

## Review

# Novel Diagnosis of Lyme Disease: Potential for CAM Intervention

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Lyme disease (LD) is the most common tick-borne disease in the northern hemisphere, producing a wide range of disabling effects on multiple human targets, including the skin, the nervous system, the joints and the heart. Insufficient clinical diagnostic methods, the necessity for prompt antibiotic treatment along with the pervasive nature of infection impel the development and establishment of new clinical diagnostic tools with increased accuracy, sensitivity and specificity. The goal of this article is 4-fold: (i) to detail LD infection and pathology, (ii) to review prevalent diagnostic methods, emphasizing inherent problems, (iii) to introduce the usage of *in vivo* induced antigen technology (IVIAT) in clinical diagnostics and (iv) to underscore the relevance of a novel comprehensive LD diagnostic approach to practitioners of Complementary and Alternative Medicine (CAM). Utilization of this analytical method will increase the accuracy of the diagnostic process and abridge the time to treatment, with antibiotics, herbal medicines and nutritional supplements, resulting in improved quality of care and disease prognosis.

**Keywords:** *Borrelia burgdorferi* – *in vivo*-induced antigen technology – Lyme disease – multi-peptide ELISA

## Introduction

Bacterial infection with specific spirochetes from the genus *Borrelia*, usually acquired from the bite of an infected *Ixodes* tick, is the cause of Lyme disease (LD). The species complex *B. burgdorferi sensu stricto* is predominantly responsible for LD cases in the United States while *B. burgdorferi sensu lato*, *B. afzelii* and *B. garinii* are the responsible pathogenic species in Europe and Asia (1,2).

Presentation and destructive effects of LD vary widely depending on the stage and magnitude of spirochete dissemination. Initial effects may include rashes or flu-like symptoms, while later stages may be characterized by arthritic, neurological, psychiatric and cardiac symptoms.

Due to the varying nature of symptom appearance there exist a number of DNA technologies and serological methods that may be utilized, in conjunction with clinical examination, to support and confirm a diagnosis of LD; however, the techniques maintain certain pitfalls. Divergent symptoms between infected individuals and flawed clinical diagnostics hinder accurate diagnosis and prompt antibiotic treatment, which are essential for a good prognosis and the prevention of chronic infection (3).

The conventional treatment for LD is a course of antibiotic therapy, such as doxycycline, amoxicillin, penicillin, erythromycin and other antibiotics listed in Table 1. Maximal efficacy of oral antibiotic administration is rendered in the early stages of infection, typically days or weeks following pathogen introduction. At the chronic stage of the disease, administration of a combination of these medications orally, intramuscularly and intravenously is required for several months before an attenuation of infection is apparent (4–8).

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**Table 1.** CAM treatment of Lyme disease with a combination of different antibiotics, herbal remedies, and nutritional supplements, with pertinent references

Antibiotics used for treatment of Lyme disease (Refs 6–8, 10, 11)	Herbal remedies	Ref	Nutritional supplements	Ref
Doxycycline	Samento	(24)	Omega-3 Fatty Acids	(26)
Minocycline	Cumada	(24)	Coenzyme-Q10	(26)
Amoxicillin	Burbur	(24)	S-Adenosylmethionine	(8, 24, 25)
Augmentin	Allicin	(25)	Grapeseed Extract	(26)
Azithromycin	Dragon's blood	(25)	Magnesium	(24)
Biaxin	Cat's claw	(25)	L-Carnitine	(4, 8)
Erythromycin	Devil's claw	(24)	$\alpha$ -Lipoic acid	(14, 15)
Vaucomycin	Echinacea	(25–28)	Dimethylglycine	(27)
Clarithromycin (biaxin)	Citriodiol (lemon eucalyptus extract)	(9)	Vitamin B-complex	(4, 8)
Cefuroxime axetil	Astragalus	(29)	Methylcobalamin	(4, 8)
Penicillin	Nettle	(24)	Vitamin C and D	(12, 13)
Ceftriaxone	Ginkgo biloba	(4, 8)	Hydrolytic enzymes	(16, 17)
Rifampin	Curcumin	(19–20)	Mushroom extracts and Beta-glucan	(22)
Flagyl	Oregano tea	(25)	Thymic peptides	(29)
Plaquenil	Boswellia	(26)	Pycnogenol	(27)
Taurox SB <sup>TM</sup>	Parsley extract	(24)	N-Acetylcysteine and glutathione	(26)
Colloidal silver	Red chili pepper (capsaicin)	(18)	Probiotics	(23)
Imipinem	Quercetin	(21)	Dehydroepiandrosterone	(12)
			Royal Jelly	(26)

