Alberg, Doreen & Blank, Denise

Doreen Alberg & Denise Blank
Poster Presentation
Improved Diagnostics for Lyme Borreliosis Using a New Stripe Immunoblot Test System
Sharon Hospital Laboratory
Sharon, CT

2017 Poster Presentation

Improved Diagnostics for Lyme Borreliosis Using a New Stripe Immunoblot Test System

Introduction: Lyme Borreliosis, caused by the spirochete Borrelia burgdorferi, is the most commonly reported vector-borne infection in the United States. In the United States, the CDC prescribes a two-tier testing algorithm for determining if a patient is infected1. The primary step is a highly sensitive screen assay, using either an IFA (Indirect fluorescence assay) or an ELISA/EIA derived from Borrelia lysate or highly immunogenic C6 peptide. Patient samples that are positive or equivocal on screen assay are further confirmed using a highly specific immunoblot assay. Traditionally this was performed using western blots (WB) derivedfrom B. burgdorferi lysate. Although this is a highly sensitive and effective test system, the interpretation of results is complicated by immunoreaction of samples to non-specific portions of the B. burgdorferi proteome and variation in the CDC characterized immunogenic band locations. Line immunoblot technology, in which highly purified Borrelia antigens expressed using recombinant systems are striped on predefined locations on membranes, has simplified several

procedural and interpretation challenges associated with WB 2. Moreover, the line blots are well suited for automated execution and result interpretation of the assays.

Study objective: This study compares the performance of a new line immunoblot assay to that of the traditional WB test used for serological confirmation of Lyme infection and highlights the advantages of the new format for the clinical labs.

Methods: Patient Population: Between 7/20/2016 and 8/29/2016, 274 patient samples testing equivocal or positive on a first-tier screen test (Device ref# VIDAS?) (clinical immunology laboratory at Sharon Hospital (Sharon, CT) were confirmed by MarBlot IgG test system (Trinity Biotech Plc). The same sera were further evaluated on a new LIA test system (B. burgdorferi MarStripe IgG test system, Trinity Biotech Plc).

Results: MarBlot test system provided an overall sensitivity of 47.8% . Overall agreement between the MarBlot (WB) device and the new MarStripe (LIA) was 95.6% (262/274). Positive agreement between the MarBlot (WB) device and the new MarStripe (LIA) was 94.7% (124/131) and negative agreement between the two devices was 96.5% (138/143).

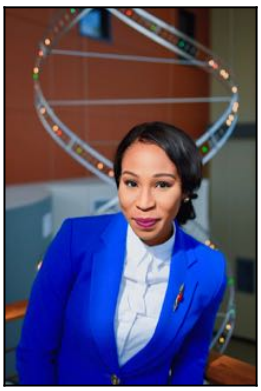
Conclusions:
•Western blot is the gold standard confirmatory device for Lyme borreliosisby feature of presenting the complete B. burgdorferi proteome.
•The MarStripe IgG test preserves the superior diagnostic performance of a western blot, and substantially improves the accuracy and ease of interpreting immunoblot reactions.
•The MarStripe IgG kit uses a next generation reagent set that is upgraded for superior performance and stability.

References:
1. Engstrom, S. M., E. Shoop, and R. C. Johnson. 1995. Immunoblot interpretation criteria for serodiagnosisof early Lyme disease. Journal of clinical microbiology 33: 419-427.

2. Louis A. Magnarelli, S.J.P. ErolFikrigand A. R. A. F. John F. Anderson. 1996. Use of Recombinant Antigens of Borreliaburgdorferiin Serologic Tests for Diagnosis of Lyme Borreliosis. Journal of clinical microbiology: 237–224.

Izuogu, Adaeze

Adaeze O. Izuogu, PhD
Poster Presentation
The Innate Antiviral Response in Peromyscus leucopus Confers a Potent Restriction to Tick-Borne Flaviviruses
Dept. of Medical Microbiology & Immunology
U of Toledo Coll of Med & Life Sci
Toledo, OH



Adaeze Izuogu is a biomedical research scientist with over 6 years of bench work experience in the study of infectious diseases. She currently works as a postdoctoral research fellow at the University of Toledo investigating the innate immune responses to Flaviviruses in reservoir host species. Adaeze completed her doctoral training in medical microbiology and immunology at the University of Toledo after receiving a bachelor’s degree in Microbiology at Covenant University Nigeria. While in Nigeria, she worked in the public health laboratory sector where she contributed to HIV/AIDS management and diagnosis of other endemic diseases. Her scientific progress has been recognized on various platforms through honors, awards, and publication of scholarly articles. In her spare time, she is a photographer and sings in a choir.

