Lyme Immunopathogenesis Kevin Driver 2004

Lyme Borreliosis is a complex multisystem illness caused by infection with *Borellia burgdorferi* sensu latu spirochetes transmitted by exposure to *Ixodes scapularis* ticks. Lyme disease was first described in the late 1970s with the causative spirochete, *Borrelia burgdorferi* cultured from *Ixodes* ticks and isolated from infected individuals in 1982 and 1983 respectively. In the quarter decade since the pathogen was discovered, much has been learned of the immunopathogenesis of Lyme Borelliosis from *in vitro* studies, *in vivo* animal models, and inferred from epidemiological study of infected individuals. However, significant gaps in knowledge remain, with crucial questions clinically relevant to both diagnosis and treatment left outstanding. The intent of this review is to outline the extent of the current knowledge regarding Lyme Disease immunopathogensis with particular emphasis on recent findings, and to evaluate current models of Lyme Borreliosis in light of the existing empirical data.

Early Infection, Immunologic Characteristics of Erythema Migrans, and Borrelial Dissemination

Borrelia burgdorferi spirochetes subsist within the gut of infected *Ixodes* ticks, both adult and nymphal. Upon tick attachment and initiation of the blood meal, *B. burgdorferi* ticks are aroused and activated, presumably by the resultant increase in temperature associated with the influx of blood. *In vitro* studies have shown that *B. burgorferi* is able to initiate molecular changes in its outer surface proteins (OSPs) in response to temperature change; specifically there is a downregulation of the dominant tick-phase OspA and replacement with upregulated OspC. This Osp switching is significant as humoral immunity to OspA has been shown to be sufficient to prevent infection and actually target and neutralize the spirochete while in the tick gut. Following the temperature induced structural changes; the spirochete migrates to the tick saliva glands and ultimately infects the host at the level of the superficial dermal tissues. The spirochete then initially spreads peripherally, activating innate immune mechanisms, resulting macroscopically in the characteristic Erythema Migrans rash. It is likely that the spirochete proliferates and attains the highest density in the dermis, as biopsy of the Erythema Migrans rash is the location most likely to yield spirochete cultures. The spirochete is able to migrate efficiently through the fibrous tissue of the dermis, with motility provided both by flagella at both axes as well as its ability to bind host matrix proteins, most notably fibronectin, a ubiquitous component of the extracellular matrix (Szczepauski et al 1986). An intrinisic collagenase activity has also been attributed to *B. burgdorferi* spirochetes *in vitro* further enabling motility and penetration through the dermis (Grab et al 1996). *Bb* additionally has been shown to bind decorin, a collagen-associated proteoglycan via two separate decorin binding proteins; interestingly mice immunized with antibodies to spirochetal decorin binding proteins prior to *Bb* inoculation are resistant to successful *Bb* infection (Hanson et al 1998). This finding indicates that *Bb* binding to host matrix associated proteins is either necessary to produce active infection, or that the immunogenic profile of such binding elements in early infection is great enough to result in successful clearance of the pathogen.

A study undertaken by Salazar et al elucidates the *ex vivo* immune components of peripheral blood and Erythema Migrans lesions of early Bb infection. This study reports that blister fluid samples from EM lesions revealed a 100 fold amplification of leukocyte population in compared to normal skin, as well as relative enrichment of T lymphocytes and Monocyte/Macrophage populations and paucity of neutrophil and B lymphocyte populations (Salazar et al 2003). EM lesional infiltrates also showed a marked increase in relative proportions of T lymphocytes compared to blister fluid samples following intradermal *Bb* lipoprotein injection. Lesional infiltrates showed increased expression of cell type-specific activity markers of CD14 and CD27 on neutrophils and T lymphocytes respectively. This study additionally revealed an unambiguous Th1 cytokine profile of both EM lesional infiltrates: IFN-γ and IL-6 levels were significantly increased (Salazar et al, 2003).

