

56 Publications for LDA-Funded Research

The LDA has a long history of funding/supporting research projects that lead to real results. Below is the list of links to peer-reviewed articles that acknowledge LDA's funding/support. Click on the publication link (or just scroll down to it) to see the authors, which authors LDA provided the funding for (**green**), and the abstract and a link to the publication itself when possible. When a journal has multiple publications, click on the year in which you are interested.

Frontiers in Neurology 2021

(2) Frontiers in Medicine 2020, 2019

(2) Antibiotics 2020, 2017

Brain, Behavior, & Immunity – Health 2020

Meta Gene 2019

Ticks and Tick-Borne Diseases 2018

Archives of Clinical Neuropsychology 2018

(2) Psychosomatics 2018, 2013

Healthcare (Basel) 2018

Bio-Protocol 2017

(6) PLOS One 2017, 2012 (2), 2011, 2010

The FEBS Journal 2017

ACS Chemical Biology 2017

Biochemistry 2017

(2) Frontiers in Microbiology October 2016, May 2016

Park Science (NPS, Department of Interior) 2016

FEMS Microbiology Letters 2015

Clinical Infectious Disease 2014

Veterinary Sciences 2014

International Journal of Medical Sciences 2013

Northeastern Naturalist, 2013

(3) Journal of Neuropsychiatry & Clinical Neurosciences 2013, 2003, 2001

Open Neurology Journal 2012

Journal of Bacteriology 2011

Genetics 2011

Journal of Medical Entomology 2010

Neurobiology of Disease 2010

Archives General Psychiatry 2009

Gene 2009

(2) Neurology 2008, 1999

Emerging Infectious Diseases July 2008

Minerva Medica October 2008

Microbial Pathogenesis 2008

Journal of International Neuropsychological Soc. 2006

Infection & Immunity 2006

Journal of Clinical Microbiology 2005
Expert Review of Anti-Infective Therapy 2004
The Proceedings of National Academy of Science 2004
(2) JSTBD 2002, 1999
Medscape Infectious Diseases April 2000
Journal of American Medical Association (JAMA) 1999
Psychiatric Clinics of North America 1998
(2) Infection 1998, 1996

Click here to see a sample of conference presentations from researchers who have received LDA funding for their work.

[Conference Presentations Resulting from LDA-Funding](#)

Journal Articles Below

56. Frontiers in Neurology, May 10, 2021

Detecting Borrelia Spirochetes: A Case Study With Validation Among Autopsy Specimens

<https://www.frontiersin.org/articles/10.3389/fneur.2021.628045/full>

Shiva Kumar Goud Gadila¹, Gorazd Rosoklija^{2,3}, Andrew J. Dwork^{2,3,4,5}, **Brian A. Fallon^{2*}** and Monica E. Embers^{1*}

1 Division of Immunology, Tulane National Primate Research Center, Tulane University Health Sciences, Covington, LA, United States

2 Department of Psychiatry, Columbia University, New York, NY, United States

3 Division of Molecular Imaging and Neuropathology, New York State Psychiatric Institute, New York, NY, United States

4 Macedonian Academy of Sciences and Arts, Skopje, Macedonia

5 Department of Pathology and Cell Biology, Columbia University, New York, NY, United States

*Correspondence: Brian A. Fallon, baf1@cumc.columbia.edu; Monica E. Embers, members@tulane.edu

Abstract: The complex etiology of neurodegenerative disease

has prompted studies on multiple mechanisms including genetic predisposition, brain biochemistry, immunological responses, and microbial insult. In particular, Lyme disease is often associated with neurocognitive impairment with variable manifestations between patients. We sought to develop methods to reliably detect *Borrelia burgdorferi*, the spirochete bacteria responsible for Lyme disease, in autopsy specimens of patients with a history of neurocognitive disease. In this report, we describe the use of multiple molecular detection techniques for this pathogen and its application to a case study of a Lyme disease patient. The patient had a history of Lyme disease, was treated with antibiotics, and years later developed chronic symptoms including dementia. The patient's pathology and clinical case description was consistent with Lewy body dementia. *B. burgdorferi* was identified by PCR in several CNS tissues and by immunofluorescent staining in the spinal cord. These studies offer proof of the principle that persistent infection with the Lyme disease spirochete may have lingering consequences on the CNS.

55. *Frontiers in Medicine* (Lausanne), Oct 27, 2020

***Borrelia miyamotoi* Serology in a Clinical Population With Persistent Symptoms and Suspected Tick-Borne Illness**

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7652925/>

Shannon L. Delaney,^{1,2,*} Lilly A. Murray,^{1,2} Claire E. Aasen,¹ Clair E. Bennett,^{1,2} Ellen Brown,^{1,2} and **Brian A. Fallon**^{1,2}

¹ Lyme & Tick-Borne Diseases Research Center, Columbia University Irving Medical Center, New York, NY, United States

² New York State Psychiatric Institute, Columbia University Irving Medical Center, New York, NY, United States

Edited by: Ying Zhang, Zhejiang University, China

Reviewed by: Pallab Ghosh, Harvard Medical School, United States; Raymond James Dattwyler, New York Medical College, United States

*Correspondence: Shannon L. Delaney sld2158@cumc.columbia.edu

Abstract: Eighty-two patients seeking consultation for long-

term sequelae after suspected tick-borne illness were consecutively tested for *Borrelia miyamotoi* antibodies using a recombinant glycerophosphodiester phosphodiesterase (GlpQ) enzyme immunoassay. Twenty-one of the 82 patients (26%) tested positive on the GlpQ IgG ELISA. Nearly all of the patients (98%) had no prior *B. miyamotoi* testing, indicating that clinicians rarely test for this emerging tick-borne pathogen. Compared to patients who solely tested positive for Lyme disease antibodies, patients with *B. miyamotoi* antibodies presented with significantly more sleepiness and pain. A prospective study is needed to ascertain the relationship between the presence of *B. miyamotoi* antibodies and persistent symptoms.

54. Antibiotics (Basel). May 25, 2020

Effect of *Borrelia burgdorferi* Outer Membrane Vesicles on Host Oxidative Stress Response

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7277464/>

Keith Wawrzeniak, Gauri Gaur, Eva Sapi, and **Alireza G. Senejani***

Department of Biology and Environmental Science, University of New Haven, West Haven, CT 06516, USA; kwawr1@unh.newhaven.edu (K.W.); ggaur2@unh.newhaven.edu (G.G.); ESapi@newhaven.edu (E.S.) *Correspondence: ASenejani@newhaven.edu

Abstract: Outer membrane vesicles (OMVs) are spherical bodies containing proteins and nucleic acids that are released by Gram-negative bacteria, including *Borrelia burgdorferi*, the causative agent of Lyme disease. The functional relationship between *B. burgdorferi* OMVs and host neuron homeostasis is not well understood. The objective of this study was to examine how *B. burgdorferi* OMVs impact the host cell environment. First, an in vitro model was established by co-culturing human BE2C neuroblastoma cells with *B. burgdorferi* B31. *B. burgdorferi* was able to invade BE2C cells within 24 h. Despite internalization, BE2C cell viability and levels of apoptosis remained unchanged, but resulted in dramatically increased

production of MCP-1 and MCP-2 cytokines. Elevated secretion of MCP-1 has previously been associated with changes in oxidative stress. BE2C cell mitochondrial superoxides were reduced as early as 30 min after exposure to *B. burgdorferi* and OMVs. To rule out whether BE2C cell antioxidant response is the cause of decline in superoxides, superoxide dismutase 2 (SOD2) gene expression was assessed. SOD2 expression was reduced upon exposure to *B. burgdorferi*, suggesting that *B. burgdorferi* might be responsible for superoxide reduction. These results suggest that *B. burgdorferi* modulates cell antioxidant defense and immune system reaction in response to the bacterial infection. In summary, these results show that *B. burgdorferi* OMVs serve to directly counter superoxide production in BE2C neurons, thereby 'priming' the host environment to support *B. burgdorferi* colonization.

53. Brain, Behavior, & Immunity – Health, January 7, 2020

<https://www.sciencedirect.com/science/article/pii/S2666354619300158?via%3Dihub>

Anti-lysoganglioside and other anti-neuronal autoantibodies in post-treatment Lyme Disease and Erythema Migrans after repeat infection

Brian A. Fallon a,b,* Barbara Strobino, a,b, Sean Reim c, Julie Stoner d, Madeleine W. Cunningham c

a Columbia Psychiatry, Columbia University Irving Medical Center, New York, USA

b New York State Psychiatric Institute, 1051 Riverside Drive, New York, USA

c Department of Microbiology and Immunology, University of Oklahoma Health Sciences Center, Oklahoma City, USA

d Department of Biostatistics, University of Oklahoma Health Sciences Center, Oklahoma City, USA

*Corresponding author: Brian A. Fallon, Columbia University, 1051 Riverside Drive, Unit 69, New York, NY, 10032, USA.

Abstract: Background: Molecular mimicry targeting neural tissue has been reported after *Borrelia burgdorferi* (Bb)

infection. Herein, we investigate whether antineuronal autoantibodies are increased and whether antibody-mediated signaling of neuronal cells is elevated in a cohort of symptomatic adults with a history of Lyme Disease (LD).

Methods: Participants (n = 179) included 24 with recent Erythema Migrans (EM) without prior LD, 8 with recent EM and prior LD (EM + prior LD), 119 with persistent post-treatment LD symptoms (PTLS), and 28 seronegative endemic controls with no prior LD history. Antineuronal immunoglobulin G (IgG) titers were measured by standard ELISA and compared with mean titers of normal age-matched sera against lysoganglioside, tubulin, and dopamine receptors (D1R and D2R). Antibody-mediated signaling of calcium calmodulin dependent protein kinase II (CaMKII) activity in a human neuronal cell line (SK-N-SH) was identified in serum.