The swiftness in diagnosis is further emphasized by the characteristic abilities of *Borrelia*, via antigenic changes on its surface, to avoid the immune system and subsequently infiltrate areas of the body maintaining low levels of drug distribution, such as the joints and nervous system. Due to this complexity, Complementary and Alternative Medicine (CAM) holds great promise for the abrogation of disease pathology through the combination and utilization of accurate diagnostic methods and effective treatment modalities (9–30).

The CAM treatment for LD, summarized in Table 1, combines intravenous antibiotic therapy, herbal medicine administration and nutritional medicine to enhance the immune system's ability to fight this intracellular microorganism (9–30).

Considering the side effects of antibiotics and the length of this treatment with CAM modalities, an effective procedure for diagnosis of LD is needed to justify such a course. A novel diagnostic approach to LD, utilizing *in vivo* induced antigen technology (IVIAT) and enzyme-linked immunosorbent assay (ELISA), may provide the data necessary to justify CAM treatment of LD and associated spirochetes. Therefore, it is imperative to review the merit of this diagnostic approach, which will expedite the accurate diagnosis of LD, in order to emphasize its importance to practitioners of CAM in areas of the world greatly affected by LD (31–34).

### Prevalent Diagnostic Techniques: The Benefits and Inherent Drawbacks

A number of diagnostic tests for LD aim to detect the presence of *Borrelia* antigens and related immune system components (35–39). While the theory behind the development of these techniques was sound, these tests suffer from false results (40); recent research has served to give an understanding as to the reasons underlying such inaccuracies.

Polymerase chain reaction (PCR) for LD attempts to detect the DNA of the spirochete. In a recent study, urine-based PCR for LD was evaluated and shown to be insufficient for a diagnosis (35).

The Center for Disease Control (CDC) proposes a two-tiered approach to LD diagnosis (36). This includes an initial screening test such as ELISA with high sensitivity, which is followed by an immunoblot with high specificity.

### ELISA and Initial Screening Test for LD

The Lyme ELISA test is intended for the quantitative detection of IgG and IgM antibodies to *B. burgdorferi* in human serum. Titers of IgG are generally low during the first weeks of illness. They peak within three to six weeks after onset but are often not detectable in symptomatic patients (37). This assay is performed based on crude

preparation of *B. burgdorferi* in culture and its binding to solid phase matrix. IgG and IgM antibodies in patients serum binds specifically to antigen coated wells resulting in color development. The intensity of color is equivalent to the level of antibodies.

### Western Immunoblot Assay for Confirmation of LD

The western blot assay has been widely used to detect the presence of antibodies in human serum and plasma to various infectious disease agents. In this procedure, component proteins of purified, inactivated bacteria are electrophoretically separated by SDS-polyacrylamide electrophoresis followed by electrotransfer to nitrocellulose sheets (38). Each strip served as the solid-phase antigen for an ELISA test. The western blot assay is more reliable since the cross-reactive antibodies are excluded and peptide specific antibodies are observed. However, if antibodies are not present in the blood, false negative results will be obtained (39).

### Drawbacks of Serodiagnosis of LD

The current problems with serodiagnosis of LD include a lack of standardization and poor evaluation criteria regarding sensitivity and specificity. These troubles are due in part to the broad heterogeneity of the immunodominant antigens from disease causing *Borrelia* strains and the utilization of a single strain in the whole-cell lysate immunoblot assay (36). It is thought that the usage of several strains of different species would increase assay sensitivity; while the usage of multiple *Borrelia* species grown in culture does provide a broader set of antigens, it is not sufficient due to antigenic variations and expression employed by *Borrelia* to avoid immune destruction in the mammalian system (40–43). Therefore, it is crucial to combine clinical symptomology with sensitive techniques such as IVIAT Multi-Peptide ELISA that take into account antigenic variation.

