Comprehensive *Borrelia burgdorferi* specific inflammatory immune response analysis in patients with Lyme disease

Pranay D. Khare, Ph.D.[1], Meenakshi Khare, Ph.D. [2], Gottfried H. Kellermann, Ph.D.[1, 2]

[1]NeuroScience, Inc., Osceola, WI USA, [2]Pharmasan Labs, Osceola, WI

Published in Cytokine. Vol. 50 (1-2) page 99. 2010.

Abstract

Background: Lyme disease is the most prevalent vector-borne infectious disorder in humans in the United States, caused by the spirochete *Borrelia burgdorferi*. The inflammatory processes induced by *B. burgdorferi* remains largely unknown.

Objective: The purpose of this study was to examine the inflammatory immune response to *B. burgdorferi* specific antigens in patients diagnosed with Lyme disease compared with asymptomatic healthy subjects.

Methods: Recombinant *B. burgdorferi* antigens (OspC, VlsE-1, p41, p100) were prepared and used in the study. Peripheral blood mononuclear cells (PBMCs) were purified from whole blood obtained from patients with Lyme disease (n = 40) and asymptomatic healthy subjects (n =10). Purified PBMCs were exposed to each of the specific *B. burgdorferi* antigens. A comprehensive multiplex cytokine analysis was performed on the supernatant of the PBMCs after the exposure of the *B. burgdorferi* specific antigens and compared to un-stimulated controls.

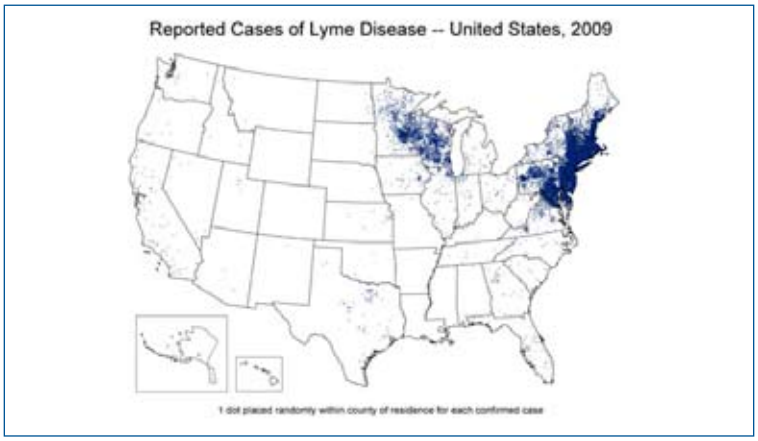
Results: A trend for elevated levels of IL-1β, IL-6, TNF-α, IFN-γ, IL-8, IL-10, G-CSF and GM-CSF was observed in patients with Lyme disease compared to healthy subjects upon spontaneous release as well as following stimulation with *B. burgdorferi* antigens. Compared to healthy subjects, significant elevated levels ($p<0.05$) of IL-6 and TNF-α were observed upon p41 stimulation, and of IL-8 upon p100 stimulation in patients with Lyme disease.

Conclusion: These findings indicate that the patients with Lyme disease had an active immune response with pronounced inflammation. The etiology of an active immune response in Lyme patients needs to be explored further to identify treatment regimens that go beyond anti-bacterial drugs. This study emphasizes the assessment of *B. burgdorferi* specific inflammatory biomarkers for Lyme disease in clinical practice.

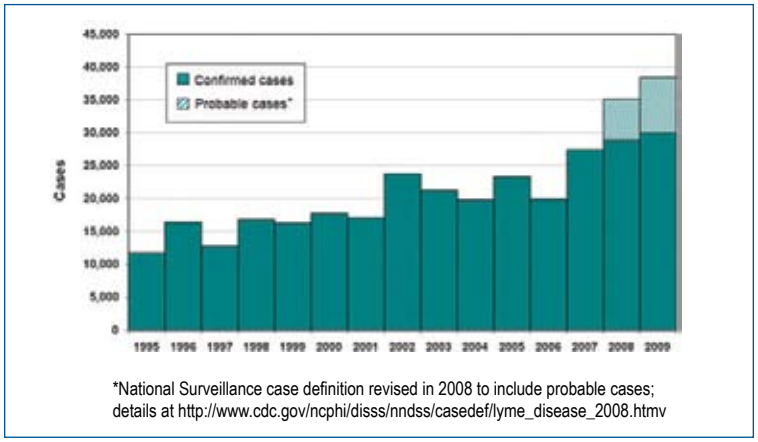
Background Information

- *Borrelia burgdorferi* is the etiologic agent of Lyme disease, a most common tick borne inflammatory disease in United States.
- Clinically, Lyme disease is divided into early and late disease. Over time, *B. burgdorferi* infection can result in multisystem complication of body including joints, skin, cardiac and nervous system (1-4).
- Immune mediated mechanisms are believed to play a major role in host defense, but also Lyme disease pathology (5-6).

