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1. Int J Med Sci 2013; 10(7):915-931. doi:10.7150/ijms.6273

Lyme Borreliosis in Human Patients in Florida and Georgia, USA

Kerry L. Clark[1,] Brian Leydet[1,2], Shirley Hartman[3]

1. Department of Public Health, University of North Florida, 1 UNF Drive, Jacksonville, Florida USA 32224;
2. Current address: Department of Pathobiological Sciences, Louisiana State University, Baton Rouge, Louisiana USA 70803;
3. Mandarin Wellness Center, Jacksonville, Florida, USA 32257.

ABSTRACT: The aim of this study was to determine the cause of illness in several human patients residing in Florida and Georgia, USA, with suspected Lyme disease based upon EM-like skin lesions and/or symptoms consistent with early localized or late disseminated Lyme borreliosis. Using polymerase chain reaction (PCR) assays developed specifically for Lyme group *Borrelia* spp., followed by DNA sequencing for confirmation, we identified *Borrelia burgdorferi* sensu lato DNA in samples of blood and skin and also in lone star ticks (*Amblyomma americanum*) removed from several patients who either live in or were exposed to ticks in Florida or Georgia. This is the first report to present combined PCR and DNA sequence evidence of infection with Lyme *Borrelia* spp. in human patients in the southern U.S., and to demonstrate that several *B. burgdorferi* sensu lato species may be associated with Lyme disease-like signs and symptoms in southern states. Based on the findings of this study, we suggest that human Lyme borreliosis occurs in Florida and Georgia, and that some cases of Lyme-like illness referred to as southern tick associated rash illness (STARI) in the southern U.S. may be attributable to previously undetected *B. burgdorferi* sensu lato infections.

2. Psychosomatics 2013

Correlates of Perceived Health-Related Quality of Life in Post-treatment Lyme Encephalopathy

Avinash M.Chandra,B.A., John G. Keilp, Ph.D., Brian A. Fallon, M.D.

Columbia University

ABSTRACT: Background – Marked functional impairment has been reported by patients with post-treatment Lyme disease syndrome (PTLDS). Objective: We sought to identify but the clinical features that contribute most strongly to the impaired health status associated with PTLDS. Methods: Enrolled patients had a well-documented history of Lyme disease, prior treatment with at least 3 weeks with intravenous ceftriaxone, a positive IgG Western blot, and objective problems with memory. An index score to capture aggregate cognitive functioning, Short-Form 36 physical and mental component summer scores, and scores on other clinical and

demographic measures were examined. Multiple linear regressions were performed to determine significant predictors of perceptions of impaired life functioning as delineated by theShort-Form36.Results: Fatigue was the most important contributor to perceived impairments in overall physical functioning, and fatigue and depression significantly predicted perceived impairments in overall mental functioning. Conclusions: Because fatigue and depression contribute prominently to reports of impaired physical functioning and mental functioning among patients with PTLDS, clinicians should assess patients for these symptoms and consider targeting these symptoms in the selection of treatment interventions. Future controlled studies should examine the effectiveness of such agents for patients with PTLDS.

3. International Journal of Medical Sciences 2013

Lyme Borreliosis in Human Patients in Florida and Georgia, USA

Kerry L. Clark-1, Brian Leydet-1,2, Shirley Hartman-3

1-University of North Florida, 2-Louisiana State University, 3-Mandarin Wellness Center, Florida

ABSTRACT: The aim of this study was to determine the cause of illness in several human patients residing in Florida and Georgia, USA, with suspected Lyme disease based upon EM-like skin lesions and/or symptoms consistent with early localized or late disseminated Lyme borreliosis. Using polymerase chain reaction (PCR) assays developed specifically for Lyme group Borrelia spp., followed by DNA sequencing for confirmation, we identified Borrelia burgdorferi sensu lato DNA in samples of blood and skin and also in lone star ticks (Amblyomma americanum)

removed from several patients who either live in or were exposed to ticks in Florida or Georgia. This is the first report to present combined PCR and DNA sequence evidence of infection with Lyme Borrelia spp. in human patients in the southern U.S., and to demonstrate that several B. burgdorferi sensu lato species may be associated with Lyme disease-like signs and symptoms in southern states. Based on the findings of this study, we suggest that human Lyme borreliosis occurs in Florida and Georgia, and that some cases of Lyme-like illness referred to as southern tick associated rash illness (STARI) in the southern U.S. may be attributable to previously undetected B. burgdorferi sensu lato infections.

4. 2013 Northeastern Naturalist 20(1):197–204

Distribution of Ticks & Prevalence of *Borrelia burgdorferi* in the Upper Connecticut River Valley of Vermont

Abigail C. Serra-1, Paul S. Warden-2, Colin R. Fricker-2, and Alan R. Giese-1,*

1-Lyndon State College VT, 2-Analytical Services, Inc. VT

ABSTRACT: *Ixodes scapularis* (Black-legged Tick) has expanded its range in recent decades. To establish baseline data on the abundance of the Black-legged Tick and *Borrelia burgdorferi* (the causative agent of Lyme disease) at the edge of a putative range expansion, we collected 1398 ticks from five locations along the Connecticut River in Vermont. Collection locations were approximately evenly distributed between the villages of Ascutney and Guildhall. Relative abundance and distribution by species varied across sites. Black-legged Ticks dominated our collections (*n* = 1348, 96%), followed by *Haemaphysalis leporispalustris* (Rabbit Tick; *n* = 45, 3%), and *Dermacentor variabilis* (American Dog Tick; *n* = 5, <1%).

Black-legged Tick abundance ranged from 6198 ticks per survey hectare (all life stages combined) at the Thetford site to zero at the Guildhall site. There was little to no overlap of tick species across sites. Phenology of Black-legged Ticks matched published information from other regions of the northeastern USA. Prevalence of *B. burgdorferi* in adult Black-legged Ticks was 8.9% (*n* = 112).

5. Microbial Pathogenesis Nov-Dec 2008

Profiling the humoral immune response to Borrelia burgdorferi infection with protein microarrays

Yun Xu, John F. Bruno, Benjamin J. Luft

ABSTRACT: To determine the cell envelope proteins of Borrelia burgdorferi recognized by immune sera of patients with late Lyme disease, we developed a Borrelia microarray containing proteins encoded by 90 cell envelope genes and their homologs described in the annotated genomic sequence of B. burgdorferi, strain B31. The protein microarray was used to profile the humoral immune response using sera from 13 patients with late Lyme disease and four normal controls. Although there was considerable heterogeneity in the individual sera responses, 25 of the cell envelope proteins were recognized by seven or more samples. Sera from non-infected individuals lacked reactivity against any of the proteins on the array. Among the most antigenic envelope proteins, BLAST search revealed little sequence homology to known microbial proteins from other species. The proteins that were highly seropositive included several members of the Erp gene families, BBA24 (decorin binding protein A (DbpA)) and members of the Borrelia gene family Pfam113 that code for the Mlp lipoprotein gene family. Several novel, uncharacterized B. burgdorferi antigens identified in this study were BBA14, BBG23, BB0108, BB0442 and BBQ03. The accurate diagnosis of Lyme disease depends on correlating objective clinical abnormalities with serological evidence of exposure to B. burgdorferi. A protein array of the envelope proteins of Borrelia burgdorferi may be very useful in specifically identifying patients with Lyme disease. This approach could contribute to a more rapid discovery of antigens not expressed in vitro that may be useful for the development of vaccine and diagnostics.