2017 Poster Presentation

The Innate Antiviral Response in *Peromyscus leucopus* Confers a Potent Restriction to Tick-Borne Flaviviruses

Adaeze O. Izuogu[1], Kristin L. McNally[2], Stephen E. Harris[3], Brian H. Youseff[1], John B. Presloid[1], Christopher Burlak[4], Jason Munshi-South[5], Sonja M. Best[2], R. Travis Taylor[1]

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[2] Innate Immunity and Pathogenesis Unit, Laboratory of Virology, Rocky Mountain Laboratories, DIR, NIAID, NIH, Hamilton, Montana, United States of America

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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-

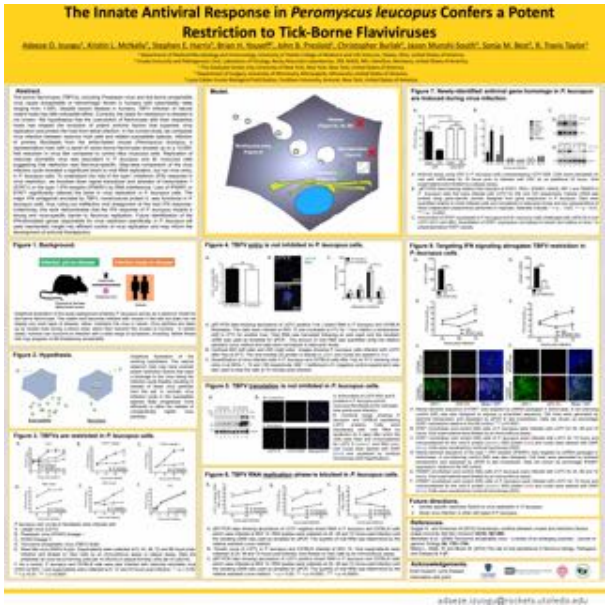
footed mouse (Peromyscus leucopus, a representative host) with a panel of vector-borne flaviviruses showed up to a 10,000-fold reduction in virus titer compared to control Mus musculus cells. Replication of vesicular stomatitis virus was equivalent in P. leucopus and M. musculus cells suggesting that 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 1 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.

Background: Graphical illustration of the study background whereby P. leucopus serves as a reservoir model for tick-borne flaviviruses. The rodent host becomes infected with viruses in the wild but does not not display any overt signs of disease rather, maintains the virus in nature. Virus particles are taken up by Ixodes ticks during a blood meal, which then transmit the viruses to humans. In certain cases, humans can succumb to infection with a wide range of symptoms, including febrile illness that may progress to life-threatening encephalitis.

Future Directions:

1. Identify specific restriction factors to virus replication in P. leucopus.
2. Study virus infection in other cell types of P. leucopus.

Acknowledgements: Grant Support Lyme Disease Association pilot grant



Horn, Liz 2

Liz Horn, PhD, MBI
Poster Presentation
Human Tissue Collection
Principal Investigator
Lyme Disease Biobank
Portland, OR
https://lymebiobank.org/

Liz has spent more than a decade building research initiatives and collaborations with non-profit organizations, with a focus on registries and biobanks. She has been working in Lyme

disease since 2013 and was part of the team that launched the Lyme Disease Biobank. The Lyme Disease Biobank was created to provide much-needed samples to researchers studying Lyme disease and other tick-borne infections, and each participant's sample donation supports up to 50 different research projects. Liz earned her doctorate in molecular pharmacology and cancer therapeutics from SUNY at Buffalo, was a National Library of Medicine fellow in biomedical informatics, and received her M.B.I. from Oregon Health & Science University. She has mentored and trained >75 advocacy organizations in the translational research enterprise, and helped these groups initiate collaborations with academia, other non-profits, and industry. Her strong foundation in basic science, informatics, registry questionnaire design, governance, and biobank planning and operations makes her well positioned to serve as the Principal Investigator.

2017 Poster Presentation

Lyme Disease Biobank and National Disease Interchange Partnership: Human Tissue Collection Program to Accelerate Biomedical Research for Lyme Disease and other Tick-Borne Infections

Liz Horn, PhD, MBI[1], Melissa VonDran, PhD[2], Honesto I. Nunez III[2], Ashley Flint[2], Saboor Shad[2], Cristina Kelly[2], Alisa McDonald[2], Thomas J. Bell, MS, PhD[2]

[1]Lyme Disease Biobank, Portland, OR; [2]National Disease Research Interchange, Philadelphia, PA