### ***In Vivo* Included Antigen Technology: The Key to Enhanced Specificity**

IVIAT is a technique that identifies pathogen antigens that are immunogenic and expressed *in vivo* during human infection. Regulating gene expression optimizes a pathogen's ability to proliferate within specific environments, inducing or repressing specific genes to enhance growth within the host during infection, thus contributing to overall pathogenicity (41–43).

IVIAT identification of these *Borrelial* gene products, especially antigens from variable regions, during infection may be utilized to develop enhanced diagnostic methods (43). The chronic nature of LD and the antigenic diversity of the spirochetes suggest the spirochete

undergoes dramatic changes in antigenic composition as it cycles between its arthropod and mammalian hosts and that this variation plays an important role in immune detection and destruction. Major proteins or lipoproteins called variable major protein (VMP) or VMP-like sequence (VlsE) are responsible for this antigenic variation strategy evolved to avoid immune detection and destruction (44, 45).

Furthermore, different membrane surface protein E (OSPE) enables the spirochete to evade the immune system and to maintain chronic infection (46–48). In this multi-peptide ELISA assay based on IVIAT (32–34), we selected peptides from different components of *Borrelia* during specific life cycles, including:

### Outer Surface Proteins and Leukocyte Function Associated Antigen

A prominent late manifestation of *B. burgdorferi* infection is Lyme arthritis. Development of antibody reactivity to outer surface protein A (OspA) occurs towards the beginning of prolonged arthritic episodes (49). The progression to an autoimmune state begins with a cross-reactive response between OspA and a self-antigen, e.g. leukocyte function associated antigen (LFA). Identification of a bacterial antigen that displays cross-reactivity with an autoantigen may provide a model for development of autoimmune disease induced by *B. Burgdorferi* and provide an important antigen to incorporate into diagnostic methods (50). Outer surface protein C (OspC) is thought to be an important virulence factor involved in the transmission and establishment of early infection (51, 52). *Borrelia burgdorferi* reduces the expression of OspC in response to the development of antibodies, indicating the importance of repressing OspC expression to avoid clearance and to maintain infection (53–55). Since humans produce highly specific antibodies against OspC, measurement of IgG and IgM antibodies against this highly conserved epitope indicate recent infection with *B. burgdorferi*. The Outer surface protein E (OspE) is expressed in both ticks and mammals, and elicits a strong antibody response. Specificity of antibody response with different OspE variants suggests that the hypervariable regions of OspE are targeted by the antibody response during infection. Consequently, it is important to assess the specificity of the antibody response to OspE epitopes during infection and incorporate this information into a testing method to target antibodies to OspE (56).

### Immunodominant Antigens

Two peptides, termed C2 and C6, are conserved across *B. burgdorferi sensu lato*, *B. burgdorferi sensu stricto* and *B. garinii* (44–47). Antigenicity of these peptides is supported by the observation that sera from humans,

monkeys and mice react with C2 and C6 peptide early and persistently through the course of infection, indicating that these peptides contain one or more epitopes that are broadly antigenic (57).

In a study of human serum samples collected in the northeast and mid-west of the US, from patients with acute or chronic LD, 35 of the 41 samples reacted with and presented significant levels of antibodies against the C6 peptide. The five serum samples that had no detectable levels of antibodies against the C6 peptide were obtained from patients who were in the early stage of infection (58).

### Variable Major Protein

The spirochetes that cause LD express the surface lipoprotein VMP or VlsE (45). Infection-induced sequence changes that alter antigenic properties of VlsE contribute to immune evasion. In an experimental model a strong VlsE IgG antibody response was apparent by the fourth week of infection, indicating that VlsE is expressed during early stage of infection. Serum samples from LD patients also possessed VlsE antibodies that were immunoreactive against all species of *Borellia* (45). This indicates that there are conserved epitopes among VlsE variants that are antigenic during infections. Based on these experiments, researchers employed recombinant VlsE in an ELISA test and demonstrated diagnostic sensitivities of 63% for culture-confirmed LD erythema migrans (EM) cases and 92% for late stage LD infections.

