SpiroFind

Technology Summary May 2013

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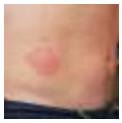
A New Way to Diagnose Lyme Disease

Unlike traditional Lyme disease diagnostics, SpiroFind™ does not look for either *Borrelia* or antibodies for *Borrelia*. Instead, the test measures the cellular immune response to *Borrelia*.



The principle behind the SpiroFind™ test is the mechanism by which the pathogen interacts with the immune system. This diagnostic assay measures the immunologic reaction throughout disease manifestation that is visible as the typical erythema migrans (EM) shortly after infection near the site of the tick bite.

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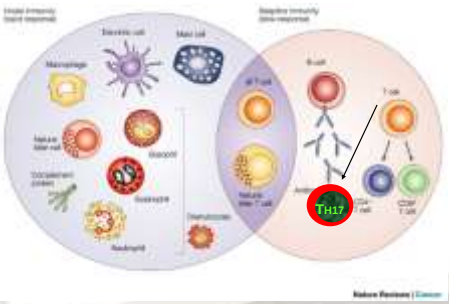
Innate Immunity Adaptive Immunity



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Lyme Disease is a very old disease

Iceman's DNA reveals health risks and relations.

Ötzi's genome hints at heart disease, bacterial infection and common ancestry with modern-day Sardinians.

Ewen Callaway

28 February 2012

Nature Communications

"Ötzi's genome also hints at other health problems: Zink's team found almost two-thirds of the genome of "*Borrelia borgdorferi,*" a bacterium that causes Lyme disease".

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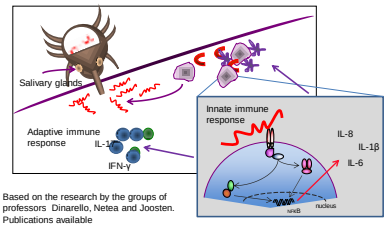
Immune Response to Lyme Disease



Based on the research by the groups of professors Dinarello, Netea and Joosten. Publications available

The primary immune response to Borrelia infection is the innate immune response and not the T-cell mediated adaptive immune response.

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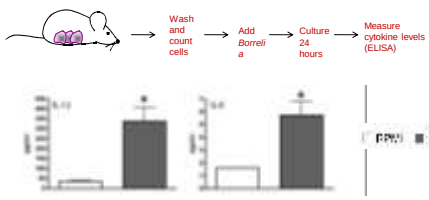
The Role of the Innate Immune System in Lyme Disease Was First Established in Mice



Isolated mouse monocytes secrete cytokines after stimulation with *B. burgdorferi*. This observation raised the question of which cellular signaling pathway causes this response.

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Evidence for the Mechanism of the Innate Immune Response to *Borrelia*

Distinct Roles for MyD88 and Toll-Like Receptors 2, 5, and 9 in Phagocytosis of *Borrelia burgdorferi and Cytokine Induction*
Ok S. Shin, Infect Immun. 2008 Jun;76(6):2341-51

Toll-Like Receptor 2 Is Required for Innate, But Not Acquired, Host Defense to *Borrelia burgdorferi*
R. Mark Wooten, J Immunol. 2002 Jan 1;168(1):348-55

Cutting Edge: Inflammatory Signaling by *Borrelia burgdorferi Lipoproteins Is* Mediated by Toll-Like Receptor 2
Matthew Hirschfeld, J Immunol. 1999 Sep 1;163(5):2382-6.

Knock-out experiments confirmed the role of Caspase 1 in *Borrelia* response.

Nature Reviews Immunology **7**, 31-40 (January 2007)

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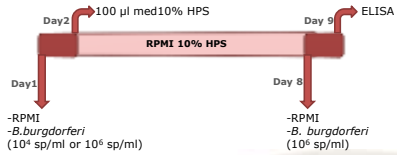
The Innate Immune System Can Be Trained

In an ex vivo model, it can be shown that adherent PBMC cells stimulated with *B. burgdorferi* on day 1 will show a much higher response to a stimulation with the organism on day 8 than those cells that were not pre-stimulated.



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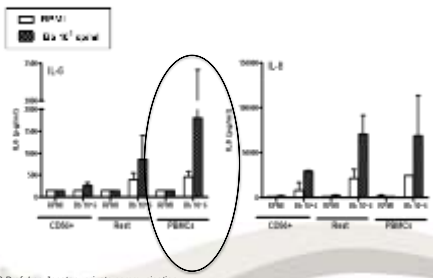
Results for Trained Immunity In Vitro

PBMCs pre-stimulated with 10^4 sp/ml *B. burgdorferi* after 9 days show a much higher IL-6 secretion upon stimulation with 10^6 sp/ml *B. burgdorferi* than cells not pre-stimulated.



2012 Prof. Leo Joosten, private communication

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Foundation for the SpiroFind™ Test

- The test measures the primary cellular immune response of monocytes after a 24 h incubation with a mix of *B. Burgdorferi, B. Afzelii, B. Garini,* without addition of serum or other immune stimulatory reagents.
- IFN-γ is not secreted as part of the primary *Borrelia* infection
- The cytokines IL-1ß , IL-6, IL-8, und IL-1a (intracellular) are secreted. Since the former three are in constant ratio to each other, the IL-1ß signal can be employed for assay read-out.
- The IL-1ra protein is also secreted, but not as a specific reaction to the *Borrelia* stimulation
- The stimulation with *Candida albicans* and the glycolipid PMA serve as assay controls

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SpiroFind™ Protocol

- The peripheral blood monocyte fraction (PBMCs) is isolated from the delivered blood sample.
- The cell number of the monocyte fraction is determined and a specified amount of monocytes is incubated with a pre-determined amount of SpiroFind *Borrelia* mix in the SpiroFind cell medium for 24 h. During this incubation, the IL-1ß cytokine is produced in relation to the added amount of *Borrelia* mix and secreted into the medium.
- The IL-1ß cytokine is quantified from the supernatant of the incubation reaction in a bead ELISA assay.
- For a negative control, a cell sample without *Borrelia* addition is treated under the same conditions. As a positive control for the vitality of the harvested monocytes, a sample is induced with the unspecific immune stimulant PMA and treated under the same assay conditions.
- The specified reference values for a positive reaction at the given MOI were determined empirically.