Introduction: The use of human biospecimens provides scientists with a direct experimental model system to advance our understanding of human biology, physiology, disease and other related areas of scientific research. When available,

biospecimens play a key role in accelerating bench-to-bedside studies identifying new medical treatment options. The Lyme Disease Biobank (LDB) currently maintains a collection of blood, serum, and urine from individuals with early, acute suspected Lyme disease (LD) and endemic controls. LDB has partnered with the National Disease Research Interchange (NDRI) to expand this collection to include a diverse range of human tissue biospecimens. NDRI has over 35 years of experience serving as a critical link between individuals wishing to donate organs and tissues for research and the nation's leading investigators who are working to find new treatments or cures for a wide range of diseases. NDRI's Donor Programs give patients and their family members an opportunity to make a significant contribution to research and development by providing a straightforward mechanism through which tissues and organs can be donated. The LDB-NDRI partnership will enable the collection of post-mortem and surgical tissue samples from individuals with LD or other tick-borne infections (TBI), providing the research community with a much needed resource to better understand these complex infections.

Methods: LDB and NDRI are developing a two pronged approach to accelerate knowledge in LD and TBI:
1) identify patients interested in donating biospecimens for research and
2) recruit biomedical investigators who can utilize these biospecimens to advance research, diagnostics, and treatment.

During the developmental phase, LDB and NDRI are creating procedures for this program by soliciting feedback from all stakeholders, including clinicians, investigators, and patients and families, to help guide the process. Once the program is launched, NDRI will maintain a registry of individuals interested in donating tissues, obtain consent for donation, develop a donation plan, and coordinate the recovery, packaging and shipping of biospecimens to the LDB for use by approved researchers. To expand the impact of this

program, customized recovery protocols will be developed by LDB and NDRI to address the major experimental needs within the Lyme research community. This approach ensures that biospecimens are suitable for the experimental objectives and procedures for numerous investigators, thus greatly amplifying the usefulness and importance of this resource.

Conclusion: Together, LDB and NDRI will provide the research community with well annotated tissue biospecimens that are suitable for state-of-the-art experimental methods. Collectively, this approach can play a key role in accelerating the bench-to-bedside pathway to develop improved diagnostics and new treatments for patients with LD and other TBI.

Funding Acknowledgement: Lyme Disease Biobank is a program of Bay Area Lyme Foundation. This program is funded by Bay Area Lyme Foundation and the Steven & Alexandra Cohen Foundation.

Horn, Liz

Liz Horn, PhD, MBI
Poster Presentation
Biorepositories
Principal Investigator
Lyme Disease Biobank
Portland, OR
https://lymebiobank.org/

Liz has spent more than a decade building research initiatives and collaborations with non-profit organizations, with a focus on registries and biobanks. She has been working in Lyme disease since 2013 and was part of the team that launched the Lyme Disease Biobank. The Lyme Disease Biobank was created to provide much-needed samples to researchers studying Lyme disease and other tick-borne infections, and each

participant’s sample donation supports up to 50 different research projects. Liz earned her doctorate in molecular pharmacology and cancer therapeutics from SUNY at Buffalo, was a National Library of Medicine fellow in biomedical informatics, and received her M.B.I. from Oregon Health & Science University. She has mentored and trained >75 advocacy organizations in the translational research enterprise, and helped these groups initiate collaborations with academia, other non-profits, and industry. Her strong foundation in basic science, informatics, registry questionnaire design, governance, and biobank planning and operations makes her well positioned to serve as the Principal Investigator.

2017 Poster Presentation

Bay Area Lyme Foundation’s Biorepository, The Lyme Disease Biobank – a Resource to Advance Our Understanding Of Lyme Disease and Other Tick-Borne Infections: Characterization of Samples Collected from 2014-2016

Liz Horn, PhD, MBI[1]; George Dempsey, MD[2]; Anna Schotthoefer, PhD[3]; U. Lena Prisco, PhD[4]; Caesar Djavaherian, MD, MS[5]; Stephanie Kumamoto[5]; Marc Golightly, PhD[6]; Cathy De Luca, BS[6]; Mel Evans, BS[6]; Bobbi Pritt, MD[7]; Elitza Theel, PhD[7]; Radha Iyer, PhD[8]; Dionysios Liveris, PhD[8]; Guiqing Wang, MD[8]; Ira Schwartz, PhD[8].

[1]Lyme Disease Biobank, Portland, OR; [2]East Hampton Family Medicine, East Hampton, NY; [3]Marshfield Clinic Research Institute, Marshfield, WI; [4]Vineyard Center for Clinical Research, Martha’s Vineyard, MA; [5]Direct Urgent Care, Mountain View, CA; [6]Stony Brook University, Stony Brook, NY; [7]Mayo

Clinic, Rochester, NY; [8]New York Medical College, Valhalla, NY.