Results: EM + prior LD cases had higher antibody titers than controls for anti-lysoganglioside GM1 (p = 0.002), anti-tubulin (p = 0.03), and anti-D1R (p = 0.02), as well as higher expression in the functional antibody-mediated CaMKII Assay (p = 0.03). The EM cases with no prior history showed no significant differences on any measures. The PTLS cases demonstrated significantly higher titers (p = 0.01) than controls on anti-lysoganglioside GM1, but not for the other measures.

Conclusion: The finding of elevated anti-neuronal autoantibodies in our small sample of those with a prior history of Lyme disease but not in those without prior Lyme disease, if replicated in a larger sample, suggests an immune priming effect of repeated infection; the CaMKII activation suggests that antineuronal antibodies have functional significance. The elevation of anti-lysoganglioside antibodies among those with PTLS is of particular interest given the established role of anti-ganglioside antibodies in peripheral and central neurologic diseases. Future prospective studies can determine whether these autoantibodies emerge after Bb infection and whether their emergence coincides with persistent neurologic or neuropsychiatric symptoms.

52. *Frontiers in Medicine*, December 6, 2019

<https://www.frontiersin.org/articles/10.3389/fmed.2019.00283/full>

The General Symptom Questionnaire-30 (GSQ-30): A Brief Measure of Multi-System Symptom Burden in Lyme Disease

Brian A. Fallon 1,2*, Nevena Zubcevik 3,4, Clair Bennett 1,2, Shreya Doshi 1,2, Alison W. Rebman 5, Ronit Kishon 1,2, James R. Moeller 1,2, Nadlyne R. Octavien 3 and John N. Aucott 5

1 Department of Psychiatry, Lyme and Tick-Borne Diseases Research Center, Columbia University Irving Medical Center, New York, NY, United States

2 Department of Psychiatry, New York State Psychiatric Institute, New York, NY, United States

3 Department of Physical Medicine and Rehabilitation, Dean Center for Tick borne Illness, Harvard Medical School, Spaulding Rehabilitation Hospital, Boston, MA, United States

4 Department of Physical Medicine and Rehabilitation, Massachusetts General Hospital, Boston, MA, United States

5 Division of Rheumatology, Department of Medicine, Lyme Disease Research Center, Johns Hopkins School of Medicine, Baltimore, MD, United States

*Correspondence: Brian A. Fallon, baf1@cumc.columbia.edu

Abstract: Introduction: The multi-system symptoms accompanying acute and post-treatment Lyme disease syndrome pose a challenge for time-limited assessment. The General Symptom Questionnaire (GSQ-30) was developed to fill the need for a brief patient-reported measure of multi-system symptom burden. In this study we assess the psychometric properties and sensitivity to change of the GSQ-30. **Materials and Methods:** 342 adult participants comprised 4 diagnostic groups: Lyme disease (post-treatment Lyme disease syndrome, n = 124; erythema migrans, n = 94); depression, n = 36; traumatic brain injury, n = 51; healthy, n = 37. Participants were recruited from clinical research facilities in Massachusetts, Maryland, and New York. Validation measures for the GSQ-30 included the

Patient Health Questionnaire-4 for depression and anxiety, visual analog scales for fatigue and pain, the Sheehan Disability Scale for functional impairment, and one global health question. To assess sensitivity to change, 53 patients with erythema migrans completed the GSQ-30 before treatment and 6 months after 3 weeks of treatment with doxycycline. **Results:** The GSQ-30 demonstrated excellent internal consistency (Cronbach α = 0.95). The factor structure reflects four core domains: pain/fatigue, neuropsychiatric, neurologic, and viral-like symptoms. Symptom burden was significantly associated with depression (r_s = 0.60), anxiety (r_s = 0.55), pain (r_s = 0.75), fatigue (r_s = 0.77), functional impairment (r_s = 0.79), and general health (r_s = -0.58). The GSQ-30 detected significant change in symptom burden before and after antibiotic therapy; this change correlated with change in functional impairment. The GSQ-30 total score significantly differed for erythema migrans vs. three other groups (post-treatment Lyme disease syndrome, depression, healthy controls). The GSQ-30 total scores for traumatic brain injury and depression were not significantly different from post-treatment Lyme disease syndrome. **Conclusions and Relevance:** The GSQ-30 is a valid and reliable instrument to assess symptom burden among patients with acute and post-treatment Lyme disease syndrome and is sensitive in the detection of change after treatment among patients with erythema migrans. The GSQ-30 should prove useful in clinical and research settings to assess multi-system symptom burden and to monitor change over time. The GSQ-30 may also prove useful in future precision medicine studies as a clinical measure to correlate with disease-relevant biomarkers.

51. Meta Gene, Volume 21, September 2019. Online May 2019
<https://www.sciencedirect.com/science/article/pii/S2214540019300489>

Genetic Variation in the ABCB1 Gene Associated with Post Treatment Lyme Disease Syndrome Status

Joanna Lyon 1, Hyunuk Seung 2

1 Advanced Clinical Pharmacist, University of Maryland School of Pharmacy, United States of America Jlyon@rx.umaryland.edu

2 Department of Pharmacy Practice and Science, University of Maryland School of Pharmacy, United States of America hseung@rx.umaryland.edu

Abstract: Post Treatment Lyme Disease Syndrome (PTLDS) poses a difficult to understand health issue. This multi-centered, randomized control trial studied the possible correlation between ABCB1 (MDR1) gene variants and the incidence of PTLDS in affected patients. Genomic DNA was isolated and analyzed for four ABCB1 gene SNPs (rs1128503, rs1045642, rs2235067, and rs4148740). Significant findings include the association of rs1128503 TC variant with PTLDS status. Additionally, the rs1128503+ rs1045642+ rs2235067 SNP combination increased rs1128503 genotype TC significance to 3.83 times the rs1128503 genotype CC. The TT variant of rs4148740 in conjunction with rs1128503 reduced the odds ratio and appeared to convey a PTLDS protective status to the rs1128503 TC variant.

50. Ticks and Tick-Borne Diseases, 2018 Nov 27

<https://www.ncbi.nlm.nih.gov/pubmed/30503356><https://www.ncbi.nlm.nih.gov/pubmed/30503356>

Regional prevalences of *Borrelia burgdorferi*, *Borrelia bissettiae*, and *Bartonella henselae* in *Ixodes affinis*, *Ixodes pacificus* and *Ixodes scapularis* in the USA.

Maggi RG 1, Toliver M 2, Richardson T 3, Mather T 4, **Breitschwerdt EB** 5

1 Intracellular Pathogens Research Laboratory, Comparative Medicine Institute, College of Veterinary Medicine, North Carolina State University (NCSU), 1060 William Moore Drive, Raleigh, NC 27607. rgmaggi@ncsu.edu

2 Public Health Pest Management Section, NC Department of Environment and Natural Resources, 10005 Waterford Court, Raleigh, NC 27613. marceetoliver@gmail.com

3 Intracellular Pathogens Research Laboratory, Comparative

Medicine Institute, College of Veterinary Medicine, North Carolina State University (NCSU), 1060 William Moore Drive, Raleigh, NC 27607. tgrichar@ncsu.edu

4 Department of Plant Sciences and Entomology, Center for Vecto-Borne Disease, College of the Environment and Life Sciences, University of Rhode Island, 231 Woodward Hall, Kingston, RI 02881. tmather@uri.edu

5 Intracellular Pathogens Research Laboratory, Comparative Medicine Institute, College of Veterinary Medicine, North Carolina State University (NCSU), 1060 William Moore Drive, Raleigh, NC 27607. ebbreits@ncsu.edu

ABSTRACT: The objective of this work was to determine the prevalence of *Borrelia* and *Bartonella* species in *Ixodes* spp. ticks collected from 16 USA states. Genus PCR amplification and sequence analysis of *Bartonella* and *Borrelia* 16SsRNA-23SsRNA intergenic regions were performed on DNA extracted from 929 questing adult ticks (671 *Ixodes scapularis*, 155 *Ixodes affinis*, and 103 *Ixodes pacificus*). Overall, 129/929 (13.9%) *Ixodes* ticks were PCR positive for *Borrelia burgdorferi sensu stricto*, 48/929 for *B. bissettae* whereas 23/929 (2.5%) were PCR positive for a *Bartonella henselae*. *Borrelia bissettae* or *B. burgdorferi* s.s. and *B. henselae* co-infections were found in *I. affinis* from North Carolina at a rate of 4.5%; in a single *I. scapularis* from Minnesota, but not in *I. pacificus*. For both bacterial genera, PCR positive rates were highly variable depending on geographic location and tick species, with *Ixodes affinis* (n = 155) collected from North Carolina, being the tick species with the highest prevalence's for both *Borrelia* spp. (63.2%) and *B. henselae* (10.3%). Based on the results of this and other published studies, improved understanding of the enzootic cycle, transmission dynamics, and vector competence of *Ixodes* species (especially *I. affinis*) for transmission of *Borrelia* spp. and *B. henselae* should be a public health research priority.

