PUBLIC FORUM**
Moderator: John Drulle, MD
Jersey Shore Medical Center
Open to the public in the area , and the place where lay people, press, (including conference attendees) can ask questions.

Friday - 3/30/90

7:00 am — Continental Breakfast

8:00 am — **CLINICAL VETERINARY ISSUES**

Comparison to Human Pathogenesis. Diagnosis, Treatment Protocols, Emerging issues as well as implications for Animal to Animal/IHuman Transmission, Livestock Ramifications, and Wildlife issues. Cats, Dogs, Horses, Cows, Goats, etc.

Moderator:
Edward M. Schneider, PhD
Director of Laboratories, Veterinary Research Associates
Edward M. Bosler, PhD
Research Scientist
New York State Department of Health
John E. Post, DVM, PhD
Professor of Pathobiology
University of Connecticut, (sabbatical in Australia)
Elizabeth C. Burgess, DVM, PhD
Associate Professor
University of Wisconsin,School of Veterinary Medicine

9:45 am — **TRANSMISSION - TICKS, HOSTS, CONTROL METHODS**

Moderator:
John F. Anderson, PhD
Director
Connecticut Agricultural Experiment Station
Julie A . Rawlings, MPH
Research Scientist
Texas Department of Health

10:30 am — **BREAK**

10:45 am — **NORTH AMERICA MOBILIZES**

Moderator:
Karen McN. B. Vanderhoof-Forschner, BS, CPCU, CLU, MBA
President , Lyme Borreliosis Foundation, Inc
1990/1991 Congressional Support for Funding
Robert L. Quackenbush, PhD
Program Officer of Bacteriology
National Institutes of Health - NIAID
Stephen P. Heyse , MD, MPH
Director, Office of Prevention, Epidemiology & Cinical
National Institutes of Health - NIAMS

11:45 am — **IMMUNE SYSTEM RESEARCH**

Potential Vaccines, Sero-Negative, Antibiotic Susceptibility, New Tests.

Moderator:
Russell C. Johnson, PhD
Professor of Microbiology
University of Minnesota
Ronald F. Schell, PhD
Professor of Microbiology /Immunology
University of Wisconsin Medical School
Thomas G. Schwan, PhD
Rocky Mountain Laboratories
Acting Head, Arthropod-Borne Diseases Section
Rocky Mountain Laboratories, NIAID

12:45 pm	Conference Close - except for those interested in specific projects.
1:30 pm	Soda Break
1:30 p.m. - 3:30 p.m.	**Special Sessions**

Meeting of the LBF Committee on Veterinary Medicine:
Open to Veterinarians and Researchers only

WHO SHOULD ATTEND THIS CONFERENCE

PRIMARY PHYSICIANS: Family Practitioners, Internists, and Pediatricians

SPECIALITY PHYSICIANS: Dermatologists, Neurologists, Cardiologists, Rheumatologists, Ophthalmologists, Intensivists, and Psychiatrists

ALLIED HEALTH: Nurses, Therapists, Psychologists, Health Officials

VETERINARIANS & VETERINARY SPECIALISTS

RESEARCHERS, ENTOMOLOGISTS, and OTHER MEDICAL PROFESSIONALS

LOCATION: ORLANDO, FLORIDA !!!

CME CREDITS HAVE BEEN APPLIED FOR. EXHIBITION AND BOOTH SPACE AVAILABLE

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Sequestration of antibody to *Borrelia burgdorferi* in immune complexes in seronegative Lyme disease

