(IDSA Project Plan: Page 3, lines 1-22)

In Canada, the Parliament of Canada has passed Bill C-442: An Act respecting a Federal Framework on Lyme Disease following lengthy and detailed hearings. This bill received Royal Assent on December 16th, 2014 and is now law. This enactment requires the Minister of Health to convene a conference with the provincial and territorial ministers responsible for health and with representatives of the medical community and patients’ groups for the purpose of developing a comprehensive federal framework to address the challenges of the recognition and timely diagnosis and treatment of Lyme disease.(1)

On November 27th, 2014, a motion was tabled in the Ontario Legislative Assembly which asked that the Minister of Health develop a provincial strategy for Lyme disease. This motion was read, debated and then carried unanimously by all three political parties on first reading. This motion specified that in developing the strategy, the minister should engage and consult with representatives of the health care community and patients’ groups, including the Ontario Medical Association, the Ontario College of Family Physicians, the Association of Local Public Health Agencies, the Ontario Lyme Alliance, the Canadian Lyme Disease Foundation, international scientific advisers and medical experts in the field of tick-borne diseases having diverse perspectives, workers’ organizations and veterinary associations, amongst others, and accept written submissions from Ontarians.(2)

These legislative acts were initiated in Canada because existing protocols have failed not only untold numbers of Canadians, but patients worldwide for decades. Therefore, although I would support a review of the IDSA Lyme guidelines if it were conducted by an independent body which could objectively evaluate the scientific evidence (or lack thereof) and the various positions being taken by all parties including organizations such as the IDSA, ILADS, other medical organizations, patient groups and a variety of other stakeholders, I do not support the IDSA's 2015 review process. Given the global spread and prevalence of tick-borne diseases, the participation of national and international scientific and medical experts with diverse perspectives and broad experience is an absolute necessity. Given the contentiousness of prior Lyme guidelines, a process that inherently promotes bias while excluding dissenting viewpoints is unacceptable.

(IDSA Project Plan: Page 1, lines 1-20, Page 4, lines 23-40)

The IDSA has decided to produce a joint multi-society consensus guideline for the prevention, diagnosis and treatment of Lyme disease. However, given that the project has been designed to include organizations that previously endorsed the earlier guidelines and panel members with long-established biases while excluding others with divergent perspectives, it is difficult to give credence to either the process or the guidelines that will emerge.

(IDSA Project Plan: Page 1, line 16)

The Canadian organization invited to participate in the 2015 panel, the Association of Medical Microbiology and Infectious Disease Canada (AMMI), endorsed the IDSA Lyme Guidelines in October 2011. At the time, they had 551 members of which 440 were in the Active Member category.(3) These Guidelines were endorsed by the AMMI Council and the Communications and Public Relations Committee which together totalled 22 members. It appears that this was by no means an open process. The review that was undertaken was not transparent and there does not appear to have been an opportunity for consideration, debate or consensus among all AMMI members. Whether AMMI members generally have knowledge of Lyme disease or an understanding of the controversy is unknown.

When AMMI announced their endorsement of the IDSA Lyme guidelines in 2011, their media release incorrectly stated, "The subsequent independent review that was initiated in 2008 by the Attorney-General of Connecticut reaffirmed all of the original IDSA guideline recommendations. This review panel included members of the International Lyme and Associated Diseases Society (ILADS)."(4) Since the composition of the review panel membership would have been a very simple fact to check, and AMMI did not verify it before publication, this causes concern with regard to the comprehensiveness and the quality of the review that was undertaken by AMMI for the Council and the Public Relations Committee to make the decision to endorse the 2006 IDSA guidelines.

(IDSA Project Plan: Page 1, line 16 and page 2, line 17)

The most recent annual report available from AMMI (2013), showed that they had 611 total members, of which 436 were active.(5) In contrast, in 2013, there were 77,674 physicians in Canada.(6) It should be noted that both the Canadian Medical Association and the College of Family Physicians of Canada endorsed Bill C-442.(7) The AMMI cannot be deemed to represent the views of all Canadian physicians nor considered to be unbiased about Lyme disease. The panelist who will represent the AMMI in the 2015 Lyme Guidelines project, Dr. Bowie, has expressed his biases in government hearings and interviews and they are a matter of public record. Canadians place a high value on the sustainability of our public healthcare system. Having ever growing numbers of patients with chronic disabilities due to late-diagnosed, undiagnosed and diagnosed but unresolved tick-borne diseases will continue to put unnecessary strains on an already burdened healthcare system. The AMMI endorsement and public advocacy for the IDSA Lyme Guidelines seems misplaced when considering the long-term healthcare needs of Canadian residents and Canadian Lyme disease patients.

(IDSA Project Plan: Page 5, line 61 through page 9, line 129)

Given the important role that clinical practice guidelines play in many aspects of health care, it is imperative that these guidelines be free of industry influence and be viewed by clinicians, policy makers, patients, and others as objective and trustworthy. To address this issue, the Institute of Medicine of the National Academies (IOM) published, "Clinical Practice Guidelines We Can Trust: Standards for Developing Trustworthy Clinical Practice Guidelines (CPGs)" in 2011.(8)

To determine whether the guidelines which will be produced as a result of the 2015 Lyme Guidelines project will ultimately be viewed as objective and trustworthy, it is important to examine the IOM standards that are currently evaluable and assess whether they are likely to be fulfilled using the process that has been outlined. Compliance with some of the IOM standards will not be evaluable until after a draft of the proposed Lyme guidelines has been issued.

(IDSA Project Plan: Page 1 lines 6-9) (Page 2 lines 1-48)

The Infectious Diseases Society of America (IDSA), the American Academy of Neurology (AAN) and the American College of Rheumatology (ACR) are jointly listed as organizational sponsors. Sponsorship would normally imply that these organizations are co-funding the project although funding is not specifically set out in the plan. According to the IOM Standard 2.4, "Funders should have no role in CPG development." However, in the case of this Lyme disease Project Plan, funders designed the plan, designated co-chairs that included a member from each sponsorship organization, designated two members from each organization for the Conflict of Interest (COI) Review Group and recruited the panel members, many of whom represent those same organizations. Clearly the funders will have an extensive and very direct role in the development of these guidelines which contravenes IOM Standard 2.4.

(IDSA Project Plan: Page 8, lines 122-123) (Page 2, lines 3-11)

In Standard 2.4, the IOM also states, "The chair or co-chairs should not be a person(s) with COI." The Project Plan also states, "The COI review group ensured that the majority of the panel and each co-chair was without potential conflicts (noted in Table 1) and the panel was approved." It is extremely difficult to give credence to the COI review group's statement. I have particular concerns regarding two of the named co-chairs with regard to their potential conflicts of interest. Another of the co-chairs appears to have less potential for conflicts of interest, but may still be perceived as problematic, considering that co-chairs should be free of any and all conflicts of interest.

(IDSA Project Plan: Page 8, lines 122-123) (Page 2, lines 4-5)

It is not possible that Dr. Bockenstedt, with eight research grants/contracts from NIH/NIAID and NIH/NIAMS related either directly or indirectly to Lyme pathogenesis, diagnostics, vaccine development, *Babesia* treatment and immunology research can be perceived as not having potential conflicts of interest for this Lyme guidelines project. Two of these projects, which list her as the Project Leader over their three and five year histories, have been provided with more than three million dollars to date.( 9)(10) Several of the other projects listed have been undertaken in collaboration with L2 Diagnostics LLC, a privately held company with a close association to the Yale School of Medicine. According to Bloomberg Business, the company's line of business includes providing commercial physical and biological research and development.(11) Beneficial self-interests and many potential conflicts with these research grants and commercial collaborative ties are obvious. Even if the personal financial benefits that might be associated with these activities were found to be limited, Dr. Bockenstedt's vested interests with regard to commercial, non-commercial and institutional benefits are sufficient to undermine public confidence in the guideline process. In addition, she is one of six panelists recruited for this 2015 Lyme Project who were also authors on the 2006 Lyme Guidelines. The appointment of panelists who served on previous guidelines panels means those members come to the table with a vested interest in maintaining both the current case definition and most, if not all, of the prior recommendations by virtue of their prior publications.

(IDSA Project Plan: Page 8, lines 122-123) (Page 2, lines 8-9)

Dr. Lantos appears to have less potential for conflicts of interest according to his submitted statement of COI. However, Dr. Lantos was a co-author of the 2010 Final Report of the Lyme Disease Review Panel of the Infectious Diseases Society of America (IDSA), and has co-authored a number of more recent Lyme related "systematic review", review and opinion articles. Therefore, he certainly is perceived as not being free of conflicts of interest and should not serve as a co-chair on this panel due to a vested interest in the outcome by virtue of prior publications.

(IDSA Project Plan: Page 8, lines 122-123) (Page 2, lines 10-11)

Dr. Rumbaugh's position as a co-chair on the panel is suspect due to conflicts of interest by virtue of his four grants/contracts with pharmaceutical companies studying medications for multiple sclerosis (MS). In many areas of the world, and particularly in Canada, Lyme disease has had little recognition, is rarely tested for in patients with neurological symptoms (and when tested for, only serology tests of limited reliability are used). Whereas multiple sclerosis is highly prevalent but Lyme is virtually unrecognized, it appears to patients that MS is a diagnosis that is too often mistakenly made for patients with late-stage Lyme disease. This co-chair is involved in MS drug research studies directly funded by several companies that profit from selling high value, long-term medications to patients with MS. The appropriateness of Dr. Rumbaugh's appointment as co-chair for guidelines that will provide diagnostic and treatment recommendations for neuroborreliosis and late-stage Lyme patients is considered highly questionable. Guidelines are needed that will help ensure patients are properly diagnosed and treated before Lyme disease is allowed to progress to a stage where CNS demyelination becomes a manifestation.(12) (13) (14)

(IDSA Project Plan: Page 3, lines16-20) (Page 8, lines 116 through Page 9, line 129)

Because there are three co-chairs who appear to have a number of unacknowledged conflicts of interest, an additional concern is that there are more panel members who also have conflicts of interest, and that they form a larger component of the panel than has been publicly accounted for. Even if these COI declarations were perceived by the COI Review Group as inconsequential, the public recognizes their potential for tainting the objectivity of the guideline recommendations. Full transparency should have been provided for all the panelists who have conflicts of interest. The three co-chairs have very clear and undeniable potential for conflicts of interest and therefore should be exchanged for panel members who do not. In addition, all panel members should be recused from drafting and voting on recommendations that are related to their conflicts of interest. I ask that this transparency be provided, that co-chairs be appointed who are genuinely free of potential conflicts of interest, and that panel members with applicable conflicts of interest be excluded from drafting and voting on related recommendations.

(IDSA Project Plan: Page 23, Table 1) (Page 9, lines 125-129)

I did not do a search of the other panelists' COI declarations. However, it was disturbing to incidentally discover that one panel recruit is a co-inventor on a patent application that was not listed in the declaration in Table 1.(15) This does not inspire confidence in the integrity of the process, the panel or the COI Review Group.

(IDSA Project Plan: Page 2, lines 13-40) (Page 8, lines 116 through Page 9, line 129)

As well as the co-chair, another five panelists also co-authored the 2006 IDSA Lyme Guidelines. Two of those panelists also served on the Lyme guidelines panel in 2000. Two co-authors of the AAN 2007, Practice parameter: treatment of nervous system Lyme disease were also recruited again for 2015; one of whom was also a co-author of the AAN 1996 guidelines. (Halperin J, Logigian E, Finkel M, Pearl R. Practice parameters for the diagnosis of patients with nervous system Lyme borreliosis (Lyme disease). Neurology 1996;46:619–627). Dr. Steere was also a co-author of the 1997 American College of Physicians clinical guideline: Guidelines for Laboratory Evaluation in the Diagnosis of Lyme Disease. (16) The objectivity of these panel members while undertaking literature reviews is called into question by virtue of their prior publications, and the likelihood that they will be reviewing studies and research that they, or close associates, may have been personally involved in.

(IDSA Project Plan: Page 2, lines 13-40) (Page 8, lines 116 through Page 9, line 129) and (Pages 17-25)

If the sponsoring organizations concluded that participants with conflicts of interest are essential to provide the necessary expertise, they should have demonstrated to the public that they made an effort in good faith, but were unsuccessful in finding individuals with the required expertise and without conflicts of interests. There was no apparent effort made to find less conflicted members when these panelists were selected. Additionally, Section 2.3 of the IOM standards states that, "Members of the GDG should divest themselves of financial investments they or their family members have in, and not participate in marketing activities or advisory boards of entities whose interests could be affected by CPG recommendations." It is not clear from Table 1, or elsewhere in the project plan, whether this has been enforced on panel members.

(IDSA Project Plan: Page 4, lines 23-40)

Standard 3 of the IOM publication states, "The GDG should be multidisciplinary and balanced, comprising a variety of methodological experts and clinicians, and populations expected to be affected by the CPG. Patient and public involvement should be facilitated by including (at least at the time of clinical question formulation and draft CPG review) a current or former patient and a patient advocate or patient/consumer organization representative in the GDG. Strategies to increase effective participation of patient and consumer representatives, including training in appraisal of evidence, should be adopted by GDGs."

This co-sponsored 2015 LD project plan has demonstrated a clear lack of balance by not including the very important populations who will be most affected by the CPG: Lyme disease patients (particularly the patients, who after guidelines recommended treatments are used, continue to be debilitated with chronic symptoms and very possibly chronic disease), the physicians and other healthcare professionals who treat and manage patient care and the moderate-minded researchers undertaking new avenues of study. The choice of a "consumer user" who has no experience with Lyme disease shows poor judgement and a complete lack of respect for the patients these guidelines are intended to serve. It also indicates yet another distinct lack of regard for the integrity of the guideline development process itself. The person who was chosen has never had Lyme disease and has no experience or knowledge of the issues affecting Lyme patients. Therefore, she has been inappropriately chosen to be a "consumer user" on the panel. Patient representatives on the panel should be legitimately regarded by members of the Lyme community as individuals who can effectively represent their interests.

The Institute of Medicine not only says that guideline panels should include populations expected to be affected by the Clinical Practice Guidelines, but also that there should be two patient representatives. One specifically is to be a patient organization representative or patient advocate. In order to restore a measure of integrity and public confidence in this guideline process, this IOM regulation must be observed and two appropriate Lyme disease patient representatives must be included as panelists along with one independent "Lyme literate" physician who regularly provides medical care for Lyme disease patients when recommended treatment has been ineffective.

(IDSA Project Plan: No page reference-transparency was not provided for this topic.)

According to the Standard 7 of Developing Trustworthy Clinical Practice Guidelines by the Institute of Medicine (IOM), " External reviewers should comprise a full spectrum of relevant stakeholders, including scientific and clinical experts, organizations (e.g., health care, specialty societies), agencies (e.g., federal government), patients, and representatives of the public" and that " The GDG should consider all external reviewer comments and keep a written record of the rationale for modifying or not modifying a CPG in response to reviewers’ comments." Standard 7.4 further states, "A draft of the CPG at the external review stage or immediately following it (i.e., prior to the final draft) should be made available to the general public for comment." However, the IDSA announcement of the LD Project Plan and public comment period ending April 9th notes only that, "After a draft of the full guideline is developed, it will also be posted on the IDSA website for a 30-day public comment period."

It is unclear if this post-draft public comment period will constitute an "external review" in compliance with IOM Standard 7, with all comments being duly considered, along with a written record kept of the rationale for modifications, or lack thereof, in response to the reviewer's comments. I would like clarification as to whether there will be a separate external review comprising a full spectrum of relevant stakeholders in keeping with the IOM Standard and whether the draft that will be supplied for the public comment period will be open to modification before a final draft is issued. I would also like to know what will be done with public comments submitted after the draft has been made available, whether they are going to be considered in the writing of the final guidelines, or whether this will simply be a pro forma (but ultimately useless and meaningless) exercise.

**(IDSA Project Plan: PICO (Questions addressed) Pages 9-16, lines 127-278)**

**Rather than providing comments on questions that will be posed to the panel outlined in the LD Project Plan, I am submitting questions I would like to be included and answered by panel members during the current Lyme guidelines review process.**

**NOTE: (Separate references for these questions are provided below.)**

Some research has been undertaken towards understanding the role of *Borrelia* genospecies and strain diversity for infectability and disease pathogenesis.1,2 Species and strain variation have also been recognized as factors affecting diagnostics,3,4 but it appears that the varied potential for immune evasion and/or host tissue damage 5,6,7,8 by differing strains has not been well-examined.

1. It is now understood that some strains of *Borrelia* are highly pathogenic and invasive while other strains do not disseminate further into blood and other tissues resulting only in an erythema migrans rash. This means an unknown number of patients diagnosed with Lyme solely on the basis of erythema migrans rashes could have been "cured" with water, or with time. When the treatment trials for early acute Lyme were undertaken, strain variability was unknown and was not considered as a factor that would affect treatment efficacy results. What effect did strain variability have on the accuracy of research results and the derived statistics related to the efficacy of antibiotic treatments for early Lyme disease which continue to be used to show that Lyme disease can be "easily" cured in the early stages with recommended treatment protocols?
2. Do different species and strains of *Borrelia* have varied abilities to evade antibiotics and/or human immune responses?
3. Do different species and strains of *Borrelia* have varied abilities to express BBHtrA causing variable levels of damage to host tissues?
4. Do different species and strains of *Borrelia* have varied abilities to elicit the production of cytokines and chemokines in human hosts?
5. Do different species and strains of *Borrelia* have varied abilities to cause immune dysregulation or dysfunction in human hosts?
6. Do some genospecies and strains (or combinations of strains) have a greater propensity to develop persister cell phenotypes than other strains of *Borrelia*?
7. If *Borrelia* species/strain variability is a factor that creates varied levels of damage in patients, how should this affect recommendations for treatment modalities?
8. If *Borrelia* species/strain variability is a factor that creates varied difficulty with clearing human infection, how should this affect recommendations for treatment modalities?

Some research has been undertaken towards understanding the role of genetic polymorphisms and other factors in human sub-populations that could possibly affect an individual patient's ability to clear infections (immune dysregulation/compromise), promote auto-immunity, exacerbate the expression of Lyme disease manifestations and/or enhance host susceptibility to disseminated Lyme disease. 9,10,11,12,13 While it seems that some of these avenues of research are likely to provide some answers for more effective treatment options for some sub-populations of patients eventually, there is much more research that needs to be done. Most research undertaken to date has relied on hosts being immunocompetent and seropositive to two-tier testing. This has meant that certain sub-populations especially those with CVID or those that have reduced immune function due to co-morbidities or treatments, (i.e. diabetes, chemotherapy, corticosteroids, etc.), have not been studied to determine treatment efficacy.

1. Which sub-populations of patients are suspected to be at greater risk for enhanced host susceptibility to disseminated Lyme disease?
2. Which sub-populations of patients have been determined to be at greater risk for enhanced host susceptibility to disseminated Lyme disease?
3. Which sub-populations of patients are suspected to be at greater risk for having increased difficulties clearing *Borrelia* infections?
4. Which sub-populations of patients have been determined to be at greater risk for having increased difficulties clearing *Borrelia* infections?
5. Which sub-populations of patients are suspected to be at greater risk for having remaining symptoms, sequelae and relapses after recommended treatment for Lyme disease?
6. Which sub-populations of patients have been determined to be at greater risk for having remaining symptoms, sequelae and relapses after recommended treatment for Lyme disease?
7. What testing is available to clinicians to determine whether their individual patients would be more at risk from a *Borrelia* infection due to having a genetic polymorphism?
8. What testing is available to clinicians to determine whether their individual patients would be more at risk from a *Borrelia* infection due to other factors?
9. Would testing for these polymorphisms or other factors be practicable, if they were available to clinicians?
10. If individual patients are suspected of being members of a sub-population that have a greater risk for enhanced host susceptibility, greater risk for having increased difficulties clearing *Borrelia* infections, or greater risk for having remaining symptoms, sequelae and relapses after recommended treatment for Lyme disease, how should this affect recommendations for treatment modalities?

Treatments have not been adequately researched that could moderate auto-immune effects and cytokine production in Lyme disease patients where immuno-suppressives would be contraindicated. Some research has been undertaken regarding the use of macrolide antibiotics for a variety of bacterial infections.14,15 In recent years, it has been established that in addition to antimicrobial activity, a number of antibiotics have significant effects on human cellular immune function. Such properties of antibiotics may have clinical significance for modulation of the immune response in patients, especially those who are immunodeficient, and in inflammatory diseases. Additionally, in vitro studies have recently been done and results suggested that treatment with a combination of antibiotics may be more efficacious for eradicating *Borrelia* persisters, although further research in animals and in humans will be needed to determine safety and efficacy results in vivo.16

1. When individual patients have remaining symptoms, sequelae or relapses after recommended treatment, are there circumstances that would suggest the use of alternate or combinations of antibiotics for either their antibiotic or immune modulating effects?
2. Will panelists who regularly treat Lyme disease patients outline their usual treatment protocols, especially where they may diverge from prior Lyme disease guidelines recommendations?17

There are currently many unknowns with regard to human borreliosis infections and their effects on human sub-populations. To state the obvious, many patients have less than adequate responses and results with the currently recommended treatments. Given that it is widely acknowledged that additional research will be critical to answering these questions, I believe to have the recommendations from past guidelines re-issued again at this juncture would be contrary to the evidential science, counter-productive and irresponsible.

Accordingly, if it is found that there are insufficient research findings to answer the above questions when the Lyme guidelines are reviewed, I would recommend:

1. that additional treatment options should be included within the guidelines where possible; and
2. that it be emphasized within the guidelines that further research on additional treatment options is necessary; and
3. that healthcare providers are encouraged to use physician judgement in treating individual patients. I would urge that an individual guideline recommendation be included, in addition to a disclaimer similar to that in the 2006 Guidelines, indicating that healthcare providers are encouraged to use their professional judgement in treating individual patients and do have latitude to use their discretion.i.e. *"It is important to realize that guidelines cannot always account for individual variation among patients. They are not intended to supplant physician judgment with respect to particular patients or special clinical situations. The Infectious Diseases Society of America considers adherence to these guidelines to be voluntary, with the ultimate determination regarding their application to be made by the physician in the light of each patient’s individual circumstances.”*

(Further submission comments: IDSA Project Plan: Page 6, line 62 through page 8, line 115)

I believe in the principles of Evidence Based Medicine (EBM). In 1996, David Sackett and colleagues clarified the definition of evidence-based medicine as "the conscientious, explicit and judicious use of current best evidence in making decisions about the care of individual patients. ... [It] means integrating individual clinical expertise with the best available external clinical evidence from systematic research." (17) Evidence-based medicine applies population-based data to the care of an individual patient, while respecting the fact that practitioners have clinical expertise reflected in effective and efficient diagnosis and thoughtful identification and compassionate use of individual patients' predicaments, rights, and preferences.

Research generally focuses on populations, but individual persons can vary substantially from population norms and certain population segments have been historically under-researched (racial minorities and people with co-morbid diseases and conditions). Unfortunately, with Lyme disease, the quality and quantity of empirical medical evidence is sadly lacking. This suggests that the knowledge gained from clinical research does not directly answer the primary clinical question for a physician of what is best for that particular patient facing them.

Ultimately, clinical practice guidelines should be providing physicians and their patients with treatment options. As explained by Johnson and Stricker, "Failure to disclose treatment options violates the principles of patient autonomy and informed consent, which require that treatment options should be disclosed to patients and that treatment decisions should be made with the patient's informed consent. Treatment guidelines should not inhibit patient access to treatment options; rather, guidelines should describe treatment options and default to the clinical judgment of treating physicians in order to maximize the ability of patients to get well."(18)

The co-sponsored LD Project Plan process for 2015 (as written) was crafted to ensure that consensus will be achieved, not by careful deliberation, but by excluding opposing viewpoints. Alternate perspectives provided by Lyme disease patients, and others who will be most affected by the CPG, will be needed to make certain that all relevant research is considered. Creating a Project Plan that excludes divergent views demonstrates a lack of confidence in the evidential science that will form the underpinnings for the resulting guidelines. If the scientific evidence truly supports the recommendations that are to be derived through the process, it should stand on its own under a full and open examination that includes input from diverse perspectives.

The IDSA 2015 Project Plan, as it stands now, will ultimately produce Lyme disease guidelines which will be considered as controversial and contentious as its predecessors. If the true purpose of the panel is to produce unbiased, evidence-based and comprehensive Lyme guidelines to serve the people they are intended to help, Lyme patients, our physicians and the public, then these recommendations will be given serious consideration and be incorporated into the 2015 IDSA Lyme Guidelines.

References submission comments:

(1) Parliament of Canada LEGISinfo:

Available from: https://openparliament.ca/bills/41-2/C-442/?tab=mentions

(2) Legislative Assembly of Ontario

First Session, 41st Parliament

Official Report Journal of Debates (Hansard)

Thursday 27 November 2014

http://www.ontla.on.ca/house-proceedings/transcripts/files\_pdf/27-NOV-2014\_L034.pdf

(3) AMMI Canada Annual Report 2011

Available from: http://www.ammi.ca/media/39706/annual%20report%20final.pdf

(4) AMMI Media Release: Endorsement Statement IDSA Lyme disease Guidelines.

Available from: http://www.ammi.ca/media/28497/idsaedorsementstatementfinalengfr24oct2011rev.pdf

(5) AMMI Canada Annual Report 2013

Available from: http://www.ammi.ca/media/63759/2013\_annualreport\_web.pdf

(6) Canadian Institute for Health Information. Physicians in Canada, 2013: Summary Report. Ottawa, ON: CIHI; 2014.

Available from: https://secure.cihi.ca/free\_products/Physicians\_In\_Canada\_Summary\_Report\_2013\_en.pdf

(7) Green Party of Canada: Bill C-442, the National Lyme Disease Strategy Act, up for its Second Reading debate in the House of Commons

http://www.greenparty.ca/en/media-release/2014-03-03/bill-c-442-national-lyme-disease-strategy-act-its-second-reading-debate-hou

(8)The Institute of Medicine of the National Academies (IOM). Clinical Practice Guidelines We Can Trust

Standards for Developing Trustworthy Clinical Practice Guidelines (CPGs)

Available from: http://www.iom.edu/Reports/2011/Clinical-Practice-Guidelines-We-Can-Trust.aspx

(9) NIH ProjectReporter:

http://projectreporter.nih.gov/project\_info\_history.cfm?aid=8839960&icde=0

4R33AI100191-03 Contact PI / Project Leader: BOCKENSTEDT, LINDA K

Title: A NEW CYTOKINE-BASED IMMUNOASSAY FOR THE DIAGNOSIS OF LYME DISEASE Awardee Organization: YALE UNIVERSITY

Total project funding amount for 3 projects is $957,363\*

\* Only NIH,CDC,and FDA funding data.

(10) NIH ProjectReporter:

http://projectreporter.nih.gov/project\_info\_history.cfm?aid=8618858&icde=0

Project Number: 5R01AI085798-05 Contact PI / Project Leader: BOCKENSTEDT, LINDA K

Title: REAL-TIME IMAGING ANALYSIS OF VECTOR-BORNE LYME BORRELIOSIS PATHOGENESIS & PERSIS Awardee Organization: YALE UNIVERSITY

 Total project funding amount for 5 projects is $2,112,424\*

\* Only NIH,CDC,and FDA funding data.

(11) Bloomberg Business Company Profile:

http://www.bloomberg.com/profiles/companies/0721103D:US-l2-diagnostics-llc

(12) Jolanta Chmielewska-Badora, Ewa Cisak, Jacek Dutkiewicz. LYME BORRELIOSIS AND MULTIPLE SCLEROSIS: ANY CONNECTION?

A SEROEPIDEMIC STUDY. Ann Agric Environ Med 2000, 7, 141–143

Available from: http://www.aaem.pl/pdf/aaem0024.pdf

(13) Chang BL, Shih CM, Ro LS, Huang CC, Lyu RK, Chen RS, Lee JD, Chao LL, Kuo HC. Acute neuroborreliosis with involvement of the central nervous system. J Neurol Sci. 2010 Aug 15;295(1-2):10-5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20561635

(14) H Schober, B Simma, J Lütschg, A Blassnig-Ezeh. Central nervous system Lyme disease – Presentation of two cases. Neuropediatrics 2012; 43 - PS17\_04

Available from: https://www.thieme-connect.com/products/ejournals/abstract/10.1055/s-0032-1307132

(15) U.S.Patent Application #20130302329

http://www.freshpatents.com/-dt20131114ptan20130302329.php

Diagnostic tests for immune reactivity with human endothelial cell growth factor

The present invention provides for methods and compositions for identifying and detecting autoantigens. Candidate autoantigens are identified by obtaining a subject sample from which HLA-DR-presented peptides are collected and identified using mass spectometry, then synthesized and reacted with the same subject peripheral blood or effected tissue. In particular, the present invention provides for endothelial cell growth factor as a novel autoantigen biomarker for Lyme disease-associated arthritis.

USPTO Applicaton #: #20130302329 - Class: 4241341 (USPTO) - 11/14/13 - Class 424

Inventors: Allen C. Steere, Elise Drouin, Catherine Costello, Robert Seward

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 61/348,733, filed Sep. 21, 2010, and incorporated fully herein by this reference.

FEDERAL FUNDING

This invention was made with Federal funding under grants AR-20358, P41 RR10888, S10 RR15942, S10 RR20946 and Contract N01 HV28178, awarded by the National Institutes of Health. The U.S. Government has certain rights in the invention.

FIELD OF THE INVENTION

The present invention provides for methods and compositions for identifying and detecting humoral and cellular autoimmune responses to disease-related autoantigens. In particular, the present invention provides for a novel autoantigenic biomarker, endothelial cell growth factor, for Lyme disease-associated arthritis.

(16) American College of Physicians Clinical Guideline: Guidelines for Laboratory Evaluation in the Diagnosis of Lyme Disease. Ann Intern Med. 1997:127:1106-1108.

Authors available from: http://annals.org/article.aspx?articleid=711029

(17) Sackett David L, Rosenberg William M C, Gray J A Muir, Haynes R Brian, Richardson W Scott. Evidence based medicine: what it is and what it isn't BMJ 1996; 312 :71. Available from: http://www.bmj.com/content/312/7023/71

(18) Lorraine Johnson and Raphael B Stricker. The Infectious Diseases Society of America Lyme guidelines: a cautionary tale about the development of clinical practice guidelines. Philosophy, Ethics, and Humanities in Medicine 2010, 5:9

Available from: http://www.peh-med.com/content/5/1/9

**(Separate references for PICO questions)**

1. Hanincova K, Mukherjee P, Ogden NH, Margos G, Wormser GP, et al. (2013) Multilocus

Sequence Typing of *Borrelia burgdorferi* Suggests Existence of Lineages with Differential

Pathogenic Properties in Humans. PLoS ONE 8(9): e73066

Available from: http://www.plosone.org/article/info:doi/10.1371/journal.pone.0073066

2. Qiong Wu, Guiquan Guan, Zhijie Liu, Youquan Li, Jianxun Luo and Hong Yin. RNA-Seq-based

 analysis of changes in *Borrelia burgdorferi* gene expression linked to pathogenicity. Parasites & Vectors (2015) 8:155

Available from: http://www.parasitesandvectors.com/content/pdf/s13071-014-0623-2.pdf

3. Gary P. Wormser, Aimee T. Tang, Natasha R. Schimmoeller, Susan Bittker, Denise Cooper, Paul Visintainer, Maria E. Aguero-Rosenfeld, Katarina Ogrinc, Franc Strle, Gerold Stanek. Utility of serodiagnostics designed for use in the United States for detection of Lyme borreliosis acquired in Europe and vice versa. Medical Microbiology and Immunology. Feb 2014; 203 (1): 65-71.

Available from: http://link.springer.com/article/10.1007/s00430-013-0315-0

4. Wormser GP, Liveris D et al. Effect of *Borrelia burgdorferi* Genotype on the Sensitivity of C6

and 2-Tier Testing in North American Patients with Culture-Confirmed Lyme Disease.

Clin Infect Dis. Oct 1, 2008; 47(7): 910–914. Available from:

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2773679/#!po=71.8750

5. Russell TM, Delorey MJ, Johnson BJ. *Borrelia burgdorferi* BbHtrA degrades host ECM

proteins and stimulates release of inflammatory cytokines in vitro. Mol Microbiol. 2013

Oct;90(2):241-51. Available from: https://www.ncbi.nlm.nih.gov/pubmed/23980719

6. Gherardini FC. *Borrelia burgdorferi* HtrA may promote dissemination and irritation. Molecular

Microbiology, Volume 90, Issue 2, pages 209–213, October 2013.

Available from: http://onlinelibrary.wiley.com/doi/10.1111/mmi.12390/full

7. Patent Application - Publication number WO2013110026 A1

Available from:

https://www.google.com/patents/WO2013110026A1?cl=en&dq=johnson+lyme&hl=en&sa=X&ei=5tiyU8iuIsb\_oQTJooCoDg&ved=0CBwQ6AEwAA

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FIELD OF THE INVENTION

[0002] Generally described herein are compositions and methods of the present invention relating to detection, diagnosis and/or treatment of Lyme disease, also known as Lyme borreliosis. Specifically described are compositions and methods of the present invention relating to B. burgdorferi HtrA sensu lato (BbHtrA) protease activity, its substrates, cleavage products, biological effects and use in detection, diagnosis and/or treatment of Lyme disease.

8. Geeta Ramesh, Andrew G. MacLean and Mario T. Philipp. Cytokines and Chemokines at the Crossroads of

Neuroinflammation, Neurodegeneration, and Neuropathic Pain. Hindawi Publishing Corporation.

Mediators of Inflammation Volume 2013, Article ID 480739, 20 pages.

Available from: http://dx.doi.org/10.1155/2013/480739

9. Alexia A. Belperron, Nengyin Liu, Carmen J. Booth, and Linda K. Bockenstedt. Dual role for Fcγ receptors in host defense and disease in *Borrelia burgdorferi*-infected mice. Front Cell Infect Microbiol. 2014; 4: 75.

Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4052197/

10. Marije Oosting, Anneleen Berende, Patrick Sturm, Hadewych J. M. ter Hofstede, Dirk J. de Jong, Thirumala-Devi Kanneganti, Jos W. M. van der Meer, Bart-Jan Kullberg, Mihai G. Netea, and Leo A. B. Joosten. Recognition of *Borrelia burgdorferi* by NOD2 Is Central for the Induction of an Inflammatory Reaction. J Infect Dis. (2010) 201 (12): 1849-1858 doi:10.1086/652871

Available from: http://jid.oxfordjournals.org/content/201/12/1849.long

11. Bramwell KKC, Teuscher C, Weis JJ. Forward genetic approaches for elucidation of novel regulators of Lyme arthritis severity. Frontiers in Cellular and Infection Microbiology. 2014;4:76. doi:10.3389/fcimb.2014.00076.

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4046100/

"Thus, the identification of genes important in Lyme arthritis also illuminated previously unrecognized pathways in RA. This linkage to a gene associated with Sly syndrome, an overt congenital lysosomal storage disease (LSD), strongly implicated a common pathogenic mechanism involving accumulation of undigested glycosaminoglycans (Tomatsu et al., 2009). This possibility was confirmed by detection of pronounced Alcian blue staining of sulfated GAGs in the inflamed joint tissues of B. burgdorferi infected and K/BxN treated mice with partial or severe Gusb deficiencies (Figure ​(Figure5).5). The association of Gusbh with increased disease severity in both Lyme-associated and rheumatoid arthritis identifies Gusb as a shared immunopathology disease gene (Teuscher, 1985; Sudweeks et al., 1993; Ma et al., 2002).

The novelty of the beta-Glucuronidase polymorphism highlights the power and added value of forward genetic approaches. Gusb is most often cited in the recent scientific literature as a housekeeping gene, primarily used as a reference to study something more interesting, making it a most unlikely candidate for a hypothesis-driven reverse genetics study. Allelic variants of the Gusb gene were found not to be differentially expressed under baseline conditions, and no changes in Gusb expression were detected by microarray analysis of joint tissue from naïve and infected C3H and B6 mice (Crandall et al., 2006; Bramwell et al., 2014). Thus, Gusb and other similar genes associated with LSD are not likely to be picked up by a microarray or RNA-Seq study in Lyme arthritis patients. Gusb was also not included in the ImmunoChip used in human RA and juvenile RA studies, because it had not yet been identified as a potential regulator (Eyre et al., 2012; Hinks et al., 2013).

More recently, Steere and colleagues have confirmed the association of two Class II alleles (DRB1\*0101 and 0401) for the subgroup of patients with treatment refractory Lyme disease but not in the larger group of patients that respond to antibiotic treatment, and have proposed an auto-immune mechanism in this treatment refractory group (Steere et al., 2006; Drouin et al., 2013).

A number of investigators found association of MHC haplotypes with antibody recognition of individual B. burgdorferi antigens using MHC congenic mouse lines. However, use of MHC congenics in our studies and in those of other investigators led to the conclusion that MHC alleles were not determinants for the differences in arthritis severity found 4 weeks following infection in C3H-H2k, C57BL/6-H2b, and DBA-H2d mice (Yang et al., 1992; Brown and Reiner, 2000). Thus, studies with mice are consistent with patient studies failing to show association with early Lyme arthritis. Interestingly, mice expressing the H2k allele do not develop collagen-induced arthritis, a contrast with their development of severe Lyme arthritis (Wooley et al., 1981).

Identification of TLR1/TLR2 in the host response to B. burgdorferi in humans and mice

Early seminal studies into the host-pathogen interaction of B. burgdorferi revealed the potential of the spirochete and its lipoproteins to induce inflammatory cytokine production in a variety of human and mouse cell types (Radolf et al., 1991; Wooten et al., 1996; Sellati et al., 1998). The association of NF-κB with these inflammatory responses directed numerous laboratories to investigate the involvement of Toll-like receptors as these molecules were discovered as central components of inflammatory responses to microbial pathogens (Wooten et al., 1996; Sellati et al., 1998). These studies documented the interactions between B. burgdorferi lipoproteins with TLR2 and TLR1, both with mouse knock-out and cell culture transfection studies and in patients, and established a critical role for TLR signaling through MyD88 in host defense to this pathogen (Aliprantis et al., 1999; Brightbill et al., 1999; Hirschfeld et al., 1999, 2000; Alexopoulou et al., 2002). More recent studies by Schroder et al. identified a human variant in TLR2, Arg753Gln, with reduced pro-inflammatory signaling in patient samples (Schroder et al., 2005). Cells from mice heterozygous for this variant also displayed reduced inflammatory responses to B. burgdorferi lysate. Notably, this TLR2 allele was significantly underrepresented within a cohort of late stage Lyme disease patients, suggesting that it has a protective effect.

Oosting et al. found that N248S and S602I polymorphisms in TLR1 were associated with reduced in vitro responsiveness to B. burgdorferi and TLR1/TLR2 agonist stimulation (Oosting et al., 2011a). Using a similar experimental approach, the same group also reported that peripheral blood mononuclear cells (PBMCs) for individuals bearing an IL-23R Arg381Gln polymorphism exhibited a reduced Th17 response following in vitro stimulation with B. burgdorferi (Oosting et al., 2011b). However, there was no association between the IL-23R polymorphism and the persistence of symptoms among patients in the study population, arguing against a role for this SNP in disease pathogenesis.

Strle et al. recently described the frequency and impact of several polymorphisms in the TLR1 gene within a cohort of Lyme disease patients (Strle et al., 2012). This study found a skewed inheritance pattern of TLR1 1805GG polymorphisms within an antibiotic-refractory Lyme arthritis patient population. They also recognized a synergy between inheritance of this host polymorphism and infection with a particular invasive isolate (termed RST1) of B. burgdorferi. Importantly, patients carrying TLR1 1805GG exhibited higher serum levels of CXCL9 and CXCL10 chemokines, consistent with a functional role for this polymorphism. This effect was reproduced through in vitro activation of PBMCs with a B. burgdorferi RST1 isolate, arguing that heightened production of these IFNγ-inducible chemokines may set the stage for antibiotic refractory arthritis."

12. M. Ahmed, N. Zlotnikov, A. Arya, M. Parikh, J. Gananam, R. Ebady, A. Bansal, A. Koh, F. Lakschevitz, A. Javid, M.C. Lima, T. Tang, Y.R. Kim, F. Matar, C. Sun, M. Santana-Sosa, I. Talior-Volodarsky, N. Gupta, P. Song, G. Wormser, I. Schwartz, C.A. McCulloch, M. Glogauer, T.J. Moriarty. Obese Mice have Higher Numbers of Lyme Disease Spirochetes in Tissues. International Conference on Lyme Borreliosis. Boston, MA, United States. Aug. 18-21, 2013.

13. Moriarty Lab/Matrix Dynamics

Available from: http://piil.matrixdynamics.ca/lab\_projects.html

"4. Role of diet-induced obesity in enhanced host susceptibility to disseminated Lyme disease

Lyme disease is the most common vector-borne disease in the northern hemisphere, and its incidence is increasing rapidly throughout the industrialized world (nearly a three-fold increase over the last twenty years). Although climate warming and associated expansion of tick habitat are thought to contribute to increasing Lyme disease incidence, the reasons for the rising prevalence of this disease are not yet fully understood. Increasing Lyme disease incidence has occurred in parallel with rising rates of obesity in the industrialized world. One of the major groups affected by Lyme disease is the middle-aged, in whom obesity and associated conditions such as diabetes and cardiovascular disease are now widely prevalent. We recently found that high fat diet-induced obesity in mice is associated with a significantly elevated bacterial burden in many tissues to which Borrelia disseminates. Currently, we are investigating the mechanisms responsible for enhanced Borrelia infectivity in obese hosts, and are examining the effects of hyperglycemia and systemic vascular inflammation to disseminated Lyme disease."

14. Khan, AnisA. et al. Effect of clarithromycin and azithromycin on production of cytokines by human monocytes.

International Journal of Antimicrobial Agents , Volume 11 , Issue 2 , 121 - 132

Available from: http://www.ijaaonline.com/article/S0924-8579(98)00091-0/fulltext

15. Kanoh S, Rubin BK. Mechanisms of Action and Clinical Application of Macrolides as Immunomodulatory Medications. Clinical Microbiology Reviews. 2010;23(3):590-615. doi:10.1128/CMR.00078-09.

Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2901655/

16. Feng J, Auwaerter PG, Zhang Y (2015) Drug Combinations against *Borrelia burgdorferi* Persisters In Vitro: Eradication Achieved by Using Daptomycin, Cefoperazone and Doxycycline. PLoS ONE 10(3): e0117207.

Available from: http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0117207

17. Walsh, Nancy. Worst Summer Yet for Lyme Disease? MedPage Today. June 18, 2012.

Available from: http://www.medpagetoday.com/InfectiousDisease/GeneralInfectiousDisease/33337