### Peptides from Complement Regulator Acquiring Surface Protein-1 (CRASP-1)

It is well known that the three different genospecies of *B. burgdorferi* exhibit distinct disease manifestations and tissue tropisms during mammalian infection considering the ability of pathogenic *Borrelia* sp. to survive intracellular in the mammalian host, these spirochetes must have developed efficient strategies for circumventing the innate immune response (59–61). Along these lines, it has been suggested that CRASPs are key mediators that allow *B. burgdorferi* to evade the innate immune response and complement-mediated destruction during the acute stage of infection. Thus CRASP-1 expression is necessary for *B. burgdorferi* to resist killing by human serum (59, 61).

### Peptides from Decorin Binding Protein for Species-Specific Serodiagnosis of Lyme Arthritis and Neuroborreliosis

Among individual *Borrelial* proteins from different species, sequence heterogeneity varies up to 40% (62, 64). Decorin binding protein A, a *Borrelial* outer surface protein, is one of the key proteins. This antigen elicits a strong antibody response during experimental murine borreliosis and has been suggested as a potential

vaccine protein (65). Decorin binding protein-peptide from *B. sensu stricto*, *B. garinii* and *B. afzelii* were produced and tested as antigens by ELISA.

One hundred percent of patients with neuroborreliosis (NB) and 93% of patients with Lyme arthritis (LA) reacted positively. Sera from the majority of patients reacted with one rDbpA only and had no or low cross-reactivity to other variant proteins. In patients with culture-positive EM, the sensitivity of rDbpA immunoglobulin G (IgG) or IgM ELISA was low. The DbpA seems to be a sensitive and specific antigen for the serodiagnosis of LA or NB, but not of EM (65).

### Arthritis-Related Protein or Glutathione-S-Transferase

In addition, a 37-kDa arthritis-related protein of *B. burgdorferi* outer-surface protein, termed arthritis-related protein (Arp), has been shown to prevent or reduce the severity of arthritis. Antibody responses to this single protein resembling glutathione-S-Transferase were tested on 124 antibiotic-treated patients with early or late manifestations of LD (66). Among the 124 antibiotic-treated patients, 53% with culture-proven EM had IgG responses to recombinant glutathione S-transferase (GST)-Arp, as did 59% of the patients with facial palsy and 68% of those with LA. Therefore, measurement of antibody against recombinant GST may be additional tool for detection of *B. burgdorferi* involvement in patients with accompanying arthritis during initial episodes of joint pain or during the maximal period of arthritis (66).

### Antigens from Cross-Reactive Spirochetes and Other Bacteria

Three genera, *Treponema*, *Borrelia* and *Leptospira* contain pathogenic spirochetes that cause disease in humans. All spirochetal disease share remarkable similarities in clinical manifestations including spirochetemia, in the early stages of the disease, dissemination of spirochetes to body organs, skin lesions, one or more stages of the disease are frequently interrupted by a period of latency, and neurological and cardiac involvement (67). In addition to similarities of their clinical conditions, cross-reactivity in all antibody assays including ELISA and western blot has been observed with treponema and leptospira (67). This cross-reactivity in an antibody assay could be related to *Borrelia hermsii*, which is a cause of relapsing fever (68), *Babesia microti*, which causes Babesiosis (69), Ehrlichia or the agent of human granulocytic ehrlichiosis (*Anaplasma phagocytophilia*) (69, 70) and *Bartonella henselae*, which can cause Cat-Scratch disease in a human (71, 72). In addition, serological cross-reactivity between *B. burgdorferi* and bacteria such as a *Yersinia enterocolitica* outer membrane protein was shown in a recent study (73).