From the dermis the spirochete gains access to the vascular system via local inflammatory processes yielding peripheral vasculature increasingly permeable. Both proinflammatory cytokines, including IFN-γ, TNF-α, and IL-6, as well as borrelial binding and utilization of host enzymes, notably metalloproteases and plasminogen, contribute to spirochete crossing the endothelium and accessing the intravascular compartment (Coleman et al 1995). The spirochete is then disseminated hematogenously to its various target organs. Interestingly, the spirochete seems to be only pathogenic in several specific locations corresponding to the usual clinical manifestations of Lyme Borreliosis. In the skin the spirochete induces Erythema Migrans and, in the case of Eurasian Borrelial species *B. garinii* and *B. afzelii*, Acrodermatitis Atrophicans. *B. burgdorferi* also is competent to infect the heart and pericardial cavity in early disseminated infection; where it is responsible for causing AV block, cardiomyopathy, and pericarditis. Spirochetes have been demonstrated histopathologically in cardiac biopsy sections. Spirochetes also localize to the synovia of principally large joints, as well as central and peripheral nervous tissues. A fortuitous biopsy sample also yielded *Bb* cultures from the iris as well as the posterior chamber of the eye (Meier et al, 1999). But the most devastating clinical symptoms of fatigue, encephalopathy, and neuritis result from Bb infection of neural tissues, specifically the Central Nervous System.

Immunopathogenesis of Neuroborreliosis

B. burgdorferi spirochetes have been shown to enter and actively infect the human Central Nervous System compartment: *Bb* spirochetes have been successfully cultured from cerebrospinal fluid and spirochetal structures have been demonstrated from neurohistological preparations from cerebral biopsy (Pachner et al 1989). Increases in proinflammatory cytokines induced by infecting *Bb* spirochetes as well as induced upregulation of metalloproteinases MMP-9 and MMP-1 by PBMCs and neutrophils likely contribute to spirochetal access to the CNS compartment by permeabilizing the blood brain barrier (Gebbia et al 2001). Once in the CNS, typical CSF finding of neuroborreliosis include elevated intrathecal protein, increased intrathecal antibodies to *Bb*, as well as lymphocytic pleocytosis. The lymphocytic infiltration in turn produces the CSF cytokines upregulated in neuroborreliosis: Th1 cytokines IFN-γ, TNF-α, IL-1, and IL-6. Post-mortem autopsy of a patient with CSF *Bb* culture positivity and persistent encephalopathy and neuritis despite several courses of antibiotic therapy (Liegner et al

1997) revealed perivascular fibrosis of cerebral vasculature as well as meningeal infiltration by lymphocytes and plasmacytes, with particular damage localized to the leptomeninges (Libian unpublished observations).

Several models have been proposed to elaborate mechanisms of injury in neuroborreliosis, and the debate centers on whether the continued presence of the spirochete is necessary for neurological injury resulting in the encephalopathic symptoms of clinical Lyme Disease. In the first case *B. burgdorferi* may be both sufficient and necessary to directly cause neurological injury of neuroborreliosis, either through direct action of Borrelial proteins or induction of pathogenic inflammation. A borrelia neurotoxin has been invoked as a possible mediator of neurological injury, and clinicians treating 'chronic lyme disease' have used this hypothesis as a rationale for diverse treatments including GI binding agent Cholestyramine to sequester putative borrelial toxins. However, this reviewer failed to find documentation in the peer-reviewed literature for any Lyme neurotoxin, and it seems that the hypothesis of a pathogenic borrelial neurotoxin is speculative at best.

Recent results from Ramesh et al revealed the potential borrelia lipoprotein to induce astrogliosis, a non-specific neuropathology marked by astrocyte proliferation and apoptosis *in vivo* as well as inducing astrocyte proliferation and apoptosis *in vitro* via concurrently upregulating IL-6 and TNF-α production in the rhesus monkey astroglial cultures utilized (Ramesh et al 2003). The authors believe that the finding of lipoprotein induced astrogliosis and proinflammatory cytokine upregulation could be sufficient to bring about neuroborrelial signs and symptoms. *Bb* has been shown to adhere to and produce *in vitro* cytotoxic effects to rat glial cells as early as 1991, so brain parenchyma cytotoxicity has already been suspected in *Bb* neuropathology (Garcia-Monco and Benach 1997). Models of direct *Bb* involvement have hypothesized that Nitric Oxide mediates the inflammatory cytotoxic effects of Neuroborreliosis (Ibid).