Grant Title: Profiling the humoral response to Borrelia burgdorferi infection with protein microarrays

Presented at LDA Columbia University Lyme & Other Tick-Borne Diseases: Solutions through Cutting Edge Science 2008

Benjamin J Luft PhD Stony Brook University

New Insights from the Borrelia Genome

ABSTRACT: Dr. Luft spoke about studies including studies of different strains of Bb to identify virulence markers, which have investigated gene expression in B. Burgdorferi. He reported that under laboratory conditions around half of the potential 1400 Borrelia proteins are expressed. Conditions such as pH and temperature can be varied and the effects on gene expression can be studied.

7. PLoS ONE 7(10): e48277. doi:10.1371/journal.pone.0048277

Characterization of Biofilm Formation by *Borrelia burgdorferi In Vitro*

Sapi E, Bastian SL, Mpoy CM, Scott S, Rattelle A, et al. (2012)

University of New Haven, CT

ABSTRACT: Borrelia burgdorferi, the causative agent of Lyme disease, has long been known to be capable of forming aggregates and colonies. It was recently demonstrated that Borrelia burgdorferi aggregate formation dramatically changes the in vitro response to hostile environments by this pathogen. In this study, we investigated the hypothesis that these aggregates are indeed biofilms, structures whose resistance to unfavorable conditions are well documented. We studied Borrelia burgdorferi for several known hallmark features of biofilm, including structural rearrangements in the aggregates, variations in development on various substrate matrices and secretion of a protective extracellular polymeric substance (EPS) matrix using several modes of microscopic, cell and molecular biology techniques. The atomic force microscopic results provided evidence that multilevel rearrangements take place at different stages of aggregate development, producing a complex, continuously rearranging structure. Our results

also demonstrated that Borrelia burgdorferi is capable of developing aggregates on different abiotic and biotic substrates, and is also capable of forming floating aggregates. Analyzing the extracellular substance of the aggregates for potential exopolysaccharides revealed the existence of both sulfated and non-sulfated/carboxylated substrates, predominately composed of an alginate with calcium and extracellular DNA present. In summary, we have found substantial evidence that Borrelia burgdorferi is capable of forming biofilm in vitro. Biofilm formation by Borrelia species might play an important role in their survival in diverse environmental conditions by providing refuge to individual cells.

8. The Open Neurology Journal, 2012, 6, (Suppl 1-M2) 79-87

A Reappraisal of the U.S. Clinical Trials of Post-Treatment Lyme Disease Syndrome

Brian A. Fallon*,1, Eva Petkova2, John G. Keilp3 and Carolyn B. Britton4

Columbia University

ABSTRACT: Four federally funded randomized placebo-controlled treatment trials of post-treatment Lyme syndrome in the United States have been conducted. Most international treatment guidelines summarize these trials as having shown no acute or sustained benefit to repeated antibiotic therapy. The goal of this paper is to determine whether this summary conclusion is supported by the evidence.

Methods: The methods and results of the 4 U.S. treatment trials are described and their critiques evaluated.

Results: 2 of the 4 U.S. treatment trials demonstrated efficacy of IV ceftriaxone on primary and/or secondary outcome measures.

Conclusions: Future treatment guidelines should clarify that efficacy of IV ceftriaxone for post-treatment Lyme fatigue was demonstrated in one RCT and supported by a second RCT, but that its use was not recommended primarily due to adverse events stemming from the IV route of treatment. While repeated IV antibiotic therapy can be effective, safer modes of delivery are needed.

9. Northeast Natural History Conference, Syracuse, NY 2012

A Survey of Tick Populations Along the Connecticut River in Vermont

A.C. Serra, A.R. Giese, Lyndon State College (VT)

ABSTRACT (see publication above)

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10. PLOS One 2012

Genome Stability of Lyme Disease Spirochetes: Comparative Genomics of Borrelia burgdorferi PlasmidsLyme disease is the most common tick-borne human illness in North America. In order to understand the molecular pathogenesis, natural diversity, population structure and epizootic spread of the North American Lyme agent, Borrelia burgdorferi sensu stricto, a much better understanding of the natural diversity of its genome will be required. Towards this end we present a comparative analysis of the nucleotide sequences of the numerous plasmids of B. burgdorferi isolates B31, N40, JD1 and 297. These strains were chosen because they include the three most commonly studied laboratory strains, and because they represent different major genetic lineages and so are informative regarding the genetic diversity and evolution of this organism. A unique feature of Borrelia genomes is that they carry a large number of linear and circular plasmids, and this work shows that strains N40, JD1, 297 and B31 carry related but non-identical sets of 16, 20, 19 and 21 plasmids, respectively, that comprise 33–40% of their genomes. We deduce that there are at least 28 plasmid compatibility types among the four strains. The B. burgdorferi ~900 Kbp linear chromosomes are evolutionarily exceptionally stable, except for a short ≤20 Kbp plasmid-like section at the right end. A few of the plasmids, including the linear lp54 and circular cp26, are also very stable. We show here that the other plasmids, especially the linear ones, are considerably more variable. Nearly all of the linear plasmids have undergone one or more substantial inter-plasmid rearrangements since their last common ancestor. In spite of these rearrangements and differences in plasmid contents, the overall gene complement of the different isolates has remained relatively constant.

Sherwood R. Casjens1*, Emmanuel F. Mongodin2, Wei-Gang Qiu3, Benjamin J. Luft4, Steven E. Schutzer5, Eddie B. Gilcrease1, Wai Mun Huang1, Marija Vujadinovic1, John K. Aron1, Levy C. Vargas3, Sam Freeman3, Diana Radune6, Janice F. Weidman6¤a, George I. Dimitrov6¤b, Hoda M. Khouri6¤c, Julia E. Sosa6, Rebecca A. Halpin6, John J. Dunn7, Claire M. Fraser2

1-University of Utah School of Medicine, 2-University of Maryland School of Medicine, 3-Hunter College of the City University of New York, 4-Stony Brook University, NY, 5- New Jersey Medical School, 6-J. Craig Venter Institute, MD, 7-Brookhaven National Laboratory, NY

ABSTRACT: Lyme disease is the most common tick-borne human illness in North America. In order to understand the molecular pathogenesis, natural diversity, population structure and epizootic spread of the North American Lyme agent, Borrelia burgdorferi sensu stricto, a much better understanding of the natural diversity of its genome will be required. Towards this end we present a comparative analysis of the nucleotide sequences of the numerous plasmids of B. burgdorferi isolates B31, N40, JD1 and 297. These strains were chosen because they include the three most commonly studied laboratory strains, and because they represent different major genetic lineages and so are informative regarding the genetic diversity and evolution of this organism. A unique feature of Borrelia genomes is that they carry a large number of linear and circular plasmids, and this work shows that strains N40, JD1, 297 and B31 carry related but non-identical sets of 16, 20, 19 and 21 plasmids, respectively, that comprise 33–40% of their genomes. We deduce that there are at least 28 plasmid compatibility types among the four strains. The B. burgdorferi ~900 Kbp linear chromosomes are evolutionarily exceptionally stable, except for a short ≤20 Kbp plasmid-like section at the right end. A few of the plasmids, including the linear lp54 and circular cp26, are also very stable. We show here that the other plasmids, especially the linear ones, are considerably more variable. Nearly all of the linear plasmids have undergone one or more substantial inter-plasmid rearrangements since their last common ancestor. In spite of these rearrangements and differences in plasmid contents, the overall gene complement of the different isolates has remained relatively constant.