*Yersinia enterocolitica* and *Yersinia pseudotuberculosis* have been identified as causative organisms of reactive arthritis in humans. Cross-reactivity between *Yersinia* and *B. burgdorferi* indicates that molecular mimicry between bacteria that does not belong to genus *Borrelia* with *B. burgdorferi* is possible (74). This includes other arthrogenic bacteria such *Salmonella* and *Shigella*, which antibodies against them are detected in 67% of patients with LD (75). Due to the extensive evidence of cross-reactivity between *Treponema*, *Leptospira*, *Babesia*, *Ehrlichia*, *Bartonella* and other bacteria that do not belong to the genus *Borrelia*, particularly *Yersinia* with *B. burgdorferi*, the diagnosis of LD should be based on clinical findings and complete serological analysis of *B. burgdorferi* antibodies and the exclusion of its cross-reactive microorganisms (74, 75). Furthermore, antibodies against these spirochetes may result in autoimmune and neurological disorders, which are summarized below:

#### *Autoimmunity Induced by Molecular Mimicry of Peptides from B. burgdorferi and Human Tissue Antigens*

Antigenic mimicry of infectious agents and auto-antigens is a proposed patho-mechanism for autoimmune diseases. For example, multiple auto-antigen mimotopes of infection agents (*Shigella*, *Rotavirus* and *Klebsiella*), were shown to induce autoimmune arthritis and uveitis in rats (73, 75), multiple microbial peptides (76) and *Chlamydia pneumoniae*, specific peptides in multiple sclerosis (77, 78), *Campylobacter jejuni* in Guillain-Barre Syndrome (79) and *Y. enterocolitica* and autoimmune thyroid disease (80). In this regard spirochete *B. burgdorferi* could be another trigger, which is an amino acid sequence homology with self antigen that may trigger multi-organ system disorder in genetically predisposed individuals (80). For example, using a basic local alignment search (BLAST), four matched with *Y. enterocolitica*, and five matched with *B. burgdorferi* and thyroid stimulating hormone receptor (TSH-R), up to 50% identity and similarity were detected. Through this, it was concluded that *Borrelia* and *Yersinia* as environmental triggers can induce autoimmune thyroid disease in a genetically predisposed person through molecular mimicry mechanisms (80). Another target antigen for autoimmune reaction downstream of the original bacterial infection is cytokeratin-10 (81). These auto-antibodies in synovial lesions of chronic antibiotic treatment-resistant LA are due to cross-reactivity between cytokeratin 10 and a prominent outer surface protein A of *B. burgdorferi* (81). This cross-reactivity suggests a degree of antigenic mimicry that might contribute toward setting the stage for autoimmunity, sustaining the response originally elicited by *B. burgdorferi* long after elimination of the spirochete.

This autoimmune reaction in LA may occur through different mechanism of action and by utilization of

proteases from its mammalian hosts to degrade extra cellular matrix proteins. Binding of *B. burgdorferi* to the host plasminogen and its activator Urokinase, is able to induce the production of the matrix metalloproteinases (MMPs) and degradation of extra cellular matrix proteins that allows *B. burgdorferi* to disseminate in the tissue (82).

This up regulation of MMPs not only plays a role in dissemination of the organism through extra cellular matrix tissue, but it can also result in destructive pathology (83). Among the MMPs that have been suggested to participate in cartilage degradation in patients with rheumatoid arthritis are collagenases (MMP-1, -8 and -13), stromelysins (MMP-3, -10 and -11), and gelatinases (MMP-2 and -9) (84).

*Borrelia burgdorferi* is dependent upon the mammalian or tick host for the provision of amino acids and other required components. An ABC oligopeptide transporter system, (OPP) has been well characterized as an aminopeptidase. This and other proteases fulfill important roles in both the basic physiology and pathogenesis of many microbial pathogens including the ability to provide required amino acids. These proteases, by providing required amino acids, enhance the growth of spirochetes and their motility through mammalian host tissues (82–84).

#### *Neurological Manifestations of LD*

Neurologic involvement in LD occurs in 10–40% of patients within several weeks to months of the tick bite. Both the peripheral and the central nervous system (CNS) can be involved, although disease in the former occurs more frequently. Early neuroborreliosis manifests as lymphocytic meningitis (sometimes meningoencephalitis), cranial neuropathies and peripheral radiculoneuropathies (85). Patients with meningitis have headaches and meningeal signs. Examination of the CSF shows a lymphocytic pleocytosis with normal glucose. There is often evidence of intrathecal antibody reactive with *B. burgdorferi* antigens, and oligoclonal bands may be present. Direct invasion of *B. burgdorferi* into the subarachnoid space with a secondary immune response is believed to be the cause of meningitis. As with other manifestations of LD, meningitis can revolve spontaneously despite persistent infection (85–87).