In the second model of neurological injury, *B. burgdorferi* spirochetes function indirectly; initiating an autoimmune reaction in the CNS compartment. Development of autoantibodies to peripheral nervous tissues was noted by Sigal and Tatum (1998) and Aberer et al (1989), however CNS autoimmunity has been more difficult to establish. This view is supported both by the clinical observation of the difficulty to demonstrate active spirochetal infection in patients with encephalopathic signs concurrent with definite Lyme Disease, and by *ex vivo* isolation of autoreactive and cross-reactive T cell lines. A nonpathogenic strain of *Bb* had previously been shown to induce production of IgM autoreactive to gangliosides *in vivo* in rats (Garcia-Monco et al 1995), and more importantly subsets of neuroborreliosis patients were found to have autoreactive antibodies by ELISA: with IgG reactive to cardiolipin and IgM reactive to gangliosides. Martin et al report the isolation of *ex vivo* T cell lines from *Bb* infected individuals, one from peripheral blood capable of cross reacting with *Bb* antigens and galactocerebrosides and several more lines from two separate patients capable of cross reacting between *Bb* antigens and myelin basic protein (Martin et al, 1988). Whether these putative cross reactive T cell lines represent a single authentic clonal line or several variants from an oligoclonal T cell population was not determined. Additionally this study did not specify the Borrelial antigen(s) responsible for presumably inducing this autoreactivity through molecular mimicry. However, Steere et al have elucidated human lymphocyte function associated antigen-1 (LFA-1) as a potential self protein target for autoreactivity in T cells

with Bb OspA recognition (Steere et al 2001). The worry is that such an interaction could result in a positive feedback loop of inflammation: IFN-γ production is upregulated in T cells recognizing OspA antigen, IFN-γ stimulation of T cells upregulates hLFA-1 and its ligand ICAM-1. There is no indication that such a target would have significance in neurological injury.

Immune Evasion by *B. burgdorferi*

        While persistent chronic Lyme disease remains controversial, several lines of evidence point to specific spirochetal strategies to evade immune responses as well as antibiotic therapy. These strategies may include immune avoidance, evasion through molecular morphology mediated by altered gene expression, direct cytotoxicity, as well as immune modulation.
        As described previously, the specific localization pattern of the *Bb* spirochete is not well understood. However, its clinical effects seem to be limited to the dermis, cardia, synovia, as well as peripheral and central nervous tissues. Such locations may indicate that the spirochete may preferentially exist in sites relatively inaccessible to immune surveillance. It is possible that these sites are negatively selected for spirochetal survival due to the inefficiency of immune penetration.
        In contrast, spirochetal morphology has been thoroughly studied and several critical insights have been established. First, the *Bb* spirochete exists in three different morphological forms: the corkscrew shaped active spirochete, spheroplast (L-form), and a cystic form (Brorson and Brorson 1998, Preac-Mursic et al 1996). Concurrent to changes in morphology the spirochete alters gene expression, presumably to effect the morphological change. Structural change is enacted through transcriptional changes; in the case of *Bb* alterations of expression are believed to triggered by diverse stimuli including temperature changes, factors contained within serum media, culture density, as well as potentially in response to antibody binding (Indest et al 1997, Ramamoorthy and Philipp 1998, Alban et al 2000). The latter possibility is a potential explanation for the finding that OspC expressing spirochetes are found in the absence of anti-OspC antibody in vivo, while spirochetes cultured from anti-OspC producing animals have undetectable levels of OspC expression; $OspC^+$ phenotypic Bb could be rescued from the latter animals with ablation of OspC antibody (Fang et al 2002). These findings are believed to be due to selection and expansion of an $OspC^-$ subset of the *Bb* population in the presence of antibody mediated selective pressure. In addition to its ability to alter morphology and gene expression in response to environmental stimuli the spirochete is able to move into the intracellular compartment of various mammalian cell types. This function was first noted in fibroblasts, but has been additionally observed in synovial cells, and lymphocytes (Dorward et al 1997). Intracellular localization would be sufficient to evade immune surveillance, protect spirochetes from immune effector functions: antibody binding and phagocytosis, as well as potentially modulating the immune response; the latter because spirochetal antigens would be presented to lymphocytes via MHC I rather than MHC II in the presence of different costimulatory factors.
        In addition to invading lymphocytes, two separate groups have documented spirochetal cytotoxicity specifically to lymphocytes. Sandra et al documented a four fold