	Control	*Borrelia* cocktail: *afzelii, garini, burgdorferi*				Unspecific
Stimulant	RPMI	MOI 1	0.1	0.01	0.001	PMA
Ref. Value	<100	>2800	>1400	>100	>100	>100

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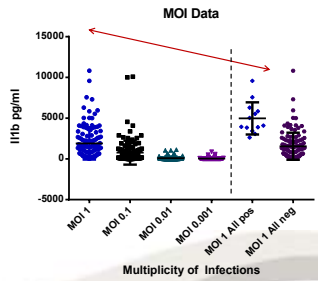
Results of the modified cellular test (SpiroFind™)



PBMCs stimulations, 24h.

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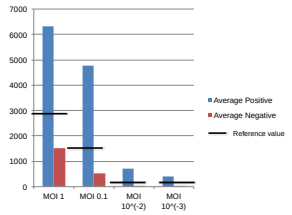
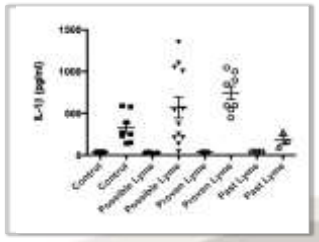
SpiroFind™ Test Results: IL-1ß (pg/ml)



The average values of the positive samples are significantly higher than those of the negative samples. However, due to the inherent individual variability of cellular immune reactions, only a positive reaction at all four MOI concentrations gives a reliable positive result.

SpiroFind™ Correlation with Clinical Diagnosis

An early study at the University of Nijmegen showed the IL-1ß signal correlates well with clinical diagnosis and may provide a diagnostic method to confirm effectiveness of treatment.



Nijmegen Borferon study vs. Serology

Table 3. Comparison of SpiroFind versus Borrelia *ELISA*

Number of patients tested		59	
Number of positives SpiroFind		15	25%
Number of patients tested serology		55	
Number of positives (both ELISA/Western Blot)		17	31%
Number of evaluated samples (SpiroFind vs serology)		55	
positive SpiroFind + serology		7	13%
negative SpiroFind + serology		31	56%
positive SpiroFind, negative serology	FP	8	15%
negative SpiroFind, positive serology	FN	10	18%

Abbreviations: FP, false-positive; FN, false-negative

The relatively high number of false positives and false negatives compared to serology does not necessarily mean the test has lower sensitivity. Serology can be positive in patients who are not currently infected, but had the disease in the past. The SpiroFind test only identifies current infections.

SpiroFind™ Specificity

Case-ID	il1b_B_Mix_V1 Ref=5900	il1b_B_Mix_V2 Ref=3000	il1b_B_Mix_V3 Ref=900	il1b_B_Mix_V4 Ref=100	SpiroFind Score	IGG	IGM	patient's history
13-DE-00051	7,943.24	1,729.47	368.7	191.37	0	0	0	HIV, under Therapie, no Viremia
13-DE-00052	8,635.38	1,630.02	694.1	63.06	0	0	0	Brain infection under Antibiosis, Diabetes
13-DE-00053	3,512.62	1,301.05	168.06	878.99	0	0	0	Liver infection
13-DE-00054	9,478.64	2,567.34	202.54	20.86	0	2	0	Foot Ulcer with Osteomyelitis, HepC
13-DE-00055	6,788.89	2,294.33	424.83	172.61	0	2	0	Urinary tract infection, CRP 10, D-Dimere
13-DE-00056	4,766.96	1,487.13	182.76	337.13	0	0	0	healthy
13-DE-00057	4,260.09	1,523.93	805.86	237.95	0	0	0	healthy
13-DE-00058	3,010.48	1,529.68	384.49	56.23	0	1	0	slight respiratory Infektion, otherwise healty
13-DE-00059	9,018.88	5,128.82	803.13	71.51	0	2	0	HIV, under Therapy, no viremia, October tick bite with redness, no therapy
13-DE-00060	8,426.44	1,142.63	96.05	5.91	0	0	0	HIV under therapy
13-DE-00061	4,632.99	1,102.71	1,085.63	320.71	0	0	0	Respiratory infection, influenza, but Patient had past borreliosis, possibly active now?
13-DE-00073	10,985.21	4,123.24	2,298.59	186.23	2	2	0	possible borreliosis

The SpiroFind assay is specific for *Borrelia* infection. Other infections do not interfere.

Case 0059 is questionable.

Case 0073 is an example of a Lyme positive patient.

An additional cohort of five *Coxiella* patients (Q-fever) also tested negative for Lyme Disease.

Ongoing Clinical Studies

- University of Erlangen/University of Nijmegen:
  - Correlation between SpiroFind results and treatment response, evaluated among others by SF36 score
  - Correlation between SpiroFind results and PCR (where available)
  - Specificity of SpiroFind by testing related and unrelated diseases
- University Clinic Cluj
  - Correlation between SpiroFind results and EM
- US clinical trials to support FDA submission
  - Principle investigator Dr. Brian Fallon, Columbia U