Geographic of Lyme Disease[7]

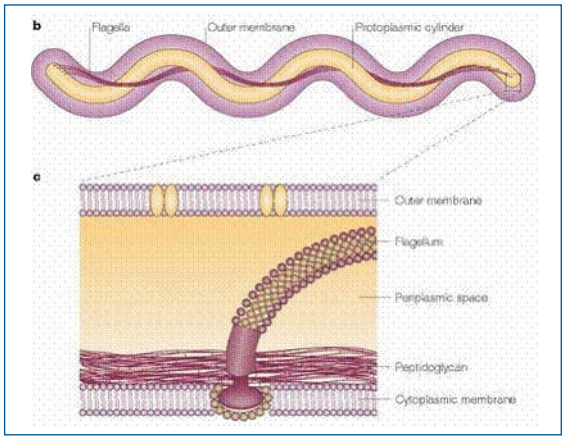


Frequency of Lyme Disease[7]



*National Surveillance case definition revised in 2008 to include probable cases; details at http://www.cdc.gov/ncphi/disss/nndss/casedef/lyme_disease_2008.htmv

Schematic of *B. burgdorferi*



Ref. #8

Objective

To evaluate the inflammatory immune response to *Borrelia burgdorferi* specific antigens in patients diagnosed with Lyme disease compared with asymptomatic healthy subjects.

NEUROSCIENCE
The Leader in Neuro-Endo-Immune Solutions

Methods

- Whole Blood was obtained from patients with seropositive (WB) for Lyme disease (n = 40) and asymptomatic healthy subjects (n =10).
- Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll, washed 2X
- PBMCs were cultured in 24-well plates with recombinant *Borrelia burgdorferi* specific antigens (OspC, VlsE-1, p41, p100).
- Cultures were incubated for 24 hours at 37oC, 5% CO2.
- Supernatant was collected, spun and stored at -20°C.
- Cytokines were determined using Multiplex Human-Cytokine Assay for:
  - Pro-inflammatory Cytokines: IL-1β, IL-6, IFN-γ, TNF-α
  - Anti-inflammatory Cytokines: IL-10
  - Pro-inflammatory Chemokines: IL-8 (CXCL8)
  - Growth factors: G-CSF, GM-CSF
- Statistical Analysis: Mann-Whitney test.
  Graphs: Scatter plot with Mean ± SEM

Lyme Protein Antigens

OspC: Outer surface protein C. An early antigen of the spirochete, important for virulence and appears shortly after tick bite.

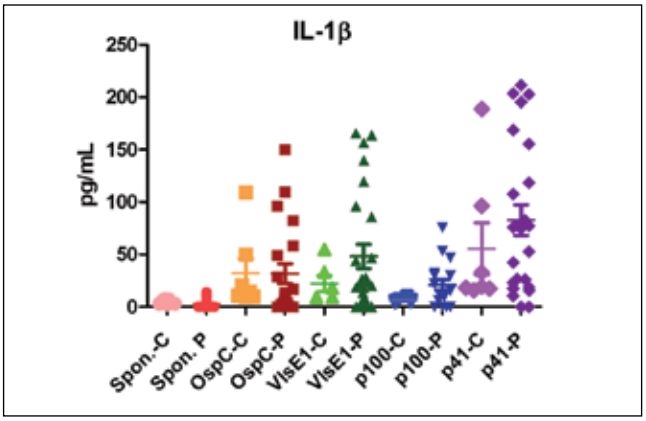
VlsE1: Outer surface lipoprotein (Variable surface antigen E). Plays a major role in the immune response to the *B. burgdorferi*.

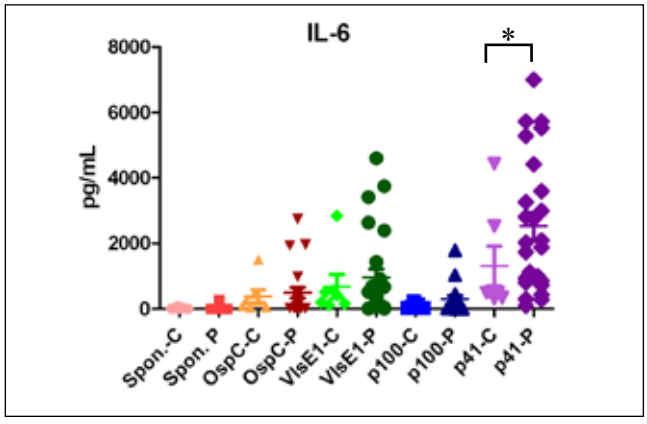
p41: Flagella protein. An immunodominant spirochete protein for early and late stage immune response.

p100: Chromosomal protein. An immunodominant protein of the late stage immune response.