increase in apoptosis of CD4+ T lymphocytes following 24 hour incubation with whole live *Bb* and antibiotic inactived *Bb*, as well as a three fold increase in apoptosis with sonicated *Bb*. They additionally found this apoptotic increase to be correlated with CD95 (*Fas*) overexpression in CD4+ T lymphocytes; this *Fas* overexpression, in conjunction with a demonstrated upregulation of *ex vivo* IL-10 production by PBMCs exposed to lipidated OspA and OspC led to their hypothesis that T lymphocyte apoptosis is mediated by the release of anti-inflammatory cytokines following *Bb* stimulation of lymphocytes Sandra et al 2003). Dorward et al reports that in a coincubation of human PBMCs and live *Bb* spirochetes, spirochetes were able to attach to lymphocytes within hours, invade via endocytic pits, and induce death via membrane permeabilization of both B and T lymphocytes, with B lymphocytes showing apoptosis to a greater degree (Dorward et al 1997). In contrasting results to the Sandra study, no cytotoxicity was observed from heat-killed *Bb* or *Bb* sonicates. In a separate presentation of the same microscopic data, Dorward indicates that *Bb* spirochetes were able to emerge from their invaded lymphocytes ensheathed in lymphocyte membrane which was maintained for an additional 24 hours *in vitro* (Dorward video). Such results demonstrate an intriguing phenomena of *Bb* cytotoxicity to lymphocytes in specific, although the mechanisms suggested by the studies differ; results from Dorward et al strongly suggest a direct cytotoxic effect of the virulent *Bb* spirochetes, while Sandra proposes a cytokine mediated apoptotic effect, in accord with *ex vivo* results demonstrating that naïve PBMCs exposed to *Bb* lysates induce greater amounts of anti-inflammatory cytokines IL-10 and G-CSF while PBMCs from patients with chronic *Bb* seropositivity and Lyme Disease symptoms released an attenuated level of proinflammatory cytokines IFN-γ and TNF-α to lipopolysaccharide endotoxins (Diterich et al 2001). Both of these studies require additional *in vivo* evidence of *Bb* cytotoxicity, as in each case spirochetes were coincubated in 50 to 100 fold excess to PBMCs to show any cytotoxic effect; the difficulty of culturing *Bb* in patients with active infection argues against an overwhelming bacterial load *in vivo*.

Autoreactivity may not be limited to the humoral arm of the specific immune; Naïve primate lymph node ex-plants generated in the presence of freeze-thawed *Bb* have been shown *in vitro* to secrete IL-6 cocultured with autologous PBMCs in the absence of *Bb*. The ability of to produce a stimulatory, proinflammatory factor in the absence of antigen represents an autoreactive response. However, the identity of the putative autoreactive cells is still undetermined; the population of cells proliferating under these conditions lacked prominent T cell markers CD3, CD4, and CD8 (Ganapamo et al 2001).

γδ T cells represent another leukocyte subset which may play a critical role in Bb immunopathogenesis. A family of γδ T cells expressing the Vδ1 T cell receptor (TCR) upregulate activity and control markers CD25 and FasLigand and simultaneously downregulate their TCR when exposed to PBMC and *Bb* (Vincent et al 1998). These γδ T cells are activated by antigen-presenting dendritic cells (DC) exclusively, and the response is dependent on the lipid moiety of *Bb* Osps: the activating response is abrogated with exposure to unlipididated Osps (Vincent et al 1998). This population of activated, FasL expressing γδ lymphocytes have been shown to induce apoptosis of CD4 T helper cells in synovia, and may be involved in the immunopathogenesis of additional organ systems (Vincent et al 1998).