Bell's palsy, due to the involvement of cranial nerve VII, is the most common cranial neuropathy, may be bilateral, and is often associated with other peripheral nervous system manifestations. The most common peripheral nervous system manifestation is a mild, patchy, distal sensorimotor (axonal) neuropathy (86). Neurophysiology testing of individuals with radicular symptoms is abnormal, and in some cases, lymphocytic pleocytosis in the CSF is demonstrable

and associated with intrathecal anti-*B. burgdorferi* antibody production (86).

CNS involvement is relatively uncommon, usually late, and occurs in either of two ways: an acute, disseminated encephalomyelitis and a more indolent, late, slowly progressive parenchymal neurologic disorder resembling multiple sclerosis. Patients with the acute form present with depressed mental status, cognitive deficits and multifocal abnormalities on neurologic examination. Magnetic resonance imaging (MRI) scans of the brain reveal predominantly white matter lesions, and the CSF examination can exhibit both lymphocytic pleocytosis and intrathecal anti-*B. burgdorferi* antibody production. These patients respond well to antibiotic therapy with little, if any, residual deficit (85, 86). In contrast, patients with slowly progressive inflammatory CNS neuroborreliosis have often been misdiagnosed with multiple sclerosis and have received immunosuppressive treatment to stabilize their neurologic deficits. These patients have white matter lesions on brain MRI scans, which had led to the speculation that this form of neuroborreliosis is a demyelinating disorder (86).

The presence of decorin binding protein B of *B. burgdorferi* and antibody against it has been detected in cerebrospinal fluid of patients with neurologic LD. This indicates that *B. burgdorferi* may enter the CNS after infection. To infect the brain, the circulating spirochetes must first cross the blood-brain barrier (BBB). This barrier is composed of tightly apposed brain microvascular endothelial cells held together by that tight junction (87). Several bacteria express their own proteases that digest the extracellular matrix in order to invade tissues, but other bacteria, like *B. burgdorferi*, appear to utilize the fibrinolytic system of the host to disseminate (87, 88). *Borrelia burgdorferi* and *Borrelia crocidurae* spirochetes use the fibrinolytic system it disseminates into host tissues, including the brain. At the same time *B. burgdorferi* induces the expression and secretion of the urokinase-type plasminogen activator (uPA) and expression of the uPA receptor (uPAR) by a variety of cell types. As the fibrinolytic system can directly digest components of the extracellular matrix, it can also activate other proteases, including MMPs. Not only do these enzymes naturally occur in the blood circulation, but they are also expressed as a result of the interaction between the host cells and *B. burgdorferi* (89). To prove the transversal of *B. burgdorferi* across the human BBB and systematic endothelial cell barriers *in vitro* model systems constructed of human brain microvascular endothelial cells (BMEC), a human umbilical vein endothelial cell (HUVEC) line were used (90). Results showed that *B. burgdorferi* differentially crosses human BMEC and HUVEC and that the human BMEC from a barrier to traversal. Importantly, traversal of *B. burgdorferi* across human BMEC induces the expression of plasminogen activators, activator receptors and MMPs.

This fibrinolytic system linked by an activation cascade may lead to focal and transient degradation of tight junction proteins that allows *B. burgdorferi* to invade CNS (90).

In addition to the activation of the fibrinolytic system, entry of spirochetes into the CNS may result in a severe inflammatory reaction, producing proinflammatory cytokines such as interferon- $\gamma$  and TNF- $\alpha$ . The cytokine reaction with neuronal cells may result in a neural cell antigen release and entry in circulation and induction of both cellular and humoral immune reaction against myelin ganglioside, sulfatides and many other self-antigens. Therefore, the complementary approach to the diagnosis and treatment of LD could not be based only on ELISA, western blot or Multi-Peptide ELISA assay for detection of antibodies against *Borrelia*-specific antigens (32–34), but against other cross-reactive microorganisms and different tissue antigens mentioned in this chapter. Measurements of IgG, IgM and IgA antibodies against these important antigens that are related to LD are summarized in Table 2.