Results (Pro-inflammatory Cytokines)

PBMC from Lyme disease patients produce more IL-1β and IL-6

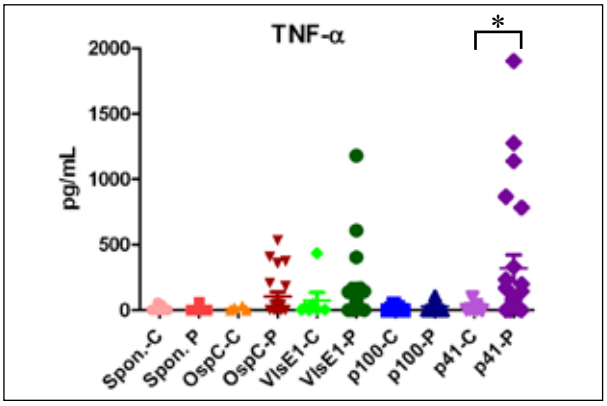


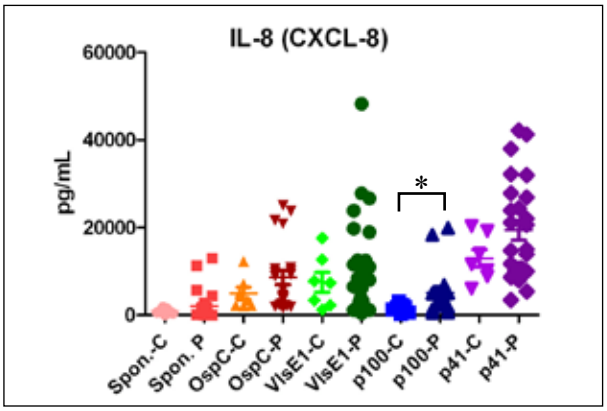


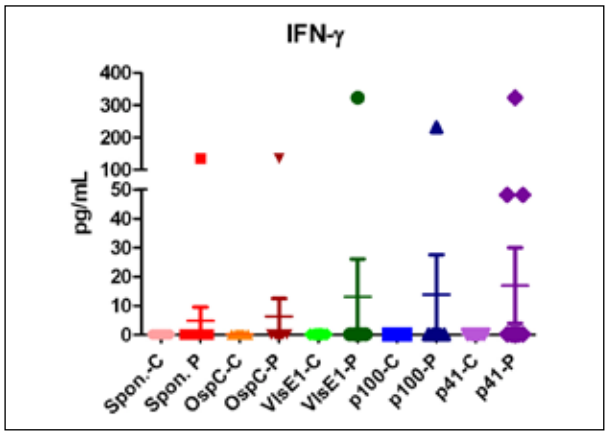
*** A significantly high (p <0.05) amount of IL-6 was observed in PBMC from patients with Lyme disease upon p41 stimulation.**

Results (Pro-inflammatory Cytokines and Chemokines)

In Patients with Lyme disease, PBMC produce increased TNF-α, IL-8, and IFN-γ





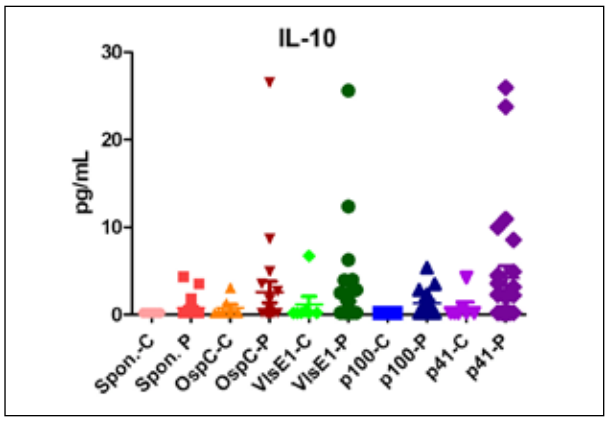


*** Significantly elevated levels (p<0.05) of TNF-α were observed upon p41 stimulation, and of IL-8 upon p100 stimulation in patients with Lyme disease.**

KEY
Spon: Spontaneous release (baseline)
C: Asymptomatic healthy population
P: Patients with seropositive (WB) for Lyme disease