11. Journal of Bacteriology 2011

Whole-Genome Sequences of Thirteen Isolates of *Borrelia burgdorferi*

Steven E. Schutzer1,*, Claire M. Fraser-Liggett2, Sherwood R. Casjens3,*, Wei-Gang Qiu4, John J. Dunn5, Emmanuel F. Mongodin2, and Benjamin J. Luft6

1-University of Medicine and Dentistry of New Jersey–New Jersey Medical School, 2-Institute for Genome Sciences, University of Maryland, School of Medicine, 3-University of Utah Medical School, 4- Hunter College of the City University of New York, 5-Brookhaven National Laboratory, Upton, New York 6-Stony Brook University

ABSTRACT: Borrelia burgdorferi is a causative agent of Lyme disease in North America and Eurasia. The first complete genome sequence of B. burgdorferi strain 31, available for more than a decade, has assisted research on the pathogenesis of Lyme disease. Because a single genome sequence is not sufficient to understand the relationship between genotypic and geographic variation and disease phenotype, we determined the whole-genome sequences of 13 additional B. burgdorferi isolates that span the range of natural variation. These sequences should allow improved understanding of pathogenesis and provide a foundation for novel detection, diagnosis, and prevention strategies.

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12. PLoS ONE 6(2): e17287. doi:10.1371/journal.pone.0017287 2011

Distinct Cerebrospinal Fluid Proteomes Differentiate Post-Treatment Lyme Disease from Chronic Fatigue Syndrome

Steven E. Schutzer-1#*, Thomas E. Angel-4#, Tao Liu-4#, Athena A. Schepmoes-4, Therese R. Clauss-4, Joshua N. Adkins-4, David G. Camp II-4, Bart K. Holland-3, Jonas Bergquist-5, Patricia K. Coyle-6, Richard D. Smith-4, Brian A. Fallon-7, Benjamin H. Natelson-2,8

1-Department of Medicine, University of Medicine and Dentistry of New Jersey-New Jersey Medical School, Newark, New Jersey, United States of America, 2 Department of Neurology, University of Medicine and Dentistry of New Jersey-New Jersey Medical School, Newark, New Jersey, United States of America, 3 Division of Biostatistics and Epidemiology, University of Medicine and Dentistry of New Jersey-New Jersey Medical School, Newark, New Jersey, United States of America, 4 Biological Sciences Division, Pacific Northwest National Laboratory, Richland, Washington, United States of America, 5 Department of Physical and Analytical Chemistry, Uppsala University, Uppsala, Sweden, 6 Department of Neurology, State University of New York-Stony Brook, Stony Brook, New York, United States of America, 7 Department of Psychiatry, Columbia University Medical Center, New York, New York, United States of America, 8 Department of Pain Medicine and Palliative Care and Beth Israel Medical Center, Albert Einstein School of Medicine, Bronx, New York, United States of America

ABSTRACT: Neurologic Post Treatment Lyme disease (nPTLS) and Chronic Fatigue (CFS) are syndromes of unknown etiology. They share features of fatigue and cognitive dysfunction, making it difficult to differentiate them. Unresolved is whether nPTLS is a subset of CFS.

Pooled cerebrospinal fluid (CSF) samples from nPTLS patients, CFS patients, and healthy volunteers were comprehensively analyzed using high-resolution mass

spectrometry (MS), coupled with immunoaffinity depletion methods to reduce protein-masking by abundant proteins. Individual patient and healthy control CSF samples were analyzed directly employing a MS-based label-free quantitative proteomics approach. We found that both groups, and individuals within the groups, could be distinguished from each other and normals based on their specific CSF proteins ($p<0.01$). CFS ($n = 43$) had 2,783 non-redundant proteins, nPTLS ($n = 25$) contained 2,768 proteins, and healthy normals had 2,630 proteins. Preliminary pathway analysis demonstrated that the data could be useful for hypothesis generation on the pathogenetic mechanisms underlying these two related syndromes. nPTLS and CFS have distinguishing CSF protein complements. Each condition has a number of CSF proteins that can be useful in providing candidates for future validation studies and insights on the respective mechanisms of pathogenesis. Distinguishing nPTLS and CFS permits more focused study of each condition, and can lead to novel diagnostics and therapeutic interventions.

13. Genetics: Published Articles Ahead of Print, published on September 2, 2011 as 10.1534/genetics.111.130773 Copyright 2011

Pervasive Recombination and Sympatric Genome Diversification Driven by Frequency-Dependent Selection in Borrelia burgdorferi, the Lyme disease Bacterium

James Haven-1,†, Levy C. Vargas2, Emmanuel F. Mongodin-3, Vincent Xue-4, Yozen Hernandez-2, Pedro Pagan-2, Claire M. Fraser-Liggett-3, Steven E. Schutzer-5, Benjamin J. Luft-6, Sherwood R. Casjens-7, and Wei-Gang Qiu-1,2,8

ABSTRACT: How genomic diversity within bacterial populations originates and is maintained in thepresence of frequent recombination is a central problem in understanding bacterial evolution. Natural populations of Borrelia burgdorferi, the bacterial agent of Lyme disease, consist of diverse genomic groups co-

infecting single individual vertebrate hosts and tick vectors. To understand mechanisms of sympatric genome differentiation in B. burgdorferi, we sequenced and compared 23 genomes representing major genomic groups in North America and Europe. Linkage analysis of over 13,500 single nucleotide polymorphisms revealed pervasive horizontal DNA exchanges. Although three times more frequent than point mutation, recombination is localized and weakly affects genome-wide linkage disequilibrium. We show by computer simulations that, while enhancing population fitness, recombination constrains neutral and adaptive divergence among sympatric genomes through periodic selective sweeps. In contrast, simulations of frequency-dependent selection with recombination produced the observed pattern of a large number of sympatric genomic groups associated with major sequence variations at the selected locus. We conclude that negative frequency-dependent selection targeting a small number of surface-antigen loci (ospC in particular) sufficiently explains the maintenance of sympatric genome diversity in B. burgdorferi without adaptive divergence. In fact, pervasive recombination makes it unlikely for local B. burgdorferi genomic groups to achieve host specialization. B. burgdorferi genomic groups in northeastern United States are thus best viewed as constituting a single bacterial species, whose generalist nature is a key to its rapid spread and

human virulence.

14. Journal of Medical Entomology 47(1):89-94. 2010

Extraction of Total Nucleic Acids from Ticks for the Detection of Bacterial & Viral Pathogens

Chris D. Crowder-1, Megan A. Rounds-1, Curtis A. Phillipson-1, John M. Picuri-1, Heather E. Matthews-1, Justina Halverson-1, Steven E. Schutzer-2, David J. Ecker-1, and Mark W. Eshoo-1

1 -Ibis Biosciences, 1896 Rutherford Road, Carlsbad, CA 92008 (e-mail: meshoo@ibisbio.com), 2 -University of Medicine and Dentistry of New Jersey, Dept. of Medicine, 185 South Orange Ave., Newark, NJ 07103.

ABSTRACT: Ticks harbor numerous bacterial, protozoal, and viral pathogens that can cause serious infections in humans and domestic animals. Active surveillance of the tick vector can provide insight into the frequency and distribution of important pathogens in the environment. Nucleic-acid based detection of tick-borne bacterial, protozoan, and viral pathogens requires the extraction of both DNA and RNA (total nucleic acids) from ticks. Traditional methods for nucleic acid extraction are limited to extraction of either DNA or the RNA from a sample. Here we present a simple bead-beating based protocol for extraction of DNA and RNA from a single tick and show detection of Borrelia burgdorferi and Powassan virus from individual, infected Ixodes scapularis ticks. We determined expected yields for total nucleic acids by this protocol for a variety of adult tick species. The method is applicable to a variety of arthropod vectors, including fleas and mosquitoes, and was partially automated on a liquid handling robot.