STEVEN E. SCHUTZER P. K. COYLE ANITA L. BELMAN
MARC G. GOLIGHTLY JOHN DRULLE

To find out whether apparent seronegativity in patients strongly suspected of having Lyme disease can be due to sequestration of antibodies in immune complexes, such complexes were isolated and tested for antibody to *Borrelia burgdorferi*. In a blinded analysis the antibody was detected in all 10 seronegative Lyme disease patients with erythema chronicum migrans (ECM), in none of 19 patients with other diseases, and in 4 of 12 seronegative patients who probably had Lyme disease but had no ECM. These findings were confirmed by western blot, which also showed that immune complex dissociation liberated mainly antibody reactive to the 41 kD antigen and sometimes antibody to an approximate 30 kD antigen. Complexed *B burgdorferi* antibody was also found in 21 of 22 (95%) of seropositive patients with active disease, 3 additional seronegative but cell mediated immune reactive patients, and 3 other seronegative patients who eventually became seropositive. Apparent *B burgdorferi* seronegativity in serum immune complexes may thus be due to sequestration of antibody in immune complexes.

Lancet 1990; 335: 312–15.

Introduction

Lyme disease, which is caused by the spirochaete, *Borrelia burgdorferi*,[1,2] may affect the musculoskeketal, cardiovascular, nervous, and cutaneous systems.[3] Seronegativity is an unexplained feature and is a major obstacle to diagnosis when the hallmark, erythema (chronicum) migrans (ECM), is not observed,[4,5] as happens in up to 50% of patients with Lyme disease. The main laboratory test for the disease, the detection of antibody to *B burgdorferi*, may also be negative in many instances.[4] A negative test despite clinical features of the disease may perhaps be due to sequestration of the antibody in complexed form, as can occur in diseases such as hepatitis B, multiple sclerosis (MS), systemic lupus erythematosus, and syphilis, in which immune complexes (IC) often contain the relevant antigen and antibody, even in the absence of the free component.[6-10] To examine this possibility we have isolated and analysed IC from seronegative Lyme disease patients.

Patients and methods

Patients

The seronegative group consisted of 22 symptomatic patients who had been negative for *B burgdorferi* antibody by commercial enzyme linked immunosorbent assay (ELISA) before entry into the study ('Lyme FAST', 3M Santa Clara, California, USA) (table 1). Patients came from Monmouth and Ocean Counties, New Jersey, an area endemic for Lyme disease. All samples were received

ADDRESSES: Division of Allergy and Immunology, Department of Medicine, University of Medicine and Dentistry-New Jersey Medical School, Newark, New Jersey; and Departments of Neurology and Pathology, Health Sciences Center-State University of New York at Stony Brook, New York State, USA (S. E. Schutzer, MD, P. K. Coyle, MD, A. L. Belman, MD, M. G. Golightly, PhD, J. Drulle, MD). Correspondence to Dr S. E. Schutzer, Department of Medicine, UMDNJ, 185 South Orange Avenue, Newark, NJ 07103, USA.

in a blinded manner, and the clinical histories, including past occurrence of ECM, were unknown to those of us doing the experiments until completion of all tests. For this study, Lyme disease was taken as definite when there was a history of unequivocal ECM (n = 10), and as possible when symptoms suggestive of Lyme disease were not accompanied by a history of ECM (n = 12). Many of the symptoms listed in table I were transient and occurred some time during the course of the disease. All but 1 patient had been prescribed antibiotics by other physicians for a diagnosis other than Lyme disease; none received a currently recommended regimen[3] for Lyme disease. No patient had a history or evidence of immune deficiency.

Controls

The controls were 22 seropositive Lyme disease patients; 9 patients, matched for IC levels, with a variety of other diseases (asthma, urticaria, upper respiratory infection, polyradiculoneuropathy, allergy, multiple sclerosis, polymyositis, stroke); 10 seronegative patients from an endemic area with chronic fatigue as a prominent symptom; and seronegative healthy individuals, also from an endemic area. To further evaluate specific IC formation, additional unblinded samples were analysed from 3 cell mediated immune positive[11] individuals and from individuals with definite and possible Lyme disease.