49. Archives of Clinical Neuropsychology, Nov 12, 2018
<https://academic.oup.com/acn/advance-article-abstract/doi/10.1093/arclin/acy083/5173742?redirectedFrom=PDF>

Neurocognition in Post-Treatment Lyme Disease and Major Depressive Disorder

Keilp JG 1,2,3*, Corbera K 1, Gorlyn M 2, Oquendo MA 2,3,4, Mann JJ 2,3 **Fallon BA 1,2,3**

*Corresponding author at: New York State Psychiatric Institute and Department of Psychiatry, Columbia University College of Physicians and Surgeons, Box 42, NYSPI, 1051 Riverside Drive, New York, NY 10032, USA. Tel.: 1-646-774-7509. E-mail: jgk13@cumc.columbia.edu (J.G. Keilp)

1 Lyme Disease Research Center, Columbia University College of Physicians and Surgeons, New York, NY

2 Department of Psychiatry, Columbia University College of Physicians and Surgeons, New York, NY

3 Department of Molecular Imaging and Neuropathology, New York State Psychiatric Institute, New York, NY

4 Department of Psychiatry, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA

Dr. John Keilp, senior neuropsychologist for the Columbia Lyme & Tick-borne Diseases Research Center', reports that the cognitive profile of patients with post treatment Lyme disease is meaningfully different from the profile of patients with major depression. This is a neurocognitive biomarker or fingerprint of post-treatment Lyme disease. Although both groups might have fatigue and mental foginess, the Lyme group more often reports problems with verbal memory and verbal fluency while the depressed (non-Lyme) group more often reports slower processing speed and poor attention. These results highlight the value of neurocognitive testing in helping to tease out the potential causes of cognitive problems in patients with post-treatment Lyme disease.

48. Psychosomatics 59(5) · Sept/Oct 2018
<https://www.ncbi.nlm.nih.gov/pubmed/29606281>

Depressive Symptoms and Suicidal Ideation Among Symptomatic Patients with a History of Lyme Disease Versus Two Comparison Groups

Shreya Doshi M.A 1*., John G. Keilp Ph.D 2., Barbara Strobino Ph.D. 1, Martin McElhiney Ph.D. 1, Judith Rabkin Ph.D. 1, **Brian A. Fallon M.D. 1**

1 Division of Clinical Therapeutics, Department of Psychiatry, New York State Psychiatric Institute, New York, NY

2 Division of Molecular Imaging and Neuropathology, Department of Psychiatry, New York State Psychiatric Institute, New York, NY

*sd2698@tc.columbia.edu

ABSTRACT: Background: Depression has been reported in 8–45% of patients with posttreatment Lyme symptoms (PTLS), but little is known about suicidal ideation in these patients. Method: Depression and suicidal ideation were assessed using the Beck Depression Inventory (BDI-II). Scores from the PTLS group (n = 81) were compared to those from 2 other groups: HIV+ patients being treated for fatigue (n = 70), and a nonpatient comparison group (NPCG; n = 44). ANOVA and t-tests were used to compare groups; logistic regression was used to identify the strongest correlates of suicidal ideation. Results: Mean BDI-II scores fell in the mildly depressed range for PTLS and HIV+ patients, with both groups having higher depression scores than the NPCG. Suicidal ideation was reported by 19.8% of the PTLS patients and 27.1% of the HIV+ patients, a nonsignificant difference. Among those with mild or no depression, suicidal ideation was uncommon (6.5% PTLS and 11.9% HIV+). Among the patients with moderate-to-severe depression, suicidal ideation was more common (63.2% of 19 PTLS and 50% of 28 HIV+); among these, 2 with PTLS and 1 with HIV+ expressed suicidal intent. Further, 4.5% (n = 2) of the NPCG had suicidal ideation, each had scores in the moderate-to-severe depression range. Higher scores on the cognitive symptoms subscale of the BDI-II predicted greater likelihood

of suicidal ideation across patient groups. Conclusion: As expected, suicidal ideation is increased among patients who are depressed. The fact that 1 in 5 patients with PTLs reported suicidal ideation highlights the importance of screening for depression and suicidality to optimize patient care.

47. Healthcare (Basel) 2018 Jun; 6(2): 69.

A Community Study of Borrelia burgdorferi Antibodies among Individuals with Prior Lyme Disease in Endemic Areas

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6023339/>

Barbara Strobino 1,2,* Katja Steinhagen 3, Wolfgang Meyer 3, Thomas Scheper 3, Sandra Saschenbrecker 3, Wolfgang Schlumberger 3, Winfried Stöcker 3, Andrea Gaito 4 and **Brian A. Fallon** 1.1 Department of Psychiatry, Lyme and Tick-Borne Diseases Research Center, Columbia University Irving Medical Center, New York, NY 10032, USA; baf1@cumc.columbia.edu

2 Research Foundation for Mental Hygiene, Inc., New York, NY 10032, USA

3 Institute for Experimental Immunology, Euroimmun, 23560 Lübeck, Germany;

k.steinhagen@euroimmun.de (K.S.); w.meyer@euroimmun.de (W.M.);

t.scheper@euroimmun.de (T.S.);

s.saschenbrecker@euroimmun.de (S.S.);

w.schlumberger@euroimmun.de (Wo.S.);

w.stoecker@euroimmun.de (Wi.S.)

4 Independent Researcher, Basking Ridge, NJ 07920, USA;

adg0339@gmail.com

* Correspondence: barbara.strobino@nyspi.columbia.edu; Tel.: +1-646-774-8052

Abstract: The objective was to examine the prevalence of Borrelia antibodies among symptomatic individuals with recent and past Lyme disease in endemic communities using standard assays and novel assays employing next-generation antigenic substrates. Single- and two-tiered algorithms included

different anti-Borrelia ELISAs and immunoblots. Antibody prevalence was examined in sera from 32 individuals with recent erythema migrans (EM), 335 individuals with persistent symptoms following treatment for Lyme disease (PTLS), and 41 community controls without a history of Lyme disease. Among convalescent EM cases, sensitivity was highest using the C6 ELISA (93.8%) compared to other single assays; specificity was 92.7% for the C6 ELISA vs. 85.4–97.6% for other assays. The two-tiered ELISA-EUROLINE IgG immunoblot combinations enhanced case detection substantially compared to the respective ELISA-IgGWestern blot combinations (75.0% vs. 34.4%) despite similar specificity (95.1% vs. 97.6%, respectively). For PTLS cohorts, two-tier ELISA-IgG-blot positivity ranged from 10.1% to 47.4%, depending upon assay combination, time from initial infection, and clinical history. For controls, the two-tier positivity rate was 0–14.6% across assays. A two-tier algorithm of two-ELISA assays yielded a high positivity rate of 87.5% among convalescent EM cases with specificity of 92.7%. For convalescent EM, combinations of the C6 ELISA with a second-tier ELISA or line blot may provide useful alternatives to WB-based testing algorithms.

46. Bio-Protocol. Vol 7, Iss 23, December 05, 2017 DOI: 10.21769/BioProtoc.2643.

<https://bio-protocol.org/e2643>

Lentiviral Knockdown of Transcription Factor STAT1 in Peromyscus leucopus to Assess Its Role in the Restriction of Tick-borne Flaviviruses

Adaeze O. Izuogu,¹ and **R. Travis Taylor**^{1,*}

*travis.taylor@utoledo.edu

¹ Department of Medical Microbiology and Immunology, University of Toledo College of Medicine and Life Sciences, Toledo, Ohio, USA

ABSTRACT: Cellular infection with tick-borne flaviviruses (TBFVs) results in activation of the interferon (IFN)

signaling pathway and subsequent upregulation of numerous genes termed IFN stimulated genes (ISGs) (Schoggins et al., 2011). Many ISGs function to prevent virus pathogenesis by acting in a broad or specific manner through protein-protein interactions (Duggal and Emerman, 2012). The potency of the IFN signaling response determines the outcome of TBFV infection (Best, 2017; Carletti et al., 2017). Interestingly, data from our lab show that TBFV replication is significantly restricted in cells of the reservoir species *Peromyscus leucopus* thereby suggesting a potent antiviral response (Izuogu et al., 2017). We assessed the relative contribution of IFN signaling to resistance in *P. leucopus* by knocking down a major transcription factor in the IFN response pathway. Signal transducer and activator of transcription 1 (STAT1) was specifically targeted in *P. leucopus* cells by shRNA technology. We further tested the impact of gene knockdown on the ability of cells to respond to IFN and restrict virus replication; the results indicate that when STAT1 expression is altered, *P. leucopus* cells have a decreased response to IFN stimulation and are significantly more susceptible to TBFV replication.

45. FEBS J. 2017 Nov;284(21):3662-3683. Epub 2017 Sep 30.

<https://www.ncbi.nlm.nih.gov/pubmed/28892294>

Nuclease activity gives an edge to host-defense peptide piscidin 3 over piscidin 1, rendering it more effective against persisters and biofilms.

Libardo MDJ1, Bahar AA2, Ma B3, Fu R4, McCormick LE5, Zhao J6, McCallum SA7, Nussinov R3,8, Ren D2,9,10,11, **Angeles-Boza*** AM1, Cotten* ML12.

*alfredo.angeles-boza@uconn.edu, *mcotten@wm.edu

1 Department of Chemistry, University of Connecticut, Storrs, CT, USA.

2 Department of Biomedical and Chemical Engineering, Syracuse University, NY, USA.

3 Basic Science Program, Leidos Biomedical Research, Inc.

Cancer and Inflammation Program, National Cancer Institute, Frederick, MD, USA.

4 National High Magnetic Field Laboratory, Tallahassee, FL, USA.

5 Hamilton College, Department of Chemistry, Clinton, NY, USA.

6 Cancer and Inflammation Program, National Cancer Institute, Frederick, MD, USA.

7 Rensselaer Polytechnic Institute, Center for Biotechnology & Interdisciplinary Studies, Troy, NY, USA.

8 Sackler Institute of Molecular Medicine, Department of Human Genetics and Molecular Medicine, Sackler School of Medicine, Tel Aviv University, Israel.