Introduction: Biorepositories are increasingly important resources for advancing scientific research and knowledge. The Lyme Disease Biobank is a collection of human biological samples to facilitate research in the field of Lyme disease (LD) and other tick-borne infections (TBI).

Materials and Methods: This multisite initiative, launched in 2014, collects whole blood, serum, and urine samples from individuals with suspected acute Lyme disease presenting with or without an erythema migrans (EM) or annular rash (cases) and unaffected individuals (endemic controls). Participants with suspected Lyme disease (all cases) are given an optional opportunity to provide a second sample 2-3 months after the first visit, and provide updated clinical information at 3, 6, and 12 months after enrollment. Briefly, after informed consent was obtained, samples (i.e., whole blood, serum and urine) were collected from participants according to IRB-approved protocols and shipped to a centralized biorepository for processing and storage. Associated clinical information was also collected to better characterize the samples. Additional validation and testing was performed to confirm the presence of tick-borne pathogens (including Borrelia burgdorferi, Anaplasma phagocytophilum, Babesia microti, and Borrelia miyamotoi) in the samples. Samples from Wisconsin were also tested for strains specific to the Midwest, including Borrelia mayonii and Ehrlichia muris eauclairensis. A subset of samples has been tested by culture followed by PCR for Borrelia burgdorferi. Serology using the two-tiered testing algorithm for Lyme disease (i.e., ELISA followed by IgM and IgG immunoblots) was performed on all samples.

Results and Discussion: 267 participants were enrolled from 2014-2016 from three sites: East Hampton, NY, Marshfield, WI, and Martha’s Vineyard, MA. The biobank contains samples from participants with confirmed LD (41; [+] 2-tier serology OR [+]

qPCR OR [+] culture), probable/ suspected LD (50; EM or annular rash; [-] 2-tier serology; [-] qPCR), symptomatic no rash (SNR; 50; symptomatic; [-] 2-tier serology; [-] qPCR), and endemic controls (97; [-] serology). 29 additional controls had at least one positive serology result. Samples from convalescent draws from 62 cases are also available.

Samples are available to qualified investigators through an application process that includes a rigorous peer-review. If approved, the biobank will provide blinded, de-identified samples to the investigator followed by associated clinical information. Samples collected in 2017, from East Hampton, Marshfield, and from the Bay Area, California, will be available in Spring 2018 once all testing is completed.

Conclusion: Each participant's donation provides samples for ~50 research projects, with aliquots of whole blood (1 and 2 ml), serum (250 µl), and urine (1 ml). Through the availability of samples with well-annotated clinical information, investigators will have additional tools available to advance the study of LD and other TBI.

Funding Acknowledgement: Lyme Disease Biobank is a program of Bay Area Lyme Foundation. This program is funded by Bay Area Lyme Foundation and the Steven & Alexandra Cohen Foundation.

Smith, Patricia V.

Patricia V. Smith, BA
President, Lyme Disease Association, Inc.
Advisory Board Member, Columbia University Lyme & Tick-Borne Diseases Research Center
Programmatic Panel Member, TBD Research Program, DoD Congressionally Directed Medical Research

Program
Conference Organizer/Program Committee, Jackson, NJ

Welcome/Overview of Lyme/Introductions

Patricia V. Smith, a Monmouth University graduate, is in her 20th year as President of the all-volunteer run national non-profit Lyme Disease Association, LDA and is a member of Columbia University's Lyme & Tick-Borne Diseases Research Center Advisory Committee, member of the Food & Drug Administration's (FDA) PESP Partnership to promote avoidance of tick exposure, and member of the Tick IPM Working Group with federal and non federal members, from the IPM Institute of North America, to eradicate tick-borne diseases. She was appointed in 2016 as a member of the US Army Medical Research and Materiel Command (USAMRMC) Tick-Borne Disease Research Program (TBDRP) as a member of the Congressionally Directed Medical Research Program Programmatic Panel.

Ms. Smith is also former Chair, (NJ) Governor's Lyme Disease Advisory Council. She was EPA's PESP 2011 Lyme prevention conference session co-chair with CDC. In 2011 she presented a Lyme session to the New Jersey Education Association's Annual Meeting. She is a member & former officer of ILADS, International Lyme & Associated Diseases Society, a professional medical and research organization.

Ms. Smith is former President/12-year member of the Wall NJ Board of Education where she earned state board member-certified status. She is a former officer of Monmouth County School Boards Assn. and was a member of the Federal Relations Network for New Jersey School Boards Association/National School Boards Assn.