Antibodies to B burgdorferi

Samples were retested by ELISA[11] for anti-*B burgdorferi* antibodies to confirm the pre-study tests indicating apparent seronegativity. The 96 flat-bottomed-well microtitre plates were coated with a sonicate of *B burgdorferi* (B31) (5 μg/ml). Serum was diluted 1/500. Optical density readings greater than 3 SD above the mean for a standard panel of 10 healthy controls without a history of *Borrelia* infection was taken as a positive result. These 10 controls came from an endemic area and were used in each ELISA run. This assay was also used to probe isolated serum IC (diluted 1/10) for *B burgdorferi* antibodies. In this case the control panel used to define the optical density cutoff (mean + 3 SD) consisted of isolated serum IC from 10 individuals without a history of *Borrelia* infection.

Immunoglobulin concentration

An ELISA was used to measure the Ig isotype contents of the serum samples and isolated IC as previously described.[10]

Rheumatoid factor (RF)

The presence of RF, which could potentially bind *Borrelia* reactive antibody, was screened for in all samples by latex agglutination ('Rheuma-Fac', ICL Scientific, Fountain Valley, California, USA).

Immune complex detection

ICs have different sizes and physicochemical properties so two different assays[12] were used—anti C3 and anti-C1q assays ('Raji' cell replacement EIA and CIC EIA, Cytotech, San Diego, California, USA).

Analysis of immune complexes

Polyethylene glycol precipitation (PEG), modified[13] from the method of Digeon et al,[14] was used to isolate and analyse IC. Briefly, 100 μl of the sample was added to an equal volume of 7% PEG in 0·1 mol/l sodium borate, pH 8·4, incubated overnight at 4°C, then centrifuged at 8320 *g* for 15 min. Pellets were washed twice with 3·5% PEG-borate and resuspended in 100 μl of 0·1 mol/l sodium borate, pH 10·2. This dissociates IC into its components, which can be analysed by ELISA and western blot for *Borrelia* antibody and antigen. The precipitate was also probed for IgG, and IgM and IgA in appropriate cases, and the optical densities read on the microELISA reader. The ELISA described above was used to probe for anti-*Borrelia* antibody in the dissociated serum IC preparations.

TABLE I—CHARACTERISTICS OF SERONEGATIVE PATIENTS

Feature	ECM positive (n = 10)	ECM negative (n = 12)
Mean (SD, range) age (years)	35·4 (22·2, 8–86)	38 (9·2, 23–52)
Sex (M/F)	3/7	7/5
Mean (SD range) time from onset of sample (mo)	25·8 (26·7, 1–81)	22·4 (28·8, 4–78)
Clinical manifestations during course of disease		
Minor CNS (headache, cognitive, mood)	10	12
Fatigue	10	12
Paraesthesias	7	7
Arthralgias	9	11
Arthritis	2	2
Myalgias	8	10
Palpitations, tight chest, without conduction defects	5	8
Flu-like onset	7	2
Laboratory findings		
Positive Lyme (free Ab) tests		
Pre-study ELISA	0	0
ELISA*	0	0
Rheumatoid factor*	2	1
Immune complex assays* (mean)		
Anti C3 (+ > 15)	3·3 μg Eq/ml	2·4 μg Eq/ml
C1q binding (+ > 4)	5.1 μg Eq/ml	6·1 μg Eq/ml

CNS = central nervous systems.
*Tests performed after entry into study.

To confirm the ELISA results western blots were done as previously described,[11,15-17] with relevant modifications. Sonicates of cultured B31 strain *B burgdorferi* were used as the antigenic source (25 μg/85 mm of membrane) and run on a preparative 10% sodium dodecyl sulphate-polyacrylamide gel (SDS-PAGE) with a 4% stacking gel electrophoresis ('Mighty Small II' system, Hoeffer, San Francisco, California, USA). The separated proteins and molecular weight standards were transferred to nitrocellulose membranes, which were developed in a 28 lane 'Miniblotter' (Immunetics, Cambridge, Massachusetts, USA) by previously reported methods.[11,17] A 1/100 dilution of serum, and 1/10 dilution of the IC preparation was addded to each lane as the source of the first antibody. To better compare bound to free antibody proportions, serum was also diluted to a concentration of IgG equivalent to that of the isolated IC and then used in the western blot. This would also serve to control for any possible concentration of immunoglobulin during PEG precipitation. Controls included known positive sera, conjugate alone, and known seronegative sera from patients with other diseases and matched for IC levels.