15. PLoS ONE 5(5) 2010

Genotypic variation and Mixtures of Lyme Borrelia in Ixodes Ticks from North America and Europe

Chris Crowder-1, Heather Mathews -1, Steven Schutzer-2 et al

1 -Ibis Biosciences, 1896 Rutherford Road, Carlsbad, CA 92008 (e-mail:

meshoo@ibisbio.com), 2 -University of Medicine and Dentistry of New Jersey, Dept. of Medicine, 185 South Orange Ave., Newark, NJ 07103.

ABSTRACT: Lyme disease, caused by various species of Borrelia, is transmitted by Ixodes ticks in North America and Europe. Studies have shown the genotype of Borrelia burgdorferi sensu stricto (s.s.) or the species of B. burgdorferi sensu lato (s.l.) affects the ability of the bacteria to cause local or disseminated infection in humans.

Methodology/Principal Findings: We used a multilocus PCR electrospray mass spectrometry assay to determine the species and genotype Borrelia from ticks collected in New York, Connecticut, Indiana, Southern Germany, and California and characterized isolates from parts of the United States and Europe. These analyses identified 53 distinct genotypes of B. burgdorferi sensu stricto with higher resolution than ospC typing. Genotypes of other members of the B. burgdorferi sensu lato complex were also identified and genotyped including B. afzelii, B. garinii, B. lusitaniae, B. spielmanii, and B. valaisiana. While each site in North America had genotypes unique to that location, we found genotypes shared between individual regions and two genotypes found across the United States. Significant B. burgdorferi s.s. genotypic diversity was observed between North America and Europe: only 6.6% of US genotypes (3 of 45) were found in Europe and 27% of the European genotypes (3 of 11) were observed in the US. Interestingly, 39% of adult Ixodes scapularis ticks from North America were infected with more than one genotype of B. burgdorferi s.s. and 22.2% of Ixodes ricinus ticks from Germany were infected with more than one genotype of B. burgdorferi s.l.

Conclusions/Significance: The presence of multiple Borrelia genotypes in ticks increases the probability that a person will be infected with more than one genotype of B. burgdorferi, potentially increasing the risks of disseminated Lyme disease. Our study indicates that the genotypic diversity of Borrelia in ticks in both North America and Europe is higher then previously reported and can have potential clinical consequences.

16. Kerry Clark, PHD University of N. Florida

Investigations of Human Borreliosis in the Southern US

Presented at 2009 Columbia University/LDA national Lyme & Tick-Borne Diseases: 34 Years From Lyme Connecticut Across the Nation scientific conference and at Jacksonville State University Spring 2010 conference.

ABSTRACT: This study address the hypothesis that lone star ticks (*Amblyomma americanum*), in addition to blacklegged ticks (*Ixodes scapularis*), serve as vectors of Bbsl to humans. The study results are expected to confirm a previously unrecognized genetic group of Bbsl as the cause of a significant portion of LB cases in the U.S., to estimate the rate of infection in ticks biting humans in the southern U.S., to provide evidence of the tick species responsible for transmitting Lyme *Borrelia* to humans in southern states, and to provide improved methods for DNA testing and identification of Lyme Borrelia in human patient samples.

History: Dr. Clark's research is focused on the ecology and epidemiology of Lyme disease and other tick-borne diseases in the southern U.S. He collaborated with investigators at Georgia Southern University in several studies, including those leading to the first isolations and characterizations of *B. burgdorferi* in South Carolina. Dr. Clark and colleagues have documented the presence of several Lyme *Borrelia* species infecting small mammals, ticks, and lizards in Florida and South Carolina. He was the first to ever report finding Lyme disease spirochetes infecting wild reptiles. More recently, he has focused his investigative efforts on the cause of Lyme-like illness in humans in the southern U.S.

Objectives: The primary objectives of his research and service activities are the following: (1) to learn more about the ecology and epidemiology of Lyme and other tick-borne diseases affecting humans in the U.S.; (2) to improve early detection and diagnosis by developing better diagnostic tests; and (3) to educate clinicians, public health personnel and the general public about the presence, identification, and prevention of tick-borne infections.

17. Neurobiology of Disease 2010

Inflammation and central nervous system Lyme disease.

Brian Fallon, MD, David Hardesty, MD et al

Columbia University

ABSTRACT: Neurologic manifestations of Lyme disease occur in 10-15% of individuals with untreated Lyme. This paper discusses the symptoms of neurologic Lyme and reviews experimental studies that provide insight into the possible mechanisms of inflammation following ***Borrelia*** infection and contributing risk factors.

18. Arch Gen Psychiatry. 2009;66(5):554-563.

Regional Cerebral Blood Flow and Metabolic Rate in Persistent Lyme Encephalopathy

Brian A. Fallon, MD; Richard B. Lipkin, BA; Kathy M. Corbera, MD; Shan Yu, PhD; Mitchell S. Nobler, MD; John G. Keilp, PhD; Eva Petkova, PhD; Sarah H. Lisanby, MD; James R. Moeller, PhD; Iordan Slavov, PhD; Ronald Van Heertum, MD; Brett D. Mensh, MD, PhD; Harold A. Sackeim, PhD

Columbia University

ABSTRACT

Main Outcome Measures:

Patients with persistent Lyme encephalopathy were compared with age-, sex-, and education-matched controls. Fully quantified assessments of rCBF and rCMR for glucose were obtained while subjects were medication-free using positron emission tomography. The CBF was assessed in 2 resting room air conditions (without snorkel and with snorkel) and 1 challenge condition (room air enhanced with carbon dioxide, ie, hypercapnia).

Results:

Statistical parametric mapping analyses revealed regional abnormalities in all rCBF and rCMR measurements that were consistent in location across imaging methods and primarily reflected hypoactivity. Deficits were noted in bilateral gray and white matter regions, primarily in the temporal, parietal, and limbic areas. Although diminished global hypercapnic CBF reactivity ($P < .02$) was suggestive of a component of vascular compromise, the close coupling between CBF and CMR suggests that the regional abnormalities are primarily metabolically driven. Patients did not differ from controls on global resting CBF and CMR measurements.

Conclusions:

Patients with persistent Lyme encephalopathy have objectively quantifiable topographic abnormalities in functional brain activity. These CBF and CMR reductions were observed in all measurement conditions. Future research should address whether this pattern is also seen in acute neurologic Lyme disease.

19. Gene 2009

Fast, adaptive evolution at a bacterial host-resistance locus: The PFam54 gene array in Borrelia burgdorferi"

*Wywial E. Haven J, Casjens SR, Hernandez YA, Singh S, Mongodin EF, Fraser-Liggett CM, Luft BJ, Schutzer SE, Qiu WG.

ABSTRACT: Microbial pathogens have evolved sophisticated mechanisms for evasion of host innate and adaptive immunities. PFam54 is the largest paralogous gene family in the genomes of Borrelia burgdorferi, the Lyme disease bacterium. One member of PFam54, the complement-regulator acquiring surface proteins 1 (BbCrasp-1), is able to abort the alternative pathway of complement activation via binding human complement-regulator factor H (FH). The gene coding for BbCRASP-1 exists in a tandem array of PFam54 genes in the B. burgdorferi genome, a result apparently of repeated gene duplications. To help elucidate the functions of the large number of PFam54 genes, we performed phylogenomic and structural analyses of the PFam54 gene array from ten B. burgdorferi genomes. Analyses based on gene tree, genome synteny, and structural models revealed rapid adaptive evolution of this array through gene duplication, gene loss, and functional diversification. Individual PFam54 genes, however, do not show high intra-population sequence polymorphisms as genes providing evasion from adaptive immunity generally do. PFam54 members able to bind human FH are not monophyletic, suggesting that human FH affinity, however strong, is an incidental rather than

main function of these PFam54 proteins. The large number of PFam54 genes existing in any single B. burgdorferi genome may target different innate-immunity proteins of a single host species or the same immune protein of a variety of host species. Genetic variability of the PFam54 gene array suggests that universally present PFam54 lineages such as BBA64, BBA65, BBA66, and BBA73 may be better candidates for the development of broad-spectrum vaccines or drugs than strain-restricted lineages such as BbCRASP-1.