During her LDA presidency, Ms. Smith has led the effort to raise funds for researchers nationally, with more than 106 research grants awarded – research acknowledged in 42 scientific journals. She has organized 18 continuing medical

education (CME) accredited Lyme scientific conferences for doctors and researchers with international faculty, held in different areas of the US, most jointly sponsored by Columbia University. She has spoken at many conferences on Lyme including those presented by the University of New Haven (CT) and the California Lyme Disease Association (now LymeDisease.org), Midcoast Maine Lyme Education and Support, Colorado Tick-Borne Awareness Association, and ILADS. She has been a speaker at hundreds of public, school, business, & government events.

She led the LDA in its effort with a partner organization, to endow the Columbia Lyme & Tick-Borne Diseases Research Center in New York, which opened in 2007. She developed the ABCs of Lyme Disease pamphlet for parents and educators and also the LymeR Primer brochure now featuring 20 tick-borne diseases, the Tick Mark bookmark, and helped design Tick Awareness cards. More than 2.5 million education items have been distributed.

Ms. Smith has testified for and secured passage of state and federal bills for Lyme research and physician's right to treat. She has been invited to state capitals in CT, MA, MD, MN, NH, NJ, NY, PA, RI, to present oral testimony and education on Lyme and has provided written testimony in many others. Based on her written testimony, LDA was recently included in ground breaking Maine legislation as a website resource on Lyme disease on Maine's DPH website. She was invited to testify on two occasions before the NY Assembly Health Care Committee and also before the Rhode Island (Governor's) Lyme Disease Advisory Commission and has spoken before the California Lyme Disease Advisory Council. Over time, she has personally met with many State Health Commissioners and with Governors in NH, RI CT on Lyme issues and with then Governor Pataki's office on many occasions along with several NY state legislators. She has also presented before the Pennsylvania House of Representatives Majority

Policy Committee and was an invited speaker for Lyme forums hosted by a member of the Massachusetts House of Representatives and the Majority Caucus Administrator for the Pennsylvania House of Representatives and the Minnesota State Senate Health Committee.

She has twice been invited to present to CDC Vector-Borne Diseases Division, Ft. Collins (2007, 2013); met with then CDC Director Dr. Julie Gerberding/5 Congressmen in DC; organized & led a team that met with HHS Asst. Sec. of Health with CDC/NIH officials teleconferenced in; met with military leaders in DC; and briefed the Senate HELP Committee Members and House Subcommittee on Health. She met several times with US Army CHPPM/Public Health Command at Aberdeen Proving Grounds. She met in DC with the NIH Program Director and research coordinator and presented educational PowerPoints on Lyme to employees at the Environmental Protection Agency (2008, 2014), to the Dept. of Energy, and to Homeland Security in 2014. In 2014, she helped develop language for a federal bill on Lyme and led the nationwide effort which successfully passed the bill through the House. Ms. Smith spoke at a 2014 press conference with Senator Charles Schumer (NY) on the doxycycline shortage for Lyme patients. In 2012, she testified before the House Foreign Affairs Committee, Africa, Global Health & Human Rights Subcommittee on issues affecting Lyme patients. In 2013, she testified before the House Energy & Commerce Health Subcommittee on HR 610 to establish a federal Lyme & Tick-Borne Diseases Advisory Committee. She co-authored an article which was read into the Congressional Record on Lyme disease research priorities from the patient perspective. In 2015, she spoke at the American Association for the Advancement of Science in DC on patient research priorities. In 2016, she spoke before the Women in Government’s annual conference. In 2016, she led the negotiations with House leadership for the Lyme language subsequently passed in the 21st Century Cures Act which creates a federal working group on tick-borne diseases with patient and advocates reps.

Chosen Jackson NJ's Chamber of Commerce 2008 Woman of the Year, she has also received commendation from the NJ legislature, a Special Congressional Recognition certificate from RI Cong. Langevin, and had a flag flown over the US Capitol by request of NJ Cong. Chris Smith in honor of her Lyme work. Ms. Smith helped to organize and presented at educational forums held by 3 congressmen (Langevin, Pitts, C. Smith). She has received awards from Dr. Brian Fallon, Columbia, from various Lyme groups, and was given the Courage in Advocacy Award in 2015 from Connecticut based Lyme Connection and Focus on Lyme Excellence in Advocacy (AZ) award in 2017.

Other activities include providing input into a NJ law requiring teacher education for staff who teach students with Lyme disease, performing school in-services for educators on Lyme disease, and working with parents of students who are classified due to Lyme disease. Working with author Amy Tan, she created LDA's LymeAid 4 Kids, a fund for children with no health coverage for Lyme, a fund that has awarded $338,400 for uninsured children to date.