9 Syracuse Biomaterials Institute, Syracuse University, NY, USA.

10 Department of Civil and Environmental Engineering, Syracuse University, NY, USA.

11 Department of Biology, Syracuse University, NY, USA.

12 Department of Applied Science, College of William and Mary, Williamsburg, VA, USA.

ABSTRACT: Host-defense peptides (HDPs) feature evolution-tested potency against life-threatening pathogens. While piscidin 1 (p1) and piscidin 3 (p3) are homologous and potent fish HDPs, only p1 is strongly membranolytic. Here, we hypothesize that another mechanism imparts p3 strong potency. We demonstrate that the N-termini of both peptides coordinate Cu^{2+} and p3-Cu cleaves isolated DNA at a rate on par with free Cu^{2+} but significantly faster than p1-Cu. On planktonic bacteria, p1 is more antimicrobial but only p3 features copper-dependent DNA cleavage. On biofilms and persister cells, p3-Cu is more active than p1-Cu, commensurate with stronger peptide-induced DNA damage. Molecular dynamics and NMR show that more DNA-peptide interactions exist with p3 than p1, and the peptides adopt conformations simultaneously poised for metal- and DNA-binding. These results generate several important conclusions. First, homologous HDPs cannot be assumed to have identical mechanisms since p1 and p3 eradicate

bacteria through distinct relative contributions of membrane and DNA-disruptive effects. Second, the nuclease and membrane activities of p1 and p3 show that naturally occurring HDPs can inflict not only physicochemical but also covalent damage. Third, strong nuclease activity is essential for biofilm and persister cell eradication, as shown by p3, the homolog more specific toward bacteria and more expressed in vascularized tissues. Fourth, p3 combines several physicochemical properties (e.g., Amino Terminal Copper and Nickel binding motif; numerous arginines; moderate hydrophobicity) that confer low membranolytic effects, robust copper-scavenging capability, strong interactions with DNA, and fast nuclease activity. This new knowledge could help design novel therapeutics active against hard-to-treat persister cells and biofilms.

44. ACS Chem. Biol., 2017, 12 (5), pp 1170–1182 Publication Date (Web): March 29, 2017

<https://pubs.acs.org/doi/abs/10.1021/acscchembio.7b00237>

Membrane Oxidation in Cell Delivery and Cell Killing Applications

Ting-Yi Wang,^{†,⊥} M. Daben J. Libardo,^{‡,⊥} **Alfredo M. Angeles-Boza**,^{*,‡} and Jean-Philippe Pellois^{*,†,§}

[†]Department of Biochemistry and Biophysics, Texas A&M University, College Station, Texas 77843, United States

[‡]Department of Chemistry, University of Connecticut, Storrs, Connecticut 06269, United States

[§]Department of Chemistry, Texas A&M University, College Station, Texas 77843, United States

*E-mail: pellois@tamu.edu, *E-mail: alfredo.angeles-boza@uconn.edu.

ABSTRACT: Cell delivery or cell killing processes often involve the crossing or disruption of cellular membranes. We review how, by modifying the composition and properties of membranes, membrane oxidation can be exploited to enhance the

delivery of macromolecular cargoes into live human cells. We also describe how membrane oxidation can be utilized to achieve efficient killing of bacteria by antimicrobial peptides. Finally, we present recent evidence highlighting how membrane oxidation is intimately engaged in natural biological processes such as antigen delivery in dendritic cells and in the killing of bacteria by antimicrobial peptides. Overall, the insights that have been recently gained in this area should facilitate the development of more effective delivery technologies and antimicrobial therapeutic approaches.

43. *Biochemistry*, 2017, 56 (10), pp 1403–1414 Publication Date (Web): February 22, 2017

<https://pubs.acs.org/doi/abs/10.1021/acs.biochem.6b01046>

Exploration of the Innate Immune System of *Styela clava*: Zn²⁺ Binding Enhances the Antimicrobial Activity of the Tunicate Peptide Clavanin A

Samuel A. Julianot†, Scott Piercet†, James A. deMayo‡, Marcy J. Balunas‡, and **Alfredo M. Angeles-Boza***†

† Department of Chemistry, University of Connecticut, Storrs, Connecticut 06269-3060, United States

‡ Division of Medicinal Chemistry, Department of Pharmaceutical Sciences, University of Connecticut, Storrs, Connecticut 06269, United States

*E-mail: alfredo.angeles-boza@uconn.edu.

ABSTRACT: Tunicates have been used as primitive models for understanding cell-mediated and humoral immunity. Clavanin A (ClavA) is one member of a family of antimicrobial peptides produced by the solitary tunicate *Styela clava*. In this work, we demonstrate that ClavA utilizes Zn²⁺ ions to potentiate its antimicrobial activity not only by reducing the concentration at which the peptide inhibits the growth of bacteria but also by

increasing the rate of killing. Membrane depolarization, β -galactosidase leakage, and potassium leakage assays indicate that ClavA is membrane active, forms small pores, but induces

cell death by targeting an intracellular component. ClavA and ClavA-Zn²⁺ added to *Escherichia coli* and imaged by confocal microscopy translocate across the cell membrane. *E. coli* mutants lacking the functional Zn²⁺ import system are less susceptible to ClavA, suggesting that the synergistic activity between ClavA and Zn²⁺ has a cytoplasmic target, which is further supported by its nucleolytic activity. Overall, these studies identify a remarkable new mechanism by which zinc contributes to the immune response in the tunicate *S. clava*.

42. PLoS One, June 26, 2017;12(6)

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5484488/>

Interferon Signaling in Peromyscus Leucopus Confers a Potent and Specific Restriction to Vector-borne Flaviviruses

Adaeze O. Izuogul¹, Kristin L. McNally², Stephen E. Harris³, Brian H. Youseff¹, John B. Presloid¹, Christopher Burlak⁴, Jason Munshi-South⁵, Sonja M. Best², R.

Travis Taylor^{1*}

¹ Department of Medical Microbiology and Immunology, University of Toledo College of Medicine and Life Sciences, Toledo, Ohio, United States of America, ² Innate Immunity and Pathogenesis Unit, Laboratory of Virology, Rocky Mountain Laboratories, DIR, NIAID, NIH, Hamilton, Montana, United States of America,

³ The Graduate Center, City University of New York, New York, New York, United States of America,

⁴ Department of Surgery, University of Minnesota, Minneapolis, Minnesota, United States of America,

⁵ Louis Calder Center-Biological Field Station, Fordham University, Armonk, New York, United States of America

* travis.taylor@utoledo.edu

Abstract: Tick-borne flaviviruses (TBFVs), including Powassan virus and tick-borne encephalitis virus cause encephalitis or hemorrhagic fevers in humans with case-fatality rates ranging from 1-30%. Despite severe disease in humans, TBFV infection of natural rodent hosts has little noticeable effect. Currently, the basis for resistance to disease is not known.

We hypothesize that the coevolution of flaviviruses with their respective hosts has shaped the evolution of potent antiviral factors that suppress virus replication and protect the host from lethal infection. In the current study, we compared virus infection between reservoir host cells and related susceptible species. Infection of primary fibroblasts from the white-footed mouse (*Peromyscus leucopus*, a representative host) with a panel of vector-borne flaviviruses showed up to a 10,000-fold reduction in virus titer compared to control *Mus musculus* cells. Replication of vesicular stomatitis virus was equivalent in *P. leucopus* and *M. musculus* cells suggesting that restriction was flavivirus-specific. Step-wise comparison of the virus infection cycle revealed a significant block to viral RNA replication, but not virus entry, in *P. leucopus* cells. To understand the role of the type I interferon (IFN) response in virus restriction, we knocked down signal transducer and activator of transcription 1 (STAT1) or the type I IFN receptor (IFNAR1) by RNA interference. Loss of IFNAR1 or STAT1 significantly relieved the block in virus replication in *P. leucopus* cells. The major IFN antagonist encoded by TBFV, nonstructural protein 5, was functional in *P. leucopus* cells, thus ruling out ineffective viral antagonism of the host IFN response. Collectively, this work demonstrates that the IFN response of *P. leucopus* imparts a strong and virus-specific barrier to flavivirus replication. Future identification of the IFN-stimulated genes responsible for virus restriction specifically in *P. leucopus* will yield mechanistic insight into efficient control of virus replication and may inform the development of antiviral therapeutics.

41. Antibiotics, March 22, 2017

<http://www.mdpi.com/2079-6382/6/1/10/pdf>

Activity of Sulfa Drugs and Their Combinations against Stationary Phase *B. burgdorferi* In Vitro

Jie Feng, Shuo Zhang, Wanliang Shi and **Ying Zhang** *

Department of Molecular Microbiology and Immunology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore,

MD 21205, USA

*Author to whom correspondence should be addressed.
yzhang@jhsph.edu

Abstract: Lyme disease is a most common vector-borne disease in the US. Although the majority of Lyme patients can be cured with the standard two- to four-week antibiotic treatment, at least 10%–20% of patients continue to suffer from prolonged post-treatment Lyme disease syndrome (PTLDS). While the cause for this is unclear, one possibility is that persisting organisms are not killed by current Lyme antibiotics. In our previous studies, we screened an FDA drug library and an NCI compound library on *B. burgdorferi* and found some drug hits including sulfa drugs as having good activity against *B. burgdorferi* stationary phase cells. In this study, we evaluated the relative activity of three commonly used sulfa drugs, sulfamethoxazole (Smx), dapsone (Dps), sulfachlorpyridazine (Scp), and also trimethoprim (Tmp), and assessed their combinations with the commonly prescribed Lyme antibiotics for activities against *B. burgdorferi* stationary phase cells. Using the same molarity concentration, dapsone, sulfachlorpyridazine and trimethoprim showed very similar activity against stationary phase *B. burgdorferi* enriched in persisters; however, sulfamethoxazole was the least active drug among the three sulfa drugs tested. Interestingly, contrary to other bacterial systems, Tmp did not show synergy in drug combinations with the three sulfa drugs at their clinically relevant serum concentrations against *B. burgdorferi*. We found that sulfa drugs combined with other antibiotics were more active than their respective single drugs and that four-drug combinations were more active than three-drug combinations. Four-drug combinations dapsone + minocycline + cefuroxime + azithromycin and dapsone + minocycline + cefuroxime + rifampin showed the best activity against stationary phase *B. burgdorferi* in these sulfa drug combinations. However, these four-sulfa-drug-containing combinations still had considerably less activity against *B. burgdorferi* stationary phase cells than the Daptomycin + cefuroxime + doxycycline used as a positive control which completely eradicated *B. burgdorferi* stationary phase cells. Future studies are needed to evaluate and optimize the sulfa drug combinations in vitro and also in animal models.