To probe for *Borrelia* antigens and further assess the specificity of the anti-*Borrelia* antibodies, dissociated PEG IC preparations

TABLE II—ANTI B. BURGDORFERI ANTIBODY IN IMMUNE COMPLEXES IN SERONEGATIVE PATIENTS AND SEROPOSITIVE ACTIVE LYME DISEASE CONTROLS

Group	IC with antibody to *B burgdorferi*	IC without antibody to *B burgdorferi*	p (compared with other diseases*)
Seronegative samples			
Definite LD: ECM + (n = 10)	10	0	5×10^{-6}
Possible LD: ECM − (n = 12)	4	8	$1{\cdot}6 \times 10^{-2}$
Seropositive Lyme disease (n = 22)	21	1	$< 10^{-6}$
Other diseases (n = 19)*	0	19	..

LD = Lyme disease.
*9 (matched for IC concentration) with asthma, urticaria, upper respiratory infection, polyradiculoneuropathy, allergy, multiple sclerosis, polymyositis, or stroke; 10 with chronic fatigue.

(undiluted, 1/10 dilutions) were run on the SDS-PAGE and then immunoblotted along with the *B burgdorferi* sonicates and molecular weight standards. Affinity purified human IgG with a very high anti-*Borrelia* antibody titre was used to probe for antigen; non-specific staining was evaluated by the use of IgG from a non-disease control as well as probing isolated IC from the controls.

Statistical analysis

Fisher's exact test was applied to results obtained from the study of *B burgdorferi* reactive antibody in the serum IC in these 22 seronegative patients suspected of having Lyme disease.

Results

*Free anti-*B burgdorferi *antibody in serum samples*

The "seronegative" status of all 22 putative seronegative samples was confirmed by the anti-*B burgdorferi* antibody ELISA (table). 21 of the 22 samples gave readings of < 1 SD above that of control panel, the other was between 1 and 2 SD above that for controls. Other samples were analysed in the same way, and the seronegative and seropositive status confirmed.

Immune complex levels

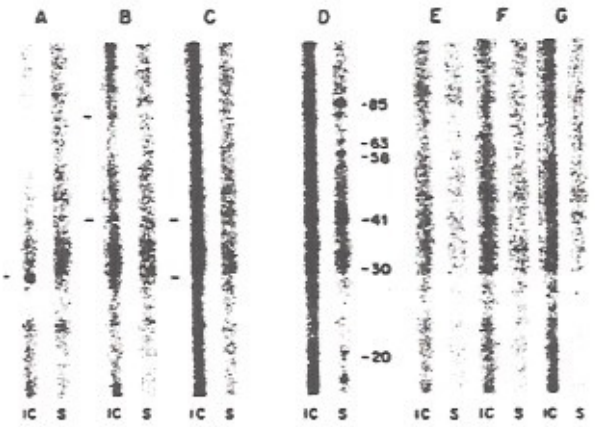
Although our primary intent was to examine the components of circulating IC in these patients, concentrations were measured so that controls could be matched for circulating IC concentrations. After decoding the samples, we found that IC levels were near normal in all groups when measured by the C1q and C'3 assays. There were no significant differences between the ECM-positive and ECM-negative groups with respect to the level of IC (table I). Specifically, among the seronegative patients, 5 of the 10 patients with ECM, and 9 of the 12 without ECM, had a low positive value in the C1q assay (above 4 µg Eq/ml); the mean concentrations were 5·1 and 6·1 µg Eq/ml, respectively. The anti-C3 IC concentrations did not rise above a positive threshold of 15 µg Eq/ml in either group—the mean values were 3·3 and 2·4 µg Eq/ml for the ECM-positive and the ECM-negative groups.