20. Emerging Infectious Diseases Volume 14, Number 7—July 2008

Wide Distribution of a High-Virulence *Borrelia burgdorferi* Clone in Europe & North America.

Wei-Gang Qiu,* John F. Bruno,† William D. McCaig,* Yun Xu,† Ian Livey,‡ Martin E. Schriefer,§ and Benjamin J. Luft†

Hunter College of the City University of New York, New York, New York, USA; †Stony Brook University, Stony Brook, New York, USA; ‡Baxter Innovations GmBH, Orth/Donau, Austria; and §Centers for Disease Control and Prevention, Fort Collins, Colorado, USA

ABSTRACT: The A and B clones of Borrelia burgdorferi sensu stricto, distinguished by outer surface protein C (ospC) gene sequences, are commonly associated with disseminated Lyme disease. To resolve phylogenetic relationships among isolates, we sequenced 68 isolates from Europe and North America at 1 chromosomal locus (16S–23S ribosomal RNA spacer) and 3 plasmid loci (ospC,dbpA, and BBD14). The ospC-A clone appeared to be highly prevalent on both continents, and isolates of this clone were uniform in DNA sequences, which suggests a recent trans-oceanic migration. The genetic homogeneity of ospC-A isolates was confirmed by sequences at 6 additional chromosomal housekeeping loci (gap, alr, glpA, xylB, ackA, and tgt). In contrast, the ospC-B group consists of genotypes distinct to each continent, indicating geographic isolation. We conclude that the ospC-A clone has dispersed rapidly and widely in the recent past. The spread of the ospC-A clone

may have contributed, and likely continues to contribute, to the rise of Lyme disease incidence.

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21. Neurology May 2008

A randomized, placebo-controlled trial of repeated IV antibiotic therapy for Lyme encephalopathy

B. A. Fallon, MD, 2. J. G. Keilp, PhD, 3 K. M. Corbera, MD, 4. E. Petkova, PhD, 5. C. B. Britton, MD, 6. E. Dwyer, MD, 7. I. Slavov, PhD, 8. J. Cheng, MD, PhD, 9. J. Dobkin, MD, 10. D. R. Nelson, PhD and 11. H. A. Sackeim, PhD

From the Department of Psychiatry (B.A.F., J.G.K., K.M.C., E.P., I.S., J.C., H.A.S.), Department of Biostatistics (E.P.), Department of Neurology (C.B.B.), Department of Medicine (E.D., J.D.), and New York State Psychiatric Institute (B.A.F., J.G.K., K.M.C., E.P., I.S., J.C., H.A.S.), Columbia University, New York; and Department of Cell and Molecular Biology, University of Rhode Island, Kingston (D.R.N.).

ABSTRACT

Background: Optimal treatment remains uncertain for patients with cognitive impairment that persists or returns after standard IV antibiotic therapy for Lyme disease.

Methods: Patients had well-documented Lyme disease, with at least 3 weeks of prior IV antibiotics, current positive IgG Western blot, and objective memory impairment. Healthy individuals served as controls for practice effects. Patients were randomly assigned to 10 weeks of double-masked treatment with IV ceftriaxone

or IV placebo and then no antibiotic therapy. The primary outcome was neurocognitive performance at week 12—specifically, memory. Durability of benefit was evaluated at week 24. Group differences were estimated according to longitudinal mixed-effects models.

Results: After screening 3368 patients and 305 volunteers, 37 patients and 20 healthy individuals enrolled. Enrolled patients had mild to moderate cognitive impairment and marked levels of fatigue, pain, and impaired physical functioning. Across six cognitive domains, a significant treatment-by-time interaction favored the antibiotic-treated group at week 12. The improvement was generalized (not specific to domain) and moderate in magnitude, but it was not sustained to week 24. On secondary outcome, patients with more severe fatigue, pain, and impaired physical functioning who received antibiotics were improved at week 12, and this was sustained to week 24 for pain and physical functioning. Adverse events from either the study medication or the PICC line were noted among 6 of 23 (26.1%) patients given IV ceftriaxone and among 1 of 14 (7.1%) patients given IV placebo; these resolved without permanent injury.

Conclusion: IV ceftriaxone therapy results in short-term cognitive improvement for patients with posttreatment Lyme encephalopathy, but relapse in cognition occurs after the antibiotic is discontinued. Treatment strategies that result in sustained cognitive improvement are needed.

22. Presented at LDA-Columbia University Lyme & Other Tick-Borne Diseases: Solutions through Cutting Edge Science 2008

Profiling the humoral response to Borrelia burgdorferi infection with protein microarrays

Benjamin J Luft, et al Stony Brook University NY

New Insights from the Borrelia Genome

ABSTRACT: Dr. Luft spoke about studies including studies of different strains of Bb to identify virulence markers, which have investigated gene expression in B. Burgdorferi. He reported that under laboratory conditions around half of the potential 1400 Borrelia proteins are expressed. Conditions such as pH and temperature can be varied and the effects on gene expression can be studied.

23. Infection and Immunity, January 2006

Identification of Borrelia burgdorferi outer surface proteins

Brooks CS; Vuppala SR; Jett AM; Akins DR Department of Microbiology and Immunology.

The University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104, USA,

ABSTRACT: Several Borrelia burgdorferi outer surface proteins have been identified over the past decade that are up-regulated by temperature- and/or mammalian host-specific signals as this spirochete is transmitted from ticks to mammals. Given the potential role(s) that these differentially up-regulated proteins may play in B. burgdorferi transmission and Lyme disease pathogenesis, much attention has recently been placed on identifying additional borrelial outer surface proteins. To identify uncharacterized B. burgdorferi outer surface proteins, we previously performed a comprehensive gene expression profiling analysis of temperature-shifted and mammalian host-adapted B. burgdorferi. The

combined microarray analyses revealed that many genes encoding known and putative outer surface proteins are down-regulated in mammalian host-adapted B. burgdorferi. At the same time, however, several different genes encoding putative outer surface proteins were found to be up-regulated during the transmission and infection process. Among the putative outer surface proteins identified, biochemical and surface localization analyses confirmed that seven (Bb0405, Bb0689, BbA36, BbA64, BbA66, BbA69, and BbI42) are localized to the surface of B. burgdorferi. Furthermore, enzyme-linked immunosorbent assay analysis using serum from tick-infested baboons indicated that all seven outer surface proteins identified are immunogenic and that antibodies are generated against all seven during a natural infection. Specific antibodies generated against all seven of these surface proteins were found to be bactericidal against B. burgdorferi, indicating that these newly identified outer surface proteins are prime candidates for analysis as second-generation Lyme disease vaccinogens.

24. J Int Neuropsychol Soc. 2006 Jan;12(1):119-29

WAIS-III and WMS-III performance in chronic Lyme disease

Keilp JG, Corbera K, Slavov I, Taylor MJ, Sackeim HA, Fallon BA.

Columbia University College of Physicians and Surgeons, Department of Psychiatry, NY, NY. New York State Psychiatric Institute, Department of Neuroscience, NY, NY.