40. *Frontiers in Microbiology*, October 19, 2016

<http://journal.frontiersin.org/article/10.3389/fmicb.2016.01744/pdf>

Ceftriaxone Pulse Dosing Fails to Eradicate Biofilm-like Microcolony *B. burgdorferi* Persisters Which Are Sterilized by Daptomycin/Doxycycline/Cefuroxime Drug Combination without Pulse Dosing

Jie Feng¹, Shuo Zhang¹, Wanliang Shi¹, **Ying Zhang^{1*}**

¹Department of Molecular Microbiology and Immunology, Bloomberg School of Public Health, Johns Hopkins University, USA

*Corresponding author: Ying Zhang, MD, PhD

Department of Molecular Microbiology and Immunology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD 21205, USA yzhang@jhsph.edu

Abstract: Although the majority of Lyme disease patients can be cured, at least 10-20% of the patients continue to suffer from persisting symptoms such as fatigue, muscular and joint pain, and neurologic impairment after standard 2-4 week antibiotic treatment. While the causes for this post-treatment Lyme disease symptoms are unclear, one possibility is due to *B. burgdorferi* persisters that are not effectively killed by current antibiotics such as doxycycline or amoxicillin used to treat Lyme disease. A previous study showed that four rounds of ceftriaxone pulse dosing treatment eradicated *B. burgdorferi* persisters in vitro using a relatively young late log phase culture (5 day old). In this study, we investigated if ceftriaxone pulse dosing could also eradicate *B. burgdorferi* persisters in older stationary phase cultures (10 day old) enriched with more resistant microcolony form of persisters. We found that ceftriaxone pulse dosing could only eradicate planktonic log phase *B. burgdorferi* spirochetal forms and round body forms but not more resistant aggregated biofilm-like microcolony persisters enriched in stationary phase cultures. Moreover, we found that not all drugs are suitable for pulse dosing, with bactericidal drugs ceftriaxone and cefuroxime being more appropriate for pulse dosing than bacteriostatic drug doxycycline and persister drug daptomycin. We also showed that drug combination pulse dosing treatment was more effective than single drug pulse dosing. Importantly, we demonstrate that pulse dosing treatment impaired the activity of the persister drug daptomycin and its drug combination against *B. burgdorferi* persisters and that the most effective way to kill the more resistant biofilm-like microcolonies is the daptomycin/doxycycline/ceftriaxone triple drug combination without pulse dosing. Our findings indicate pulse dosing may not always work as a general principle but rather depends on the specific drugs used, with cidal drugs being more appropriate for pulse dosing than static or persister drugs, and that drug combination approach with persister drugs is more effective at killing the more resistant microcolony form of persisters than pulse dosing. These observations may have implications for more effective treatment of Lyme disease. Future studies are required to validate these findings in animal models of *B. burgdorferi* persistence.

39. *Frontiers in Microbiology*, May 23, 2016

<http://journal.frontiersin.org/article/10.3389/fmicb.2016.00743/full>

A Drug Combination Screen Identifies Drugs Active against Amoxicillin-Induced Round Bodies of In Vitro *Borrelia burgdorferi* Persisters from an FDA Drug Library

Ying Zhang, Jie Feng, Wanliang Shi, Shuo Zhang, David Sullivan, Paul G. Auwaerter, Johns Hopkins

Abstract: Although currently recommended antibiotics for Lyme disease such as doxycycline or amoxicillin cure the majority of the patients, about 10–20% of

patients treated for Lyme disease may experience lingering symptoms including fatigue, pain, or joint and muscle aches. Under experimental stress conditions such as starvation or antibiotic exposure, *Borrelia burgdorferi* can develop round body forms, which are a type of persister bacteria that appear resistant in vitro to customary first-line antibiotics for Lyme disease. To identify more effective drugs with activity against the round body form of *B. burgdorferi*, we established a round body persister model induced by exposure to amoxicillin (50 µg/ml) and then screened the Food and Drug Administration drug library consisting of 1581 drug compounds and also 22 drug combinations using the SYBR Green I/propidium iodide viability assay. We identified 23 drug candidates that have higher activity against the round bodies of *B. burgdorferi* than either amoxicillin or doxycycline. Eleven individual drugs scored better than metronidazole and tinidazole which have been previously described to be active against round bodies. In this amoxicillin-induced round body model, some drug candidates such as daptomycin and clofazimine also displayed enhanced activity which was similar to a previous screen against stationary phase *B. burgdorferi* persisters not exposure to amoxicillin. Additional candidate drugs active against round bodies identified include artemisinin, ciprofloxacin, nifuroxime, fosfomycin, chlortetracycline, sulfacetamide, sulfamethoxy pyridazine and sulfathiazole. Two triple drug combinations had the highest activity against amoxicillin-induced round bodies and stationary phase *B. burgdorferi* persisters: artemisinin/cefoperazone/doxycycline and sulfachlorpyridazine/daptomycin/doxycycline. These findings confirm and extend previous findings that certain drug combinations have superior activity against *B. burgdorferi* persisters in vitro, even when pre-treated with amoxicillin. These findings may have implications for improved treatment of Lyme disease.

38. Park Science (NPS, Department of Interior) March 2016
[http://www.nature.nps.gov/ParkScience/Archive/PDF/Article_PDFs/ParkScience32\(1\)Summer2015_36-41_Ford_et_al_3819.pdf](http://www.nature.nps.gov/ParkScience/Archive/PDF/Article_PDFs/ParkScience32(1)Summer2015_36-41_Ford_et_al_3819.pdf)

Tick surveillance and disease prevention on the Appalachian Trail

(Also published in Appalachian Trail Journeys, The Magazine of the Appalachian Trail Conservancy, May/June 2014)

Karl Ford, Robyn Nadolny, Ellen Stromdahl, and Graham Hickling

Abstract: The Appalachian National Scenic Trail (AT) runs 3,520 km (2,187 mi) from northern Georgia to northern Maine, traversing 14 states where Lyme disease and other tickborne diseases are endemic or emerging. Approximately 2–3 million visitors hike the AT annually, including through-hikers who spend five to six months on the trail in spring through early fall, when common tick species are active. Disease vector tick surveillance was conducted from April through August 2013 at 42 randomly selected AT shelter areas along a south-to-north transect covering the full length of the AT. Tick abundance at shelters and tenting areas was compared with tick abundance on

the AT itself, and the collected ticks were tested for common bacterial pathogens. Human-biting tick species collected comprised *Ixodes scapularis*, *Amblyomma americanum*, *Amblyomma maculatum*, and *Dermacentor variabilis*. Human pathogens *Borrelia burgdorferi* and *Rickettsia montanensis* were detected in tested ticks. Tick abundance on the trail was low overall (2.8 ticks per 1,000 m² sampled), but exceeded tick abundance in shelters and tenting areas by 14.5 times. No ticks were collected south of Virginia or north of Massachusetts, or above 829 m (2,720 ft) in elevation, which suggests that season and elevation are significant determinants of the risk of hiker exposure to questing ticks on the AT. Such information should be included in future health messaging to hikers along with preventive measures. Management issues are discussed.

37. FEMS Microbiology Letters Advance Access, July 24, 2015

<http://femsle.oxfordjournals.org/content/early/2015/07/23/femsle.fnv120>

Biofilm formation by 1 *Borrelia sensu lato*

Arun Timmaraju^{1,2,†}, Priyanka A.S. Theophilus^{1,†}, Kunthavai Balasubramanian^{1,3}, Shafiq Shakih¹, David F. Leuckel and **Eva Sapi^{1,*}**

¹ Lyme disease research group, Department of Biology and Environmental Science, University of New Haven, West Haven, CT, USA

² Present address: Interpace Diagnostics, New Haven, CT, 06519

³ Present address: Department of Hematology, Yale School of Medicine, New Haven, CT, 06520

† Contributed equally

* Correspondence: Eva Sapi Ph.D., Department of Biology and Environmental Sciences, University of New Haven, 1211 Campbell Avenue, Charger Plaza LL16. West Haven, CT, 06516, USA. esapi@newhaven.edu

Abstract: Bacterial biofilms are microbial communities held together by an extracellular polymeric substance matrix predominantly composed of polysaccharides, proteins and nucleic acids. We had previously shown that *Borrelia burgdorferi sensu stricto*, the causative organism of Lyme disease in the United States is capable of

forming biofilms in vitro. Here, we investigated biofilm formation by *Borrelia afzelii* and *Borrelia garinii*, which cause Lyme disease in Europe. Using various histochemistry and microscopy techniques, we show that *Borrelia afzelii* and *Borrelia garinii* form biofilms, which resemble biofilms formed by *Borrelia burgdorferi sensu stricto*. High-resolution atomic force microscopy revealed similarities in the ultra-structural organization of the biofilms form by three *Borrelia* species. Histochemical experiments revealed a heterogeneous organization of exopolysaccharides among the three *Borrelia* species. These results suggest that biofilm formation might be a common trait of *Borrelia* genera physiology.