Rheumatoid factor (RF) in serum samples

Rheumatoid factor (RF), which can bind *Borrelia* reactive antibody, was rarely found.

*Anti-*B burgdorferi *antibody in isolated serum immune complexes*

Though serum IC concentrations were not raised, there was sufficient for analysis for complexed *B burgdorferi* antibody. All 10 ECM-positive seronegative Lyme disease patients had IC containing *B burgdorferi* reactive IgG antibody (table II). Included in this group was 1 patient who had not received antibiotic therapy. 4 of the 12 seronegative possible Lyme disease patients had complexed anti-*B burgdorferi* IgG antibody. 20 of 21 (95%) samples from symptomatic seropositive Lyme disease patients also had complexed IgG anti-*B burgdorferi* antibody. No complexed anti-*B burgdorferi* antibody was found in any of the 9 other disease controls, or any of the 10 seronegative chronic fatigue patients, or the 10 seronegative symptom-free healthy individuals from the endemic area. There was a significant association (Fisher's exact test, 1 degree of freedom, $p = 5 \times 10^{-8}$) between the detection of IC



Comparative western blots of IgG antibodies to *B burgdorferi* in immune complex dissociated preparations and serum samples in seronegative Lyme disease patients.

S = serum (diluted, see text); IC run undiluted. A, B, and C represent seronegative (determined by ELISA) Lyme disease patients with antibody *B burgdorferi* in the immune complex as determined by PEG-ELISA; A was ECM−, B and C were ECM+. D represents a known seropositive active Lyme disease patient. E, F, and G represent other disease controls. Not shown is conjugate run alone, which was negative. Approximate molecular weights in kilodaltons are indicated to the right of patient D. IC and serum samples from each patient were run in tandem. Not all patients were run on the same gel.

In these representative blots, IgG antibody liberated from the complex scans reacts with a polypeptide antigen of 41 kD (possibly the flagella) in most cases, as well as an approximate 30 kD one (possibly OspA- an outer surface protein) in some cases.

containing anti-*B burgdorferi* antibody and ECM-positive Lyme disease cases. The association was also significant for ECM-negative possible cases (table II).

The western blots confirmed the appearance of *B burgdorferi* reactive antibody which had been sequestered and thus not detected before PEG-borate dissociation of the IC. IgG antibody liberated from the complex reacted with a polypeptide antigen of 41 kD (flagella antigen) in most cases, and with an approximately 30 kD antigen (probably OspA) in 2 of 10 cases. Dilution of serum samples to the equivalent concentration of IgG in the IC showed that the observed results were not due to possible concentration of IgG during PEG precipitation. In other experiments dissociated IC preparations showed heavier blot staining than even serum containing 10–100 times the equivalent IgG concentrations, thus further highlighting this liberation of complexed antibody. In analysis of free antibody a 41 kD band was clearly visible in the seropositive controls but absent or barely discernible in the seronegative Lyme disease patients and other controls.

Dissociated IC samples from 5 patients with complexed *Borrelia* antibody (2 ECM+ and 1 ECM− seronegative, 2 seropositive) were analysed by western blot analysis. Polyclonal affinity purified human IgG from a seropositive Lyme disease patient stained antigens, which co-migrated with antigens from the *B burgdorferi* sonicate, most visibly in the region of 43 kD and 38 kD. Polyclonal IgG from a healthy uninfected individual showed no reactivity. 5 control samples, matched for IC levels, were negative for such antigens when similarly probed.