ABSTRACT: There is controversy regarding the nature and degree of intellectual and memory deficits in chronic Lyme disease. In this study, 81 participants with rigorously diagnosed chronic Lyme disease were administered the newest revisions of the Wechsler Adult Intelligence Scale (WAIS-III) and Wechsler Memory Scale

(WMS-III), and compared to 39 nonpatients. On the WAIS-III, Lyme disease participants had poorer Full Scale and Performance IQ's. At the subtest level, differences were restricted to Information and the Processing Speed subtests. On the WMS-III, Lyme disease participants performed more poorly on Auditory Immediate, Immediate, Auditory Delayed, Auditory Recognition Delayed, and General Memory indices. Among WMS-III subtests, however, differences were restricted to Logical Memory (immediate and delayed) and Family Pictures (delayed only), a Visual Memory subtest. Discriminant analyses suggest deficits in chronic Lyme are best characterized as a combination of memory difficulty and diminished processing speed. Deficits were modest, between one-third and two-thirds of a standard deviation, consistent with earlier studies. Depression severity had a weak relationship to processing speed, but little other association to test performance. Deficits in chronic Lyme disease are consistent with a subtle neuropathological process affecting multiple performance tasks, although further work is needed to definitively rule out nonspecific illness effects. (JINS, 2006, 12, 119-129.).

25. Daniel Cameron, MD. Presented to the 6th Annual Lyme & Other Tick-Borne Diseases: Emerging Tick-Borne Diseases Conference on October 28, 2005 in Philadelphia Pennsylvania

Results from Lyme Disease Clinical Treatment Trial

Lyme Disease Association and Columbia University, conference co-sponsors.

CONFERENCE ABSTRACT

Methods: Data were obtained from a randomized, double-blind placebo-controlled study of patients with recurrent Lyme disease. Patients received either

amoxicillin 500mg. 3 times/day or placebo for 3 months. The Short Form-36 Health Survey, administered at baseline and at the conclusion, provided a Mental Component Summary (MCS) and a Physical Component Summary (PCS) for HRQOL. Baseline HRQOL scores were compared with the general US and chronically-ill populations. Patients with Lyme disease were divided into the lowest, moderate, and higher initial quality of life as measured by SF-36.

Results: The quality of life of Lyme disease was significantly lower than the US norm and chronically-ill patients on all SF-36 scales. Compared with patients who received placebo, patients treated with amoxicillin showed greater improvement on SF-36 physical function, general health perception, vitality, social function, and emotional health. Lyme disease patient presenting with the best initial quality of life had the highest success rate.

Conclusion: Recurrent Lyme disease severely impairs quality of life. Retreatment is effective.

26. Journal of Clinical Microbiology, February 2005

Evidence of Borrelia Autoimmunity-Induced Component of Lyme Carditis and Arthritis.

Elizabeth S. Raveche,[1] Steven E. Schutzer,[1*] Helen Fernandes,[1] Helen Bateman,[1] Brian A. McCarthy,[1] Steven P. Nickell,[2] and Madeleine W. Cunningham[3].

Departments of Pathology and Medicine, New Jersey Medical School, University of Medicine and Dentistry of New Jersey, Newark, New Jersey,1 Department of Molecular Genetics and Microbiology, University of New Mexico, Albuquerque, New Mexico, 2 University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma

3.

ABSTRACT: We investigated the possibility that manifestations of Lyme disease in certain hosts, such as arthritis and carditis, may be autoimmunity mediated due to molecular mimicry between the bacterium *Borrelia burgdorferi* and self-components. We first compared amino acid sequences of *Streptococcus pyogenes* M protein, a known inducer of antibodies that are cross-reactive with myosin, and *B. burgdorferi* and found significant homologies with OspA protein. We found that *S. pyogenes* M5-specific antibodies and sera from *B. burgdorferi*-infected mice reacted with both myosin and *B. burgdorferi* proteins by Western blots and enzyme-linked immunosorbent assay. To investigate the relationship between self-reactivity and the response to *B. burgdorferi*, NZB mice, models of autoimmunity, were infected. NZB mice infected with *B. burgdorferi* developed higher degrees of joint swelling and higher anti-*B. burgdorferi* immunoglobulin M cross-reactive responses than other strains with identical major histocompatibility complex (DBA/2 and BALB/c). These studies reveal immunological cross-reactivity and suggest that *B. burgdorferi* may share common epitopes which mimic self-proteins. These implications could be important for certain autoimmunity-susceptible individuals or animals that become infected with *B. burgdorferi*.

27. Expert Review Anti-Infective Therapy 2004

International Lyme & Associated Diseases Society Lyme Disease Treatment Guidelines

ILADS Working Group: Dan Cameron, MD, MPH; Andrea Gaito, MD; Nick Harris, PhD; Gregory Bach, DO; Sandra Bellovin, MD; Kenneth Bock, MD; Steven Bock, MD; Joseph Burrascano, MD; Constance Dickey, RN; Richard Horowitz; Steven Phillips, MD; Lawrence Meer-Scherrer MD; Bernard Raxlen, MD; Virginia Sher, MD; Harold Smith, MD; Pat Smith [President Lyme Disease Association]; Ray Stricker, MD

ABSTRACT: Evidenced Based Guidelines for the Management of Lyme disease

28. Proceedings of the National Academy of Science, Sept. 2004

Genetic exchange and plasmid transfers in Borrelia burgdorferi sensu stricto revealed by three-way genome comparisons and multilocus sequence typing.

Wei-Gang Qiu [*,], Steven E. Schutzer, John F. Bruno, Oliver Attie [*], Yun Xu, John J. Dunn [¶], Claire M. Fraser [||], Sherwood R. Casjens [**] and Benjamin J. Luft

ABSTRACT: Comparative genomics of closely related bacterial isolates is a powerful method for uncovering virulence and other important genome elements. We determined draft sequences (8-fold coverage) of the genomes of strains JD1 and N40 of *Borrelia burgdorferi sensu stricto*, the causative agent of Lyme disease, and we compared the predicted genes from the two genomes with those from the previously sequenced B31 genome. The three genomes are closely related and are evolutionarily approximately equidistant (0.5% pairwise nucleotide differences on the main chromosome). We used a Poisson model of nucleotide substitution to screen for genes with elevated levels of nucleotide polymorphisms. The three-way genome comparison allowed distinction between polymorphisms introduced by mutations and those introduced by recombination using the method of phylogenetic

partitioning. Tests for recombination suggested that patches of high-density nucleotide polymorphisms on the chromosome and plasmids arise by DNA exchange. The role of recombination as the main mechanism driving *B. burgdorferi* diversification was confirmed by multilocus sequence typing of 18 clinical isolates at 18 polymorphic loci. A strong linkage between the multilocus sequence genotypes and the major alleles of outer-surface protein C (*ospC*) suggested that balancing selection at *ospC* is a dominant force maintaining *B. burgdorferi* diversity in local populations. We conclude that *B. burgdorferi* undergoes genome-wide genetic exchange, including plasmid transfers, and previous reports of its clonality are artifacts from the use of geographically and ecological isolated samples. Frequent recombination implies a potential for rapid adaptive evolution and a possible polygenic basis of *B. burgdorferi* pathogenicity.

29. The Journal of Neuropsychiatry & Clinical Neurosciences. 2002

Regional Cerebral Blood Flow and Cognitive Deficits in Chronic Lyme Disease.

Brian A. Fallon, M.D., John Keilp, Ph.D., Isak Prohovnik, Ph.D., Ronald Van Heertum, M.D. and J. John Mann, M.D.

From the Lyme Disease Research Program, The NYS Psychiatric Institute, New York, New York.