Additional evidence indicates that *ex vivo Bb*-specific T cells from peripheral blood of Lyme disease patients are able to produce significant amounts of both IFN-γ as well as IL-10 in the presence of endogenously produced IL-12 (Pohl-Koppe et al 1998). B cells also may play an active role in producing the strong Th1 cytokine profile of Lyme disease. Murine B cells from *Bb* infected mice acquire the capability of producing IFN-γ and IL-10 in response to *Bb* exposure (Ganapamo et al 2001). These cytokines are in addition to IL-18 found to be constitutively expressed by murine B cells post *Bb* infection, and IL-12 and IL-6, which *Bb* exposure can stimulate (Ganapamo et al unpublished results). All of these cytokines are stereotypic of a Th1 response and interestingly, both IL-12 and IL-18 have stimulatory properties to Natural Killer cells, IL-6 induces acute phase proteins as well as stimulating the B cell dependent humoral response, while IL-10 inhibits macrophage activity (Janeway 749).

Initial evidence points to a depression of specific non-T, non-B cell population specified by the marker CD57 in patients with chronic symptoms of Lyme disease (Stricker et al 2001). This leukocyte population was found to be lower in patients with chronic encephalopathy than predominantly musculoskeletal symptoms and appeared to resolve with prolonged antibiotic treatment. Indications are that this depressed cell population is specific to chronic Lyme disease not stereotypic of either simply chronic infectious processes or similar symptomatic conditions: CD57 levels were found to be normal in AIDs patients and increased in patients with rheumatoid arthritis. Additional clinical case evidence has indicated that CD57 levels remain depressed throughout a prolonged course of chronic Lyme disease (Stricker et al 2002). CD57 is an oligosaccharide marker present on a broad spectrum of NK cells, monocytes, and both B and T lymphocytes. Its function is currently unknown, although increased CD57 levels in T cell populations has been documented in HIV, malaria, chronic lymphocytic leukemia, and rheumatoid arthritis; its expression is believed to be induced late in an immune response (d'Angeac et al 1994).

The functional role of CD57 must be more clearly elucidated to assess the significance of this finding in chronic lyme disease, however it does potentially relate to features of the mammalian *Bb* response discussed previously. First, the cytokine profile elicited by *Bb* infection includes the prominent NK stimulatory factors IL-12 and IL-18; an autoreactive subset of non-T, non-B IL-6 secreting cells, which may represent NK functionality, has been documented as well as a depression of CD57 non-T non-B cell population in patients with previous Lyme disease and chronic symptoms. Second, chronic Lyme infection may induce or result from an inappropriate control of innate immune response mediators: NK cells, and monocyte/macrophages. Studies such as Diterich et al documenting an attenuated release of proinflammatory cytokines from chronic Lyme patients to LPS endotoxins and a relative increase in anti-inflammatory cytokines elicited by *Bb* antigenic stimulation of naïve leukocytes strongly supports this concept, although it is unclear whether the imbalanced *Bb* stimulation toward anti-inflammatory responses induces the attenuated proinflammatory response of chronic Lyme patients. Finally, as natural killer cells are key mediators of innate immune responses to altered host cells, they may be critical to eliminating residual intracellular or host cell membrane-ensheathed immune evading *Bb* spirochetes. Thus the finding of an autoreactive population of possible NK functionality may represent an inappropriate autoreactive response while depressed CD57 levels of patients with manifestations of

chronic Lyme disease may represent an inability to clear invading intracellular phase spirochetes. Such hypotheses are clearly speculative and more research is necessary to adequately assess these findings; unfortunately NK cells remain an immune population not currently well understood.

What else to talk about:

Development of a CNS immune response Re: Bb

Several insights have been elicited as to the development of an immune response to Bb in the CNS. First, the response is initiated and coordinated by activated CD4+ T lymphocytes. The antigen-specific activation state is important for intrathecal retention of T lymphocytes (Naparstek et al 1983, Nekeric et al 1986), and B cells and monocyte/macrophages fail to enter the CNS of Bb infected T cell deficient mice (Tyor et al 1989). Indeed MHC II specific T cells have been described in the CSF of acute Lyme Disease patients (Pachner et al 1985); T cells and NK cells show a preference for CSF localization while B cells and monocyte/macrophages remain within the brain parenchyma (DE Griffin in Schutzer 48). Additionally Bb must supply a stimulus to induce MHC II expression on microglia for activated T cells to recognize antigen and respond with effector functions, as MHC II is not constitutively expressed by this CNS cell type (Griffin 47). The timing of these events is still controversial; the accepted model is the peripheral humoral response preceding the CNS as spirochetes have been demonstrated in the CNS without intrathecal antibodies concurrently with peripheral anti-Bb antibodies (Pfister et al 1989). However seronegative CNS Bb infection with intrathecal anti-Bb antibody has been documented in the literature and remains an exception to this model (Lawrence et al 1995).