Zhang, Ying

Ying Zhang, MD, PhD
Professor,
Department of Molecular Biology and Immunology,
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Baltimore, MD
www.jhsph.edu/faculty/directory/profile/786/ying-zhang

Persisters

Dr. Ying Zhang is Professor at Department of Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health. Dr. Zhang is a leader in drug resistance research and has made major contributions to our modern understanding of molecular basis of drug resistance in *M. tuberculosis*, including identification of the first TB drug resistance gene *katG* (catalase-peroxidase) in isoniazid (INH) resistance, *pncA* gene in pyrazinamide (PZA) resistance, and more recently identified two new PZA targets RpsA and PanD involved in PZA action and resistance. His work on the unique persister drug PZA, which is critical for shortening TB therapy, has provided important insights into key targets of persister bacteria and has impacted the development of new drugs that further shorten the TB therapy. He has also made a number of important contributions to the mechanisms and concept of bacterial persisters. He first pointed out the link and similarity of bacterial persisters and cancer stem cells in 2007, first proposed the heterogeneity of persisters and expanded the persister definition to include viable but nonculturable (VBNC) organisms as part of the persister continuum, as well as proposed common strategies for improved treatment of persistent infections by targeting both growing and non-growing persisters More recently, Dr. Zhang applied the “PZA principle” to treatment of persistent Lyme disease, and made paradigm-shifting contributions by identifying drugs that target Borrelia persisters for more effective treatment of persistent Lyme disease. Dr. Zhang has published over 200 original articles and review articles and book chapters, serves on various editorial boards and advisory boards, and has made important contributions to mechanisms of drug resistance and persistence that impact the control of drug resistant and persistent infections of several important bacterial pathogens including TB and Lyme disease.

Conference Lecture Summary

In this presentation, the causes as to why some patients continue to suffer from post-treatment Lyme disease syndrome (PTLDS) despite antibiotic treatment will be discussed. In particular, the relevance of Borrelia persistence in animal models and in vitro to the PTLDS condition in patients will be addressed. Different strategies for treating persistent infection will be presented in terms of drug combination treatment as compared to pulse dosing. In addition, an update on the search for practical and effective drug combinations that eradicate round bodies and biofilm-like structures in vitro will be presented. A path for translating these findings for more effective treatment of persistent Lyme disease will be discussed.

Thangamani, Saravanan

Saravanan Thangamani, MSc, PhD
Associate Professor, Department of Pathology;
Vice-Chair, Institutional Animal Care and Use Committee;
Director, Insectary Services Core
Director, Arthropod Containment Laboratories
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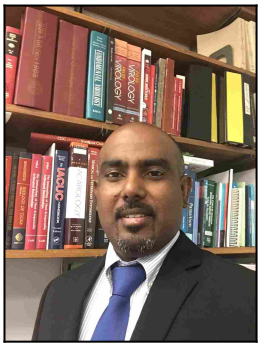

Powassan Virus: An Emerging Tick-Borne Virus of Public Health Concern in North America

Saravanan Thangamani is an Associate Professor in the Department of Pathology at the University of Texas Medical Branch (UTMB) at Galveston. Dr. Thangamani's research focuses

on understanding the role of tick feeding and tick salivary factors in the establishment of Powassan virus. His long-term goal is to develop novel strategies to control tick-borne virus transmission. To this end, he is employing cutting edge techniques to unravel the functional role of salivary factors (proteins and microRNAs) in facilitating Powassan virus transmission. Understanding the function(s) of these molecules, and how it interacts with the host immune system is vital to development of novel disease transmission control strategies. Recently, Dr. Thangamani has initiated research to understand the effect of Powassan virus co-infection on Lyme disease transmission.

Conference Lecture Summary

Powassan virus (POWV, Flaviviridae) is the only North American member of the tick-borne encephalitis serogroup of flaviviruses. It is transmitted to small- and medium-sized mammals by *Ixodes scapularis, Ixodes cookei*, and several other Ixodes tick species. Humans become infected with POWV during spillover trans- mission from the natural transmission cycles. In humans, POWV is the causative agent of a severe neuroinvasive illness with 50% of survivors displaying long-term neurological sequelae. POWV was recognized as a human pathogen in 1958 when a young boy died of severe encephalitis in Powassan, Ontario, and POWV was isolated from the brain autopsy of this case. Two distinct genetic lineages of POWV are now recognized: POWV (lineage I) and deer tick virus (lineage II). Since the index case in 1958, over 100 human cases of POWV have been reported, with an apparent rise in disease incidence in the past 16 years. This recent increase in cases may represent a true emergence of POWV in regions where the tick vector species are prevalent, or it could represent an increase in POWV surveillance and diagnosis. In the past 5 years, both basic and applied research for POWV disease has intensified, including phylogenetic studies, field

surveillance, case studies, and animal model development. This talk will provide an overview of POWV, including the epidemiology, transmission, clinical disease, and diagnosis of POWV infection. Future priorities and challenges with regard to the disease are will also be emphasized.

Telford, Sam

Sam R. Telford III, ScD
Professor, Infectious Diseases and Global Health
Tufts University
North Grafton, MA
http://vetprofiles.tufts.edu/faculty/sam-r-telford-iii

Babesia microti, B. duncani, & B. miyamotoi

Sam Telford is an epidemiologist focusing on arthropod-transmitted infections. He received his BA in ecology and evolution from Johns Hopkins in 1983; MS in tropical public health (1987) and ScD in parasitology (1990; ecology of Lyme disease) from the Harvard School of Public Health. Following postdoctoral work at Harvard on the Lyme disease vaccine, he served for 10 years as Lecturer in Tropical Public Health there, teaching parasitology and tropical medicine and continuing research on aspects of deer tick transmitted infections such as Lyme disease, babesiosis, human granulocytic ehrlichiosis, and deer tick virus. He moved to Tufts vet school in 2002, where he is currently Professor of Infectious Disease and Global Health and Director of the New England Regional Biosafety Laboratory and teaches graduate level courses on biodefense and on the epidemiology of

zoonoses. Dr. Telford has or has had federal, state, and private funding for his research on the epidemiology and ecology of tick-borne infections, and has published >220 peer reviewed reports. He advises local, state, and national organizations on public health interventions against tick and mosquito-borne infection.

Conference Lecture Summary

I will review the epidemiology, clinical picture, diagnosis, and treatment of human babesiosis, particularly with respect to the northeastern United States. Babesiosis is increasing in prevalence and distribution after having lagged that of the co-transmitted Lyme disease. Elsewhere in the U.S. and globally, babesiosis remains a rare and sporadic infection usually affecting only severely immunocompromised individuals. Babesiosis is the most important protozoal transfusion risk because infection tends to be subclinical in healthy younger individuals and donors cannot be excluded based solely on questions about exposure history. Even with treatment, case fatality rates can approach 5%; new treatment regimens need to be developed, particularly for those patients who are immunocompromised. Risk of acquiring babesiosis may be reduced by preventing tick exposure or by any of the many modalities to reduce environmental contamination by ticks. Vaccine development remains a challenge, a perspective that follows from the difficulties faced by the efforts to develop effective vaccines against the related malaria parasites.

Taylor, Travis

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http://www.utoledo.edu/med/depts/micro/faculty/Travis_Taylor.html

Host-Specific Antiviral Responses to the Tick-Borne Flaviviruses: Powassan and TBEV

Currently Dr. Taylor's research focuses on the arthropod-transmitted members of the *Flaviviridae* family. Flaviviruses are globally significant human pathogens including dengue virus (DENV), West Nile virus (WNV) and the TBEV serocomplex of viruses. Select members of the TBEV serocomplex includes the highly pathogenic TBEV-Sofjin and Powassan virus (POWV) and require biosafety level (BSL)-4 and 3 facilities respectively. The group also includes the naturally attenuated Langat virus (LGTV), which greatly simplifies studies with TBEV under BSL-2 conditions. Viruses in the TBEV serocomplex span the clinical spectrum for flaviviruses. Symptoms range dramatically from asymptomatic infection to more severe encephalitis and hemorrhagic fevers with mortality rates exceeding 30%. Treatment of infection with these viruses is hindered by the lack of effective antiviral therapies and few available vaccines. The overwhelming morbidity, in addition to the risk of emerging viruses, highlights the need for better treatment options. Fresh insight for new antiviral treatments may come from studies of the classical IFN response. IFN treatment induces the expression of hundreds of genes (interferon stimulated genes or ISGs), many of which are antiviral molecules. Though flaviviruses are highly sensitive to the antiviral effects of IFN, viral antagonism of IFN

signal transduction prevents the expression of ISGs in infected cells. Thus, virus suppression of IFN signaling renders IFN ineffective as a medicinal therapy for flavivirus infections. Identifying ISGs with virus-specific antiviral activity may reveal new methods for treating flavivirus infections.

A major accomplishment of Dr. Taylor's work has been the identification of a previously unnamed mouse-specific tripartite motif (TRIM) protein (now designated TRIM79) that targets the nonstructural 5 protein (NS5) from TBEV to inhibit virus replication. NS5 is the major IFN antagonist for flaviviruses and is an essential component of the virus replication complex and as such is a prime candidate for antiviral drug design. Focus in the lab will characterize the TRIM79-NS5 interaction, and ultimately determine the importance of the antiviral molecule to host protection in a mouse model of TBEV infection. Additionally Dr. Taylor's lab is using various screening approaches including shRNA libraries and mass spectrometry to identify new virus-host interactions that can be targeted as part of a virus-specific immune therapy. Finally, viral proteins have evolved the capacity to perform many important functions during the virus life cycle. NS5, for instance is necessary for virus replication and immune evasion. Understanding how the virus protein can maintain different functions and protein interactions in distinct cellular regions may again lead to new therapeutic targets. Previous studies by Dr. Taylor have determined that NS5 is modified by the cellular ubiquitination machinery. Understanding the importance of this post-translational modification may provide insight into NS5 regulation and/or cellular mechanisms to interfere with NS5 function.

Dr. Taylor received his Ph.D. degree at the University of Texas Southwestern Medical Center at Dallas under the mentorship of Dr. Wade Bresnahan. He then completed his

postdoctoral training at the NIH Rocky Mountain Laboratories where he studied the biosafety level (BSL)-4 tick-borne flaviviruses in the laboratories of Drs. Marshall Bloom and Sonja Best. Dr. Taylor joined the Department of Medical Microbiology and Immunology in August of 2012.

Conference Lecture Summary

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 developed an *in vitro* model to study TBFV infection in a representative natural host, the white-footed mouse *Peromyscus leucopus*. By using this model, we determined that flaviviruses indeed can infect cells from a natural host, yet replication is severely impaired. Replication of vesicular stomatitis virus was equivalent in *P. leucopus* and *M. musculus* cells suggesting that restriction was flavivirus-specific. Furthermore, by knocking down *P. leucopus* genes involved in antiviral interferon (IFN) responses, restriction was relieved and TBFV replication was restored to levels observed in more susceptible cells. Collectively, this work demonstrates that the IFN response of P. leucopus imparts a strong and virus-specific barrier to flavivirus replication and indicates that resistance in the natural reservoir to TBFV is an active process of virus suppression and not a passive lack of proviral factors. 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.

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Biofilms and Lyme Disease

Dr. Sapi received her Ph.D. degree in Genetics from the University of Eotvos Lorand (Budapest Hungary). She is a Professor and Department Chair at the University of New Haven (Connecticut) where she teaches undergraduate and graduate level biology courses and carries out Lyme disease research with her students. To date, over 80 graduate students have received training in Lyme disease related research.

Her recent studies investigate the different forms of Borrelia burgdorferi to better understand how Borrelia can hide from the immune system as well as from antimicrobial therapies. Her recent research shows that Borrelia burgdorferi is capable forming a protective layer around itself – called biofilm – which could render it to be very resistant to antibiotics and provide a logical explanation as to why extensive antibiotic

treatment for patients with a tick-bite history could fail. The goal of her research group is to fully characterize this novel form and to identify novel antibacterial agents that are effective in killing all forms of Borrelia burgdorferi.

Dr. Sapi also organized and chaired seven Lyme Disease Symposiums at the University of New Haven during the last several years.

https://www.facebook.com/UNH.LymeGroup

Conference Lecture Summary

Lyme disease patients are treated with various antibiotics though the rates of relapse and recurrence of the disease are frequent after discontinuing the antibiotic treatment. It was proposed earlier that the observed antibiotic resistance and reoccurrence of Lyme disease might be due to the formation of defensive morphological forms of *Borrelia burgdorferi*. In addition to its familiar spirochete form, *B. burgdorferi* can transform from motile spirochetes into round body and biofilm forms in the presence of unfavorable environmental conditions including the presence of antimicrobial agents. Our laboratory has demonstrated that *B. burgdorferi* biofilm formation enhances the antibiotic resistance of the organism to various antimicrobial agents, which previously showed some success against the spirochete and round body forms of *B. burgdorferi*. This data strongly suggests that Borrelia biofilm could play significant role in their survival in diverse environmental conditions by providing refuge to individual cells. However, the question remains if these structures can be found *in vivo* and whether these biofilm structures hold significant relevance for the survival strategies for *Borrelia spp*. in infected tissues.

In this presentation, we provide evidence of Borrelia biofilm presence in various human organs obtained from autopsy tissues

of a well-documented Lyme disease patient who died despite of multiple rounds of antibiotic treatments. Findings of the role of Borrelia biofilm in inflammatory processes will be also discussed as well as our recent metagenomics findings indicating potential co-infection in Borrelia biofilms.