Further support for sequestration of antibodies as a mechanism of seronegativity and IC formation as a feature even in seropositive Lyme disease patients is the finding of complexed anti *B burgdorferi* antibody in 3 seronegative

patients with positive T-cell-mediated immunity (CMI) to *Borrelia* and in 3 patients who eventually seroconverted on follow-up. Initially only complexed anti *B burgdorferi* antibody was found in all 3 seronegative patients; 2 later became seropositive and the third became borderline positive. One ECM-positive patient had complexed anti *B burgdorferi* IgM at 2 weeks, at which time treatment was started; and 2 weeks later the concentration of complexed antibody fell, but that of free, anti *B burgdorferi* IgM rose. In contrast to this rapid transition, in 1 patient in whom the diagnosis was not made and so remained untreated with antibiotics for a year, complexed IgG anti *B burgdorferi* antibody was detected in two samples a year apart; the patient seroconverted 8 months after therapy.

Discussion

Our data show that symptomatic seronegative or seropositive individuals with Lyme disease form circulating IC containing *B burgdorferi* reactive antibody, as determined by use of stringent criteria for the ELISA (>3 SD of the controls for a positive reading) and as confirmed by western blot. The specificity of the complexed antibody was also suggested in a preliminary fashion by western blot probes of the IC for *B burgdorferi* antigens.

Immune recognition of *B burgdorferi*, in the form of T cell reactivity to *B burgdorferi*, antigens, has been reported in 17 seronegative patients.[11] In 3 such patients, not part of the blinded aspect of the present study, we found complexed *B burgdorferi* antibody. Additional direct support for IC formation as a mechanism of apparent seronegativity in this disease is provided by the detection of *B burgdorferi* reactive IC in seropositive patients with active disease or in seronegative patients who later seroconverted. The absence of *B burgdorferi* reactive IC in patients with chronic fatigue, healthy symptom-free individuals, and other disease controls, all from an endemic area, indicates specificity of the IC.

Sequestration of antibody within IC occurred in all seronegative definite Lyme disease patients studied. However, these patients did not have raised levels of IC and did not show features of chronic IC disease such as those that occur in serum sickness or systemic lupus erythematosus. The near-normal levels in the patients with prolonged illness is consistent with reports[18-20] that in most patients raised C1q binding levels occurred at the start of the disease and returned to normal after 2 months. Complexes were also found in the serum of our seropositive patients.

Since early administration of antibiotics has been associated with apparent seronegativity in Lyme disease, we point out by way of observation that 3 of the patients in our study had not received antibiotics but were positive for specific IC. The other patients in the blinded part of the study had been treated with some antibiotic regimen but not any of those currently recommended for Lyme disease.[3] Though undefined mechanisms may contribute to seronegativity, in the present study, sequestration of antibody seems to be a sufficient explanation.

Western blot analysis revealed, in most cases, complexed anti-*B burgdorferi* antibody to a 41 kD antigen (probably corresponding to a flagella antigen), and in other cases to the 31 kD and higher molecular weight antigens. Antibodies to the 41 kD antigen may be found in some cases of non-Lyme disease.[11] In our study, such antibodies were absent or barely discernible in the free serum or IC of controls. In contrast these antibodies were present in the free serum of seropositive patients with Lyme disease and in the IC of seronegative patients with Lyme disease. Nevertheless, the possible occurrence of these antibodies in other diseases should be acknowledged and taken into account in interpreting positive findings.

Our study shows that, irrespective of the actual circulating concentration, the complexed antibody can be detected when simple techniques are used to isolate the complexes and dissociate antibody from its target antigen.[9] *Complexed antibodies are likely to signify disease activity, whereas free antibodies alone do not necessarily do so.*

The PEG-ELISA IC technique is simple and not time consuming and could be used as a diagnostic assay if the conventional ELISA is negative in suspected cases. In comparison the cell mediated lymphocyte stimulation assay is laborious and time consuming, and therefore only a few samples can be assayed per week. Selective use of diagnostic assays such as antibody or antigen analysis of IC components may be useful in establishing the diagnosis of Lyme disease in certain seronegative cases, as well as in variably positive cases, and in monitoring disease activity.

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