CD14 coreceptor

- CD14 levels highest in patients with early Lyme Disease, levels decline to normal in transition from late Lyme Disease to chronic Lyme Disease (Linetal et al 2000)
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Outstanding questions:

IL-6 Th1 or Th2? Is this significant?

- stimulated by Bb (Habicht et al 1991, Andersson and Andersson 1990)
- elevated in CNS infections (Schutzer 157)
- produced by microglia and astocytes in response to infection Frei et al, Righi et al 1989)
- cell extracts and whole spirochetes needed for IL-6 from glioma cells NOT Bb Lipoproteins (Schutzer 157-8).

Which models of Inflammation fit with Bb infection in Dermis/CNS: acute, chronic or granulomatous?

What to do with referencing the Libian unpublished autopsy data?

Should I disregard Discussion of CD57 in light of your unpublished data?

References:

1. Garcia-Monco JC, Benach JL. Mechanisms of Injury in Lyme Neuroborreliosis. *Seminars in Neurology* 1997; 17: 57-62.
2. Pachner AR, Duray P, Steere, AC. Central Nervous System Manifestations of Lyme Disease. *Arch. Neurol.* 1989; 46: 790-5.
3. Sellati TJ, Bouis DA, Caimano MJ, Feulner JA, Ayers C, Lien E, Radolf JD. Activation of Human Monocyte Cells by *Borrelia burgdorferi* and *Treponema pallidum* is Facilitated by CD14 and Correlates with Surface Exposure of Spirochetal Lipoproteins. *J. Immunol.* 1999; 2049-56.
4. Hu LT, Klempner MS. Host-Pathogen Interactions in the Immunopathogenesis of Lyme Disease. *J. Clin. Immunol.* 1997; 17: 354-365.
5. Liegner KB, Duray P, Agricola M, Rosenkilde C, Yannuzi LA, Ziska M, Tilton RC, Hulinska D, Hubbard J, Fallon BA. Lyme Disease and the Clinical Spectrum of Antibiotic Responsive Chronic Meningoencephalomyelitides. *J. Spirochetal and Tick-borne Diseases* 1997; 4: 61-73.
6. Ganapamo F, Dennis VA, Philipp MT. CD19+ cells produce IFN-γ in mice infected with *Borrelia burgdorferi*. Eur. J. Immunol. 2001; 31: 3460-3468.
7. Ganapamo F, Dennis VA, Philipp MT. *Borrelia burgdorferi* Stimulates In Vitro a Selective Expansion of CD3⁻CD4⁻CD8⁻ (Triple Negative) Autoreactive Cells in Naïve Rhesus Monkey Lymphocytes. *J. Infect. Diseases* 2001; 183: 1221-8.
8. Stricker RB, Burrascano JJ, Winger EE. Longterm Decrease in the CD57 Lymphocyte Subset in a Patient with Chronic Lyme Disease. *Ann. Agric. Environ. Med.* 2002; 9: 111-3.
9. Stricker RB, Winger EE. Decreased CD57 Lymphocyte Subset in Patients with Chronic Lyme Disease. *Immunol. Letters* 2001; 76: 43-8.
10. Coleman, JL, Sellati, TJ, Testa JE, Kew RR, Furie MB, Benach JL. *Borrelia burgdorferi* Binds Plasminogen, Resulting in Enhanced Penetration of Endothelial Monolayers. *Infect. Immunity* 1995; 63: 2478-84.
11. Ramesh G, Alvarez AL, Roberts ED, Dennis VA, Lasater BL, Alvarez X, Philipp MT. Pathogenesis of Lyme Neuroborreliosis: *Borrelia burgdorferi* Lipoproteins Induce both Proliferation and Apoptosis in Rhesus Monkey Astrocytes. *Eur. J. Immunol.* 2003; 33: 2539-50.
12. Salazar JC, Pope CD, Sellati TJ, Feder HM, Kiely TG, Dardick KR, Buckman RL, Moore MW, Caimano MJ, Pope JG, Krause PJ, Radolf JD. Coevolution of Markers of Innate and Adaptive Immunity in Skin and Peripheral Blood of Patients with Erythema Migrans. *J. Immunol.* 2003; 2660-70.
13. Sigal, LH. Immunologic Mechanisms in Lyme Neuroborreliosis: The Potential Role of Autoimmunity and Molecular Mimicry. *Seminars in Neurology* 1997; 17: 63-68.
14. Giambartolomei GH, Dennis VA, Lasater BL, Murthy PK, Philipp MT. Autocrine and Exocrine Regulation of Interleukin-10 Production in THP-1 Cells