ABSTRACT: This study examined brain functioning in patients with Lyme encephalopathy. Eleven patients underwent neuropsychological tests and Xenon[133]-

regional cerebral blood flow (rCBF) studies, using an external detector system. Each rCBF scan was age- and sex-matched to two archival, normal controls. While few differences were noted on gray-matter flow indices (ISI, fg), Lyme patients demonstrated significant flow reductions in white matter index (k_2) (p=.004), particularly in the posterior temporal and parietal lobes bilaterally (p=.003). Flow reductions in white matter areas were significantly associated with deficits in memory (r=.66, p=.027) and visuospatial organization (r=.62, p=.041). Results suggest that Lyme encephalopathy may be a disease primarily affecting the cerebral white matter.

30. Journal of Spirochetal and Tick-borne Diseases. Spring/Summer 2002

Borrelia burgdorferi Persists in the Gastrointestinal Tract of Children and Adolescents with Lyme Disease."

Martin Fried, MD; Dorothy Pietrucha, MD, et al.

ABSTRACT: This study documents the persistence of B burgdorferi DNA in the gastrointestinal tract of pediatric patients who have already been treated with antibiotics for Lyme disease. Ten consecutive patients between the ages of 9 and 13 years presented with an erythema migrans (EM) rash, a positive western blot for Lyme disease, chronic abdominal pain, heartburn, or bright red blood in the stool. Endoscopy assessed the gastrointestinal (GI) mucosa for inflammation and biopsies were examined for B burgdorferi using a Dieterle stain and with polymerase chain reaction (PCR) to the outer surface protein A (Osp A) of B burgdorferi. As controls, 10 consecutive patients with chronic abdominal pain

were also tested by GI biopsies and with PCR. B burgdorferi persisted in the GI tract in all 10 patients with Lyme disease as shown by Dieterle stain of biopsies and with PCR. None of the control subjects' biopsies were PCR positive for B. burgdorferi. Chronic gastritis, chronic duodenitis, and chronic colitis were found in Lyme disease patients and associated with the detection of B burgdorferi DNA in the GI tract despite prior antibiotic treatments. We have concluded that the DNA of B burgdorferi persisted in patients with Lyme disease even after antibiotic treatment.

31. Journal of Neuropsychiatry and Clinical Neurosciences. 2001 13:500-5-7

A Controlled Study of Cognitive Deficits in Children with Chronic Lyme Disease.

Felice A Tager, PhD, Brian A Fallon, MD.

Columbia University

ABSTRACT: Although neurologic Lyme disease is known to cause cognitive dysfunction in adults, little is known about its long-term sequelae in children. Twenty children with a history of new-onset cognitive complaints after Lyme disease were compared with 20 matched healthy control subjects. Each child was assessed with measures of cognition and psychopathology. Children with Lyme disease had significantly more cognitive and psychiatric disturbances. Cognitive deficits were still found after controlling for anxiety, depression, and fatigue. Lyme disease in children may be accompanied by long-term neuropsychiatric disturbances, resulting in psychosocial and academic impairments. Areas for further study are discussed.

32. A poster presentation by at the 14th International Conference on Lyme Disease & Other Tick-Borne Disorders in Farmington, Connecticut on April 21-23, 2001.

Recovery of Lyme Spirochetes in Semen Samples of Previously Diagnosed Patients

Gregory Bach, DO, PC.

ABSTRACT: The findings were that 43% of the males tested carried evidence of the Lyme disease bacterium in semen by PCR DNA testing.

Dr. Bach also presented results of the study at the American Psychiatric Association meeting in November 2000.

33. Published in Medscape Infectious Diseases April 2000

Preliminary in Vitro and in Vivo Findings of Hyperbaric Oxygen Treatment in Experimental Bb Infection."

Charles Pavia, PhD, et al

NY Medical College School of Medicine. 13th International Scientific Conference on Lyme Disease & Other Tick-borne Disorders. CONFERENCE PRESENTATION

ABSTRACT: In these studies, we evaluated repeated HBOT for its ability to kill Bb in vitro, and in vivo, in a murine model of Lyme disease. Several North American tick-derived and recently obtained patient isolates were studied separately in our assay systems. To test for in vitro susceptibility, one-half to one million Bb were cultured in a small volume (0.1 – 0.2 ml) of BSK media using small snap-cap test tubes. With the caps removed, these cultures were then exposed, for one hour (twice daily for 2 consecutive days), to pure, filtered oxygen pressurized to 2-3 times normal atmospheric conditions. This was achieved using a specially constructed, miniaturized cylindrical chamber (length = 12 inches; diameter = 8 inches), equipped to accept any pressurized gas mixture through its portal opening. After the final HBOT, all cultures received an additional 0.5 ml of BSK media (making the final volume now 0.6 – 0.7 ml), and their caps were snapped shut. Matching control cultures received no HBOT. All cultures were incubated at 33° C for 2-3 days and were examined microscopically for live Bb. Our results showed that 14 of 17 strains of Bb had their growth inhibited by 33-94%, while there was little or no inhibition of 3 Bb strains. For the in vivo studies, separate groups of C3H or CO1 mice were infected intradermally with 100,000 Bb. Two to 4 weeks later, one group of infected mice received two, 1.0-1.5 hour HBO exposures, for two consecutive or alternating days. The treated mice were sacrificed one day after the last treatment, and extract cultures of their urinary bladders were prepared in BSK media. It was found that no Bb grew out of 80% of these extract cultures, whereas live Bb organisms were recoverable from 90% of extract cultures prepared from matched, infected control mice not treated with HBO. These data suggest that HBOT may be considered as a clinically useful form of adjunct therapy in the treatment of Lyme disease.

Repeated Antibiotic Treatment in Chronic Lyme Disease.

Brian Fallon, MD, et al

Columbia University

ABSTRACT: Patients with chronic Lyme disease who experience persistent cognitive deficits despite having received the recommended antibiotic treatment pose a therapeutic dilemma. This pilot study was designed to assess whether additional antibiotic therapy is beneficial.

Enrolled in the study were 23 patients with complaints of persistent memory problems who had previously received 4-16 weeks of intravenous antibiotic therapy. Patients were tested at baseline and 4 months later. During this interval, the private physician determined treatment (intravenous, intramuscular, oral, or none). Assessments included standardized measures of cognition, depression, anxiety, and functional status.

Between times 1 and 2, 5 patients were given no antibiotics and 18 were given additional antibiotics: 7 intravenously, 4 intramuscularly, and 7 orally. At time 1, there were no statistically significant group differences in cognition, depression, or anxiety between those who later received antibiotics and those who didn’t. At time 1, the 23 patients were also functionally disabled. At time 2, compared with patients who received no antibiotics, patients given antibiotics scored better on overall and individual measures of cognition. Patients given intravenous antibiotics showed the greatest functional improvement (pain, physical functioning, energy) and the most cognitive improvement, even when controlling for baseline differences in cognition between the treatment groups. Patients who did not have a reactive Western blot currently or historically were just as likely to improve cognitively as patients with reactive Western blot

results.

This uncontrolled study suggests that repeated antibiotic treatment can be beneficial, even among patients who have been previously treated and even among patients who are currently Western blot negative, with the intravenous route of treatment being the most effective. A double-blind placebo-controlled study is needed to confirm these results.

35. JAMA, Nov. 24, 1999, Vol.282, No.20

Borrelia Burgdorferi–Specific Immune Complexes in Acute Lyme Disease.

Steven Schutzer, et al UMDNJ.

ABSTRACT: Context Diagnosis of infection with Borrelia burgdorferi, the cause of Lyme disease (LD), has been impeded by the lack of effective assays to detect active infection.