36. Clinical Infectious Diseases Advance Access, September 2, 2014

<http://www.ncbi.nlm.nih.gov/pubmed/25182244>

A comparison of Lyme disease serologic test results from four laboratories in patients with persistent symptoms after antibiotic treatment

Brian A. Fallon¹, Martina Pavlicova², Samantha W. Coffino³, Carl Brenner⁴

¹ Department of Psychiatry, Columbia University, New York NY

² Department of Biostatistics, Mailman School of Public Health, Columbia University, New York, NY

³ Department of Neurology, Columbia University, New York, NY

⁴ Lamont-Doherty Earth Observatory of Columbia University, Palisades, NY

Summary: In patients with post-treatment Lyme syndrome, rates of positive serologic test results were generally similar among a university laboratory, a commercial laboratory, and two Lyme specialty laboratories, although interlaboratory variability was high and the IgM Western Blot performed poorly.

Abstract: Background – As the incidence of Lyme disease (LD) has increased, a number of “Lyme specialty laboratories” have emerged, claiming singular expertise in LD testing. We investigated the degree of interlaboratory variability of several LD serologic tests—whole cell sonicate (WCS) enzyme-linked immunosorbent assay (ELISA), IgM and IgG Western blots (WB), and an ELISA based on the conserved sixth region of VlsE (C6)—performed at one university laboratory, one commercial laboratory and two laboratories that specialize in LD testing.

35. Veterinary Sciences, 2014 www.mdpi.com/2306-7381/1/1/5

Filarial Nematode Infection in Ixodes scapularis Ticks Collected from Southern Connecticut

Pabbati Namrata, Jamie M. Miller, Madari Shilpa, Patlolla Raghavender Reddy, Cheryl Bandoski, Michael J. Rossi and **Eva Sapi***

Department of Biology and Environmental Science, University of New Haven, West Haven, CT 06516, USA

*Author to whom correspondence should be addressed.

Abstract: It was recently demonstrated that the lone star tick *Amblyomma americanum* could harbor filarial nematodes within the genus *Acanthocheilonema*. In this study, *Ixodes scapularis* (deer) ticks collected from Southern Connecticut were evaluated for their potential to harbor filarial nematodes. Non-engorged nymphal and adult stage *Ixodes scapularis* ticks were collected in Southern Connecticut using the standard drag method. In situ hybridization with filarial nematode specific sequences demonstrated the presence of filarial nematodes in *Ixodes* ticks. Filarial nematode specific DNA sequences were amplified and confirmed by direct sequencing in *Ixodes* nymphal and adult ticks using either general filarial nematode or Onchocercidae family specific PCR primers. Phylogenetic analysis of the 12S rDNA gene sequence indicated that the filarial nematode infecting *Ixodes scapularis* ticks is most closely related to the species found in *Amblyomma americanum* ticks and belongs to the genus of *Acanthocheilonema*. Our data also demonstrated that infection rate of these filarial nematode in *Ixodes* ticks is relatively high (about 22% and 30% in nymphal and adult *Ixodes* ticks, respectively). In summary, the results from our studies demonstrated that filarial nematode infection was found in *Ixodes* ticks similar to what has been found in *Amblyomma americanum* ticks.

Vet. Sci. 2014, 1, 5-15; doi:10.3390/vetsci1010005

34. Psychosomatics 2013
<http://www.psychosomaticsjournal.com/article/S0033-3182%2813%2900078-9/fulltext>
Correlates of Perceived Health-Related Quality of Life in Post-treatment Lyme Encephalopathy

Avinash M.Chandra , B.A.-1, John G. Keilp, Ph.D.-2, **Brian A. Fallon***, M.D.-2

1 Department of Psychiatry, Columbia University, New York, NY.

2 Division of Clinical Therapeutics of the New York State Psychiatric Institute, New York, NY

*Corresponding Author: Brian A. Fallon, M.D baf1@columbia.edu

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 the Short-Form 36. 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.

33. International Journal of Medical Sciences 2013
<http://www.medsci.org/v10p0915.htm>

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

Corresponding author: Kerry L. Clark, M.P.H., Ph.D., Department of Public Health, University of North Florida,

1 UNF Drive, Jacksonville, FL 32224. Phone: (904) 620-1427. Fax: (904) 620-2848. E-mail: kclark@unf.edu.

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.

32. Northeastern Naturalist 2013; 20(1):197–204.
<http://www.bioone.org/doi/abs/10.1656/045.020.0116>

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

*Corresponding author – alan.giese@lyndonstate.edu.

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)

31. J Neuropsychiatry Clin Neurosci. 2013 Feb 27. doi: 10.1176/appi.neuropsych.12090223.

<http://www.ncbi.nlm.nih.gov/pubmed/23446551>

Post-Treatment Lyme Syndrome and Central Sensitization.

Batheja S, Nields JA, Landa A, **Fallon BA***.

Department of Psychiatry, Columbia University, and The New York State Psychiatric Institute.

*Send correspondence to Dr. Fallon; e-mail: baf1@columbia.edu

ABSTRACT: Central sensitization is a process that links a variety of chronic pain disorders that are characterized by hypersensitivity to noxious stimuli and pain in response to non-noxious stimuli. Among these disorders, treatments that act centrally may have greater efficacy than treatments acting peripherally. Because many individuals with post-treatment Lyme syndrome (PTLS) have a similar symptom cluster, central sensitization may be a process mediating or exacerbating their sensory processing. This article reviews central sensitization, reports new data on sensory hyperarousal in PTLS, explores the potential role of central sensitization in symptom chronicity, and suggests new directions for neurophysiologic and treatment research.

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

<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3474942/>

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

Brian A. Fallon*¹, Eva Petkova², John G. Keil³ and Carolyn B. Britton⁴

¹ Columbia University, Dept. of Psychiatry, Division of Clinical Therapeutics, USA

2 New York University, Dept. of Child and Adolescent Psychiatry, Division of Biostatistics, USA

3 Columbia University, Dept. of Psychiatry, Division of Neuroscience, USA

4 Columbia University, Dept. of Neurology, USA

*Send correspondence to Dr. Fallon; e-mail: baf1@columbia.edu

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.

29. PLoS ONE 7(10) 2012: e48277. doi:10.1371/journal.pone.0048277

<http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0048277>

Characterization of Biofilm Formation by *Borrelia burgdorferi* In Vitro

Eva Sapi*, Scott L. Bastian, Cedric M. Mpooy, Shernea Scott, Amy Rattelle, Namrata Pabbati, Akhila Poruri, Divya Burugu, Priyanka A. S. Theophilus, Truc V. Pham, Akshita Datar, Navroop K. Dhaliwal, Alan MacDonald, Michael J. Rossi, David F. Luecke (Lyme Disease Research Group, Department of Biology and Environmental Sciences, University of New Haven, West Haven, Connecticut, United States of America); Saion K. Sinha (Department of Physics, University of New Haven, West Haven, Connecticut, United States of America).

*Corresponding Author Email: Eva Sapi, esapi@newhaven.edu

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.

28.

PLOS

One

2012

<http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0033280>

Genome Stability of Lyme Disease Spirochetes: Comparative Genomics of *Borrelia burgdorferi* Plasmids

Sherwood R. Casjens^{1*}, Emmanuel F. Mongodin², Wei-Gang Qiu³, Benjamin J. Luft⁴, **Steven E. Schutzer**⁵, Eddie B. Gilcrease¹, Wai Mun Huang¹, Marija Vujadinovic¹, John K. Aron¹, Levy C. Vargas³, Sam Freeman³, Diana Radune⁶, Janice F. Weidman⁶, George I. Dimitrov⁶, Hoda M. Khouri⁶, 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

*Corresponding Author Email: sherwood.casjens@path.utah.edu

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.

27. Journal of Bacteriology 2011 <http://jb.asm.org/content/193/4/1018.long>

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

Steven E. Schutzer^{1,*}, Claire M. Fraser-Liggett², Sherwood R. Casjens^{3,*}, 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

*Corresponding author. Mailing address for Steven E. Schutzer: Department of Medicine, University of Medicine and Dentistry of New Jersey–New Jersey Medical School, Newark, NJ 07103. E-mail: schutzer@umdnj.edu. Mailing address for Sherwood R. Casjens: Department of Pathology, University of Utah Medical School, Room 2200 EEJMRB, 15 North Medical Dr. East, Salt Lake City, UT 84112. E-mail: sherwood.casjens@path.utah.edu

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.

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

<http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0017287>

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

*Corresponding Author Email: schutzer@umdnj.edu

#These authors contributed equally to this work

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.