Stimulated with *Borrelia burgdorferi* Lipoproteins. *Infect. Immunity* 2002; 70: 1881-8.

15. Lawrence C, Lipton RB, Lowy FD, Coyle PK. Seronegative Chronic Relapsing Neuroborreliosis. *Eur. J. Neurol*. 1995; 35: 113-7.

16. Martin R, Ortlauf J, Sticht-Groh V, Bogdahn U, Goldmann SF, Mertens HG. *Borrelia burgdorferi*-specific and Autoreactive T-Cell Lines from Cerebrospinal Fluid in Lyme Radiculomyelitis. *Ann. Neurol*. 1988; 24: 509-516.

17. Steere AC, Gross D, Meyer AL, Huber BT. Autoimmune Mechanisms in Antibiotic Treatment-Resistant Lyme Arthritis. *J. Autoimmun*. 2001; 16: 263-8.

18. Sandra P, Gianni P, Mario P, Marilena G, Rossella M, Marina C. Lymphocyte Apoptosis Co-cultured with *Borrelia burgdorferi*. *Microb. Pathogenesis* 2003; 35: 139-45.

19. Dorward DW, Fischer ER, Brooks DM. Invasion and Cytopathic Killing of Human Lymphocytes by Spirochetes Causing Lyme Disease. *Clin. Infect. Diseases* 1997; 25: 52-8.

20. Pohl-Koppe A, Balashov KE, Steere AC, Logigian EL. Hafler DA. Identification of a T Cell Subset Capable of both IFN-γ and IL-10 Secretion in Patients with Chronic *Borrelia burgdorferi* Infection. *J. Immunol*. 1998; 1804-10.

21. Lin B, Noring R, Steere AC, Klempner MS, Hu LT. *J. Infect. Diseases* 2000; 181: 1185-8.

22. Liang TL, Jacobs MB, Bowers LC, Philipp MT. An Immune Evasion Mechanism for Spirochetal Persistence in Lyme Borreliosis. *J. Exp. Med*. 2002; 195: 415-22.

23. Vincent MS, Roessner K, Sellati T, Huston CD, Sigal LH, Behar SM, Radolf JD, Budd RC. Lyme Arthritis Synovial γδ T Cells Respond to *Borrelia burgdorferi* Lipoproteins and Lipidated Hexapeptides. *J. Immunol*. 5762-71.

24. Diterich I, Harter L, Hassler D, Wendel A, Hartung T. Modulation of Cytokine Release in Ex Vivo-Stimulated Blood from Borreliosis Patients. *Infect. Immunity* 2001; 69: 687-94.

25. Meier P, Blake R, Gau M, Spencker FB, Wiedemann P. Pars Plana Vitrectomy in *Borrelia burgdorferi* Encephalomyelitis. *Klin. Monatsbl. Augenheilkd*. 1999; 213: 351-4.

26. Janeway CA, Travers P, Walport M, Schlomchik MJ. *Immunobiology* 6 ed. 2005.

27. Schutzer SS ed. *Lyme Disease: Molecular and Immunologic Approaches*. 1992.

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