Objective: To determine whether B burgdorferi specific immune complexes are detectable during active infection in LD.

Design, Setting, and Patients: Cross-sectional analysis of serum samples from 168 patients fulfilling Centers for Disease Control and Prevention surveillance criteria for LD and 145 healthy and other disease controls conducted over 8 years. Tests were performed blinded.

Main Outcome Measure Detection of B burgdorferi immune complexes by enzyme-linked immunosorbent assay and Western blot.

Results: The B burgdorferi immune complexes were found in 25 of 26 patients with early seronegative erythema migrans (EM) LD; 105 of 107 patients with

seropositive EM LD; 6 of 10 patients who were seronegative with culture-positive EM; 0 of 12 patients who were treated and recovered from LD; and 13 of 13 patients with neurologic LD without EM. Among 147 controls, B burgdorferi immune complex was found in 0 of 50 healthy individuals; 0 of 40 patients with persistent fatigue; 0 of 7 individuals with frequent tick exposure; and 2 of 50 patients with other diseases.

Conclusion: These data suggest that B burgdorferi immune complex formation is a common process in active LD. Analysis of the B burgdorferi immune complexes by a simple technique has the potential to support or exclude a diagnosis of early as well as active LD infection

Funding/Support: This work was supported in part by grants A41518, NS34092, AI31561, and AR40470 from the National Institutes of Health and grant U50/CCU206582 from the Centers for Disease Control and Prevention, and by the Lyme Disease Association of New Jersey.

Neurology, Oct 12, 1999

Absence of Borrelia Burgdorferi-specific immune complexes in chronic fatigue syndrome.”

Steven Schutzer, et al UMDNJ.

ABSTRACT: Chronic fatigue syndrome (CFS) and Lyme disease often share clinical features, especially fatigue, contributing to concern that Borrelia burgdorferi (Bb), the cause of Lyme disease, may underlie CFS symptoms. We examined 39 CFS patients and 40 healthy controls with a Bb immune complex test. Patients and controls were nonreactive. Centers for Disease Control and Prevention-defined CFS patients lacking antecedent signs of Lyme disease—erythema migrans, Bell’s palsy, or large joint arthritis—are not likely to have laboratory evidence of Bb

infection.

36. Infection. 1998 Nov-Dec:26(6):364-7

A proposal for the reliable culture of Borrelia burgdorferi from patients with chronic Lyme disease, even from those previously aggressively treated.

Phillips SE, Mattman LH, Hulinska D, Moayad H.

Greenwich Hospital, CT 06830, USA

ABSTRACT: Since culture of Borrelia burgdorferi from patients with chronic Lyme disease has been an extraordinarily rare event, clarification of the nature of the illness and proving its etiology as infectious have been difficult. A method for reliably and reproducibly culturing B. burgdorferi from the blood of patients with chronic Lyme disease was therefore sought by making a controlled blood culture trial studying 47 patients with chronic Lyme disease. All had relapsed after long-term oral and intravenous antibiotics. 23 patients with other chronic illness formed the control group. Positive cultures were confirmed by fluorescent antibody immuno-electron microscopy using monoclonal antibody directed against Osp A, and Osp A PCR. 43/47 patients (91%) cultured positive. 23/23 controls (100%) cultured negative. Although persistent infection has been, to date, strongly suggested in chronic Lyme disease by positive PCR and antigen capture, there are major problems with these tests. This new method for culturing B. burgdorferi from patients with chronic Lyme disease certainly defines the nature of the illness and establishes that it is of chronic infectious etiology. This discovery should help to reestablish the gold standard in laboratory diagnosis of Lyme disease.

37. The Psychiatric Clinics of North America Vol. 21,#3, 9/98

The Underdiagnosis of Neuropsychiatric Lyme Disease in Children and Adults.

Brian Fallon, MD, MPH et al; Columbia University

ABSTRACT: Lyme disease is a tick-borne illness caused by the spirochete Borrelia burgdorferi. Reported throughout the United States, the greatest incidence of Lyme disease occurs in certain areas, such as the Northeast, the upper Midwest, and the Pacific Coastal states. It has been dubbed “The New Great Imitator” because, like another spirochetal illness neurosyphilis-the original Great Imitator, Lyme disease has a vast array of multisystem manifestations, including neuropsychiatric ones.18 Failure to recognize Lyme disease early in its course can result in the development of a chronic illness that is only temporarily or partially responsive to antibiotic therapy. The goal of this article is to present the typical and atypical manifestations of Lyme disease in children and adults in order to help the clinician more rapidly unmask the correct diagnosis behind the puzzling presentations of some patients.

38. Abstract ACR 61st National Scientific Meeting. November 8-12 1997 Washington, DC * CONFERENCE PRESENTATION

PCR Evidence for Borrelia Burgdorferi DNA in Synovium in Absence of Positive Serology."

H. Ralph Schumacher, MD, P. Branigan, Jay Rao, H. Gerard, A. Hudson, W. Williams, T. Arayssi, M. Bayer, S. Rothfuss, G. Clayburne, M. Sieck, HR Schumacher

U of Pa, Allegheny University of Health Sciences and VAMC, Phila. PA 19104 and NIAMS, NIH, Bethesda, MD 20892

ABSTRACT: Although Borrelia burgdorferi have been identified in synovium by several groups using ilmmunohistochemistry, EM Steiner stains and PCR, there is controversy about whether they can infect joints without inducing a serologic response and whether they can persist after antibiotic treatment. We have performed PCR for Borrelia on a series of 185 synovial biopsies and synovial fluid regardless of clinical diagnosis. There were no cases included with known clinical Lyme disease or with positive Lyme ELISA serology. A positive control was from an erythema migrans lesion with known Lyme disease. PCR primers used identified Borrelia burgdorferi Osp A DNA. In 6 PCR positive cases synovium was also studied by Steiner stain and 4 had transmission EM to search for evidence of organisms.

Ten of the 185 cases studied (5, 3%) and the positive control were positive for the Osp A gene of Borrelia burgdorferi. Steiner stains were negative in all 6 studied. EM in no cases revealed any classic organisms but did show several features (including a variety of unusual membranous arrays) that have been reported before in known Lyme disease and other infections. Clinical patterns

were reviewed on the Borrelia PCR positive patients. Clinical diagnoses were RA in 4,

Adult Onset Stills Disease or JRA in 2, reactive arthritis in 2, psoriatic 1, and unclassified oligoarthritis 1. Four had received extensive antibiotics before the biopsy with improvement in 2.

PCR evidence for Borrelia has been identified in synovial biopsies of patients with clinical pictures that had not initially suggested Lyme disease. All patients were negative for antibodies to Borrelia and some were PCR positive in synovium despite previous treatment with antibiotics.

39. Infection 24 (1996) #5

Borrelia burgdorferi DNA in the Urine of Treated Patients with Chronic Lyme Disease Symptom: A PCR Study of 97 Cases

M.E. Bayer, MD et al

Author affiliation Fox Chase Cancer Center, Philadelphia, PA 19111, USA

ABSTRACT: All patients had shown erythema chronica migrans following a deer tick bite. Most of the patients had been antibiotic-treated for extended periods of time. …Of the 97 patients, 72 (74.2%) were found with positive PCR and the rest with negative PCR. The 62 healthy volunteers were PCR negative. It is proposed that a sizeable group of patients diagnosed on clinical grounds as having chronic Lyme disease may still excrete Borrelia DNA, and may do so in spite of intensive antibiotic treatment.

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Lyme Disease Association, Inc. April 2012

PO Box 1438 Jackson, NJ 08527

www.LymeDiseaseAssociation.org

888-366-6611