25. Genetics: Published Articles Ahead of Print, published on September 2, 2011 as 0.1534/genetics.111.130773 Copyright 2011

<http://www.genetics.org/content/189/3/951.long>

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

James Haven*,¹, Levy C. Vargast, Emmanuel F. Mongodin‡, Vincent Xue§, Yozen Hernandez†, Pedro Pagant, Claire M. Fraser-Liggett‡, **Steven E. Schutzer****, Benjamin J. Luft††, Sherwood R. Casjens‡‡, and Wei-Gang Qiu†*, §§, 2

*Department of Biology, The Graduate Center, City University of New York, New York, New York 10016

†Department of Biological Sciences and The Center for Gene Structure and Function and

§Department of Computer Science, Hunter College, City University of New York, New York, New York 10065

‡Institute for Genome Sciences, University of Maryland BioPark, Baltimore, Maryland 21201

**Department of Medicine, University of Medicine and Dentistry of New Jersey–New Jersey Medical School, Newark, New Jersey 07103

††Department of Medicine, Health Science Center, Stony Brook University, Stony Brook, New York 11794

‡‡Department of Pathology, Division of Molecular Cell Biology and Immunology, University of Utah School of Medicine, Salt Lake City, Utah 84112

§§National Evolutionary Synthesis Center, Durham, North Carolina 27705

1 Present address: Odum School of Ecology, University of Georgia, Athens, GA 30602.

2 Corresponding author: Department of Biological Sciences, Hunter College of the City University of New York, 695 Park Ave., New York, NY 10065. E-mail: weigang@genectr.hunter.cuny.edu

ABSTRACT: How genomic diversity within bacterial populations originates and is maintained in the presence 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.

24. Journal of Medical Entomology 47(1):89-94. 2010
<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2837073/>

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 (Mark W. Eshoo 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.

23. PLoS ONE 5(5) 2010

<http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0010650>

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

Chris D. Crowder, Heather E. Matthews, Megan A. Rounds, Curtis A. Phillipson, Feng Li, Ranga Sampath, David J. Ecker, Mark W. Eshoo* – Ibis Biosciences, Carlsbad, California, United States of America; Scott R. Campbell – Suffolk County Department of Health Services, Yaphank, New York, United States of America; Benjamin J. Luft – Department of Medicine, State University of New York at Stony Brook, Stony Brook, New York, United States of America; Oliver Nolte – Laboratory of Dr. Brunner, Constance, Germany; **Steven Schutzer** – Department of Medicine, University of Medicine and Dentistry of New Jersey, Newark, New Jersey, United States of America

*Corresponding Author Email: Mark W. Eshoo meshoo@ibisbio.com

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 than previously reported and can have potential clinical consequences.

22. Neurobiology of Disease 2010

<http://www.sciencedirect.com/science/article/pii/S0969996109003386>

Inflammation and central nervous system Lyme disease.

Brian A. Fallon*-a, d, Elizabeth S. Levin-b, Pernilla J. Schweitzer-b, David Hardestya-c

a Department of Psychiatry, Columbia University, New York, NY, USA

b College of Physicians and Surgeons, Columbia University, New York, NY, USA

c Department of Neurology, Columbia University, New York, NY, USA

d New York State Psychiatric Institute, New York, NY, USA

*Corresponding Author, Dr. Fallon, Columbia University Medical Center, 1051 Riverside Drive, New York, NY 10032, United States. Fax: +1 212 543 6515. baf1@columbia.edu

ABSTRACT: Lyme disease, caused by the bacterium *Borrelia burgdorferi*, can cause multi-systemic signs and symptoms, including peripheral and central nervous system disease. This review examines the evidence for and mechanisms of inflammation in neurologic Lyme disease, with a specific focus on the central nervous system, drawing upon human studies and controlled research with experimentally infected rhesus monkeys. Directions for future human research are suggested that may help to clarify the role of inflammation as a mediator of the chronic persistent symptoms experienced by some patients despite antibiotic treatment for neurologic Lyme disease.

21. Arch Gen Psychiatry. 2009;66(5):554-563. <http://archpsyc.jamanetwork.com/article.aspx?articleid=483068>
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

Author Affiliations: Departments of Psychiatry (Drs Fallon, Corbera, Yu, Keilp, Petkova, Lisanby, Moeller, Slavov, Mensh, and Sackeim), Radiology (Drs Van Heertum and Sackeim), and Biostatistics (Dr Petkova), College of Physicians and Surgeons of Columbia University, New York, New York; the New York State Psychiatric Institute, New York (Drs Fallon, Corbera, Yu, Nobler, Keilp, Petkova, Lisanby, Moeller, Slavov, Mensh, and Sackeim); the Department of Neural and Behavioral Science, State University of New York at Downstate, New York (Mr Lipkin); and the Department of Psychiatry and Behavioral Sciences, New York Medical College, New York (Dr Nobler).
*Corresponding Author – Brian A. Fallon, MD, Department of Psychiatry, College of Physicians and Surgeons of Columbia University, New York, New York 10032, USA. baf1@columbia.edu

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.

20. Gene 2009 <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2743244/>

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

Ewa Wywiał,^{1,2,*} James Haven,^{1,3,*} Sherwood R. Casjens,⁴ Yozen A. Hernandez,³ Shaneen Singh,^{1,2} Emmanuel F. Mongodin,⁵ **Claire M. Fraser-Liggett**,⁵ **Benjamin J. Luft**,⁶ **Steven E. Schutzer**,⁷ and Wei-Gang Qiu^{1,3,§}

1 Department of Biology, The Graduate Center of the City University of New York, 365 Fifth Avenue, New York, NY 10016, USA

2 Department of Biology, Brooklyn College, City University of New York, 2900 Bedford Avenue, Brooklyn, NY 11210, USA

3 Department of Biological Sciences and the Center for Gene Structure and Function, Hunter College, City University of New York, 695 Park Avenue, New York, NY 10065, USA

4 Department of Pathology, Division of Molecular Cell Biology and Immunology, University of Utah School of Medicine, Salt Lake City, UT 84112, USA

5 Institute for Genome Sciences, University of Maryland BioPark, 801 West Baltimore Street, Baltimore, MD 21201, USA

6 Department of Medicine, Health Science Center, Stony Brook University, Stony Brook, NY 11794, USA

7 Department of Medicine, University of Medicine and Dentistry of New Jersey-New Jersey Medical School, 185 South Orange Avenue, Newark, NJ 07103, USA

§ Corresponding Author: Weigang Qiu, Department of Biological Sciences, Hunter College of the City University of New York, 695 Park Avenue, New York, NY 10065, USA, Phone: 1-212-772-5296, Fax: 1-212-772-5227, Email: ude.ynuc.retnuh.rtceneg@gnagiew

*Both authors contributed equally to the research

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.

19. Neurology May 2008 <http://www.neurology.org/content/70/13/992.long>

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

B. A. Fallon, MD*, J. G. Keilp, PhD, K. M. Corbera, MD, E. Petkova, PhD, C. B. Britton, MD, E. Dwyer, MD, I. Slavov, PhD, J. Cheng, MD, PhD, J. Dobkin, MD, D. R. Nelson, PhD and 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.).

*Dr. Fallon – Columbia University, 1051 Riverside Drive, Unit 69, New York, NY 10032, USA. baf1@columbia.edu

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.

18. Emerging Infectious Diseases Volume 14, Number 7–July 2008

<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2600328/>

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† **Sequencing Group acknowledged (Schutzer)**

*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

Address for correspondence: Wei-Gang Qiu, Department of Biological Sciences, Hunter College of the City University of New York, 695 Park Ave, New York, NY 10065, USA; email: weigang@genectr.hunter.cuny.edu

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.

17. Minerva Medica October 2008;99(5):489-96.

<http://www.ncbi.nlm.nih.gov/pubmed/18971914>

Severity of Lyme disease with persistent symptoms. Insights from a double-blind placebo-controlled clinical trial

Daniel Cameron, MD, Northern Westchester Hospital, Mount Kisco, NY, USA.

cameron@lymeproject.com

ABSTRACT: Lyme disease is a global health concern and is the world's leading tick borne infection caused by the spirochete, *Borrelia burgdorferi*, that has been associated with numerous neurologic, rheumatologic and psychiatric manifestations. The symptoms of Lyme disease have been characterized as either severe or "related to the aches and pains of daily living." A randomized double-blind, placebo-controlled clinical trial (RCT) was conducted in a primary internal medicine practice in

Westchester County, New York, USA. A total of 84 adults with Lyme disease with persistent symptoms (LDPS) were studied; 52 received amoxicillin and 34 received placebo. The subjects received either placebo or amoxicillin 3 g per day orally for 3 months. The SF-36 was used as the outcome measure of the patient's perceived Quality of Life (QOL). For subjects enrolling in this RCT, the average SF-36 physical component summary (PCS) of QOL (40+/-9, range 29-44) and mental component summary (MCS) of QOL (39+/-14, range 23-46) were worse than the general USA population and worse than individuals with diabetes, heart disease, depression, osteoarthritis or rheumatoid arthritis. The improvements in the SF-36 measure of QOL for subjects randomized to amoxicillin vs. placebo was significant (46% vs 18%, P=0.007). It is important for clinicians to be aware that LDPS can be severe. A significant gain in the QOL for subjects randomized to amoxicillin in this RCT without serious adverse events is consistent with the goal of improving patient's QOL and consequently worthy of further study.

16. Microbial Pathogenesis Nov-Dec 2008
<http://www.sciencedirect.com/science/article/pii/S0882401008001253>

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

Yun Xu, John F. Bruno*, Benjamin J. Luft – Department of Medicine, State university of New York at Stony Brook, Stony Brook, NY 11794, USA

*Corresponding author – Department of Medicine, T-15 Room 060, SUNY at Stony Brook, Stony Brook, NY 11794-8154, USA. Tel.: +1 631 444 2054; fax: +1 631 444 2493.

jbruno@notes.cc.sunysb.edu

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.

15. Infection and Immunity, January 2006
<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1346608/>

Identification of *Borrelia burgdorferi* outer surface proteins

Brooks CS-1; Vuppala SR-2; Jett AM-2; Akins DR*-2 **Sequencing Group acknowledged (Schutzer)**

1Department of Biology, Austin Peay State University, Clarksville, Tennessee 37044,
2Department of Microbiology and Immunology, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma 731042

*Corresponding author – Mailing address: Department of Microbiology and Immunology, The University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104. Phone: (405) 271-2133 ext. 46640. Fax: (405) 271-3117. E-mail: darrin-akins@ouhsc.edu

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.

14. J Int Neuropsychol Soc. 2006 Jan;12(1):119-29
<http://www.ncbi.nlm.nih.gov/pubmed/16433951>

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

John G. Keilp a1 a2 c1 , Kathy Corbera a1 a3 , Iordan Slavov a1 a3 , Michael J. Taylor a5 , Harold A. Sackeim a1 a4, and **Brian A. Fallon** a1 a3

a1 Columbia University College of Physicians and Surgeons, Department of Psychiatry, New York, New York

a2 New York State Psychiatric Institute, Department of Neuroscience, New York, New York

a3 New York State Psychiatric Institute, Department of Therapeutics, New York, New York

a4 New York State Psychiatric Institute, Department of Biological Psychiatry, New York, New York

a5 Department of Psychiatry, University of California at San Diego, California

c1 Reprint requests to: John Keilp, Ph.D., Columbia University College of Physicians and Surgeons, Department of Psychiatry, Box 42, NYSPI, 1051 Riverside Drive, New York, NY 10032. E-mail: jgk13@columbia.edu

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.).

13. Journal of Clinical Microbiology, February 2005
<http://jcm.asm.org/content/43/2/850.long>

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

Elizabeth S. Ravechel, Steven E. Schutzer,^{1*} Helen Fernandes¹, Helen Bateman¹, Brian A. McCarthy¹, Steven P. Nickell², and Madeleine W. Cunningham³.

¹ Departments of Pathology and Medicine, New Jersey Medical School, University of Medicine and Dentistry of New Jersey, Newark, New Jersey, ² Department of Molecular Genetics and Microbiology, University of New Mexico, Albuquerque, New Mexico, ³ University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma.

*Corresponding author. Mailing address: Department of Medicine, New Jersey Medical School, University of Medicine and Dentistry of New Jersey, MSB E543, 185 S. Orange Ave., Newark, NJ 07103. Phone: (973) 972-4872. Fax: (801) 383-8534. E-mail: schutzer@umdnj.edu.

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*.

12. Expert Review Anti-Infective Therapy 2004
http://www.ilads.org/lyme/ILADS_Guidelines.pdf

Evidence-based guidelines for the management of Lyme disease

International Lyme & Associated Diseases Society Lyme Disease Treatment Guidelines

ILADS Working Group: Dan Cameron, MD, MPH-1 [Internal Medicine and Epidemiology, Mt. Kisco, New York]; Andrea Gaito, MD [Rheumatology, Basking Ridge, New Jersey]; Nick Harris, PhD [Immunology, Palo Alto, California]; Gregory Bach, DO [Family and Integrative Medicine, Colmar, Pennsylvania]; Sandra Bellovin, MD [Family Practice, Portsmouth, Virginia]; Kenneth Bock, MD [Family Practice, Rhinebeck, New York]; Steven Bock, MD [Family Practice, Rhinebeck, New York]; Joseph Burrascano, MD [Internal Medicine, East Hampton, New York]; Constance Dickey, RN [Registered Nurse, Hampden, Maine]; Richard Horowitz [Internal Medicine, Hyde Park, New York]; Steven Phillips, MD [Internal Medicine, Ridgefield, Connecticut]; Lawrence Meer-Scherrer MD [Internal Medicine, Flamatt, Switzerland]; Bernard Raxlen, MD [Psychiatry, Greenwich, Connecticut]; Virginia Sher, MD [Psychiatry, Holland, Pennsylvania]; Harold Smith, MD [Emergency Medicine, Danville, Pennsylvania]; Pat Smith [President, Lyme Disease Association]*; Ray Stricker MD [Hematology and Immunotherapy, San Francisco, California]

1-ILADS, P.O. Box 341461, Bethesda, MD 20827-1461, USA.

***Pat Smith, LDA, was co-author on article**

ABSTRACT: This report, completed in November 2003, is intended to serve as a resource for physicians, public health officials and organizations involved in the evaluation and treatment of Lyme disease.

11. Proceedings of the National Academy of Science, Sept. 2004
<http://www.pnas.org/content/101/39/14150.long>

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 §

Author Affiliations: *Department of Biological Sciences, Hunter College of the City University of New York, 695 Park Avenue, New York, NY 10021; †Department of Medicine, New Jersey Medical School, 185 South Orange Avenue, Newark, NJ 07103; §Department of Medicine, Health Science Center, Stony Brook University, Stony Brook, NY 11794; ¶Biology Department, Brookhaven National Laboratory, Upton, NY 11793; □The Institute for Genomic Research, 9712 Medical Center Drive, Rockville, MD 200850; and **Department of Pathology, Division of Molecular Cell Biology and Immunology, University of Utah Medical School, Salt Lake City, UT 84132

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 ecologically isolated samples. Frequent recombination implies a potential for rapid adaptive evolution and a possible polygenic basis of B. burgdorferi pathogenicity.

10. The Journal of Neuropsychiatry & Clinical Neurosciences. 2003
<http://neuro.psychiatryonline.org/article.aspx?articleid=101812>

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. Address correspondence to Dr. Brian A. Fallon, NYS Psychiatric Institute, 1051 Riverside Drive, #69, New York, NY 10032

ABSTRACT: This study examined brain functioning in patients with Lyme encephalopathy. Eleven patients underwent neuropsychological tests and Xenon133-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 (k2) ($p=.004$), 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.

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

<https://www.ilads.org/wp-content/uploads/2018/10/JSTBD-VOL9-SPRING-SUMMER-02-1.pdf>

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

Martin Fried, MD*; Dorothy Pietrucha, MD†, Gaye Madigan, RN‡, Aswine Bal, MD§

*Departments of Pediatric Gastroenterology, †Pediatric Neurology, ‡Academic Affairs, and §Pediatric Infectious Disease, Jersey Shore Medical Center, Neptune, New Jersey

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.

8. Journal of Neuropsychiatry and Clinical Neurosciences. 2001 13:500-5-7
<http://neuro.psychiatryonline.org/doi/pdf/10.1176/jnp.13.4.500>

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

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

From the Columbia University Department of Psychiatry, Division of Behavioral Medicine, New York, New York. Address correspondence to Dr. Tager, Columbia Presbyterian Medical Center, 622 West 168th Street, Box 427, New York, NY 10032. E-mail: ft49@columbia.edu.

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.

7. Medscape Infectious Diseases 2(1) April 2000

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

<http://www.medscape.com/viewarticle/432883#4>

Charles Pavia, PhD

NY Medical College School of Medicine. NYCOM Microbiology and Immunodiagnostic Laboratory of NYIT.

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 C01 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.

6. Journal of Spirochetal and Tick-borne Diseases Fall/Winter 1999

Repeated Antibiotic Treatment in Chronic Lyme Disease.

<https://www.ilads.org/wp-content/uploads/2018/10/JSTBD-VOL6-FALL-WINTER-99-3.pdf>

Brian A. Fallon, MD, Felice Tager, PhD, John Keilp, PhD, Nicola Weiss, PhD, Michael R. Liebowitz, MD,

New York State Psychiatric Institute and Columbia University Department of Psychiatry, New York, New York; Lesley Fein, MD, Private Practice, West Caldwell, New Jersey; Kenneth Liegner, MD, Private Practice, West Caldwell, New Jersey.

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.

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

<http://jama.jamanetwork.com/article.aspx?articleid=192130>

Borrelia Burgdorferi-Specific Immune Complexes in Acute Lyme Disease.

Steven E. Schutzer, MD; P. K. Coyle, MD; Patrick Reid, MS; Bart Holland, PhD

Author Affiliations: Department of Medicine, Division of Allergy and Immunology (Dr Schutzer and Mr Reid) and Department of Preventive Medicine (Dr Holland), University of Medicine and Dentistry of New Jersey-New Jersey Medical School, Newark (Dr Schutzer and Mr Reid); and the Department of Neurology, State University of New York, Stony Brook (Dr Coyle).

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.

4. Neurology, Oct 12, 1999 <http://www.neurology.org/content/53/6/1340.long>

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

Schutzer SE-1, Natelson BH.

1 Department of Medicine, University of Medicine and Dentistry, New Jersey Medical School, Newark 07103, USA. schutzer@umdnj.edu

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.

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

<http://www.psych.theclinics.com/article/S0193-953X%2805%2970032-0/fulltext>

The Underdiagnosis of Neuropsychiatric Lyme Disease in Children and Adults.

Brian A. Fallon, MD, MPH-1, Janice M. Kochevar, NP, Andrea Gaito, MD, Jenifer A. Niels, MD

1-Department of Psychiatry, Columbia University Medical Center and the Lyme Disease Research Program, New York, New York (BAF), 2-private practice, Armonk, New York (JMK), 3-Department of Medicine, Seton Hall University, and private practice, Basking Ridge, New Jersey (AG), 4-Department of Psychiatry, Yale University School of Medicine, New Haven, Connecticut (JAN)

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.

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

<http://www.ncbi.nlm.nih.gov/pubmed/9861561?dopt=Abstract>

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

Phillips SE-1, Mattman LH, Hulinska D, Moayad H

1Greenwich 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 immunoelectron 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.

1. Infection 24 (1996) #5 <http://www.ncbi.nlm.nih.gov/pubmed/8923044>

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

Bayer ME-1, Zhang L, Bayer MH.

1-Fox Chase Cancer Center, Philadelphia, PA 19111, USA.

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. June 2015
PO Box 1438 Jackson, NJ 08527
www.LymeDiseaseAssociation.org
888-366-6611