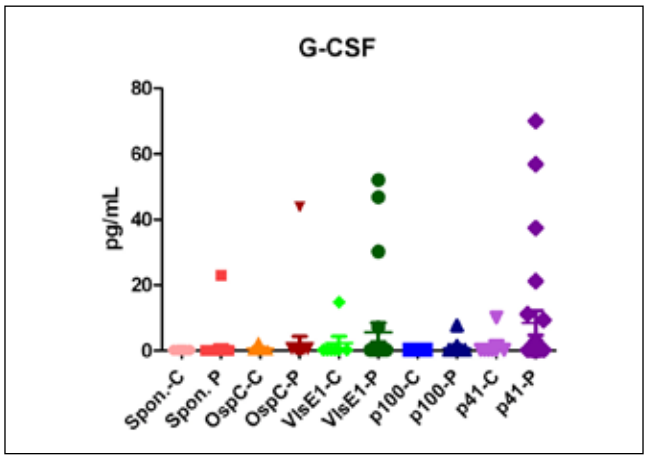
Results (Anti-inflammatory Cytokine)

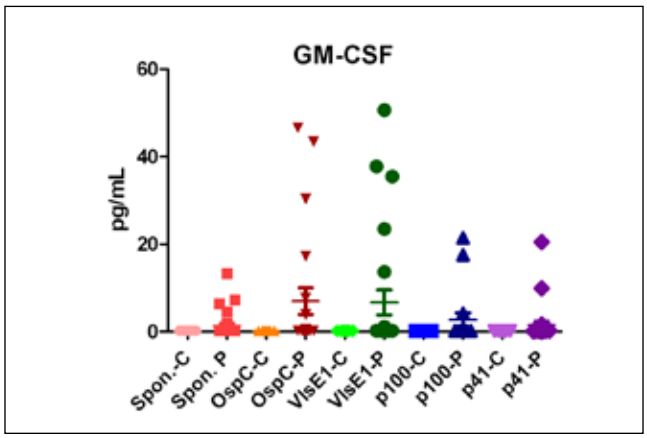
Increased production of IL-10 may indicate anti-inflammatory mechanisms to control infection and reduce bacterial burden



Results (Growth factors)

High levels of G-CSF and GM-CSF may promote inflammation in patients with Lyme disease by stimulating increased granulocyte and macrophage production





Conclusion

- *B. burgdorferi* antigens elicit cytokine production in PBMC from both Lyme disease patients and healthy subjects, consistent with earlier reports and suggestive of an innate stimulatory capacity of these antigens in vitro (9).
- Elevated PBMC cytokine production in response to *B. burgdorferi* antigens suggests an ongoing immune response in patients with Lyme disease, with both pro-inflammatory elements that may contribute to pathology, as well as IL-10 likely acting to curb inflammation.
- Unlike measurement of serum cytokine levels, assessment of cytokine production from antigen-stimulated PBMC can provide an indication of a specific instigator of inflammatory pathology, thereby informing appropriate therapeutic strategies.
- This approach is a useful method for determining active infection in Lyme disease patients who might otherwise be misdiagnosed (e.g., seronegative individuals).

References

1. Evans, J.1995.Lyme disease. Curr.Opin.Rheumatol. 7:322–8.
2. Haass, A.,and J.Treib.1996. Neurologic manifestation and classification of borreliosis. Infection 24:467–9.
3. Paparone, P.W.1997. Cardiovascular manifestations of Lyme disease. J.Am Osteopath. Assoc. 97:156–161.
4. Mullegger RR. 2004. Dermatological manifestations of Lyme borreliosis. Eur J Dermatol, 14: 296 – 309.
5. Berende A et al. 2010. Activation of innate host defense mechanism by Borrelia. Eur Cytokine Netw. 21:7-18.
6. Feder HM, Jr. et al. 2007. A critical appraisal of “chronic Lyme disease”. N Engl J Med 357:1422-30.
7. CDC Website (http://www.cdc.gov/ncidod/dvbid/lyme/ld_UpClimbLymeDis.htm)
8. Rosa PA etal. 2005. The burgeoning molecular genetics of the Lyme disease spirochete. Nat Rev Microbiol. 3:129-43.
9. Giambartolomei GH et al. 1998. *Borrelia burgdorferi* stimulates the production of IL-10 in peripheral blood mononuclear cells from uninfected humans and rhesus monkeys. Infect Immunity. 66: 2691-7.

Contact Information

NeuroScience, Inc.
Attn: Pranay D. Khare, PhD
375 280th Street
Osceola, WI 54020
Email: pranay.khare@neurorelief.com
Phone: 888-342-7272

This abstract was peer-reviewed, accepted and presented in poster format at the “International Society for Interferon and Cytokine Research” meeting October 3rd-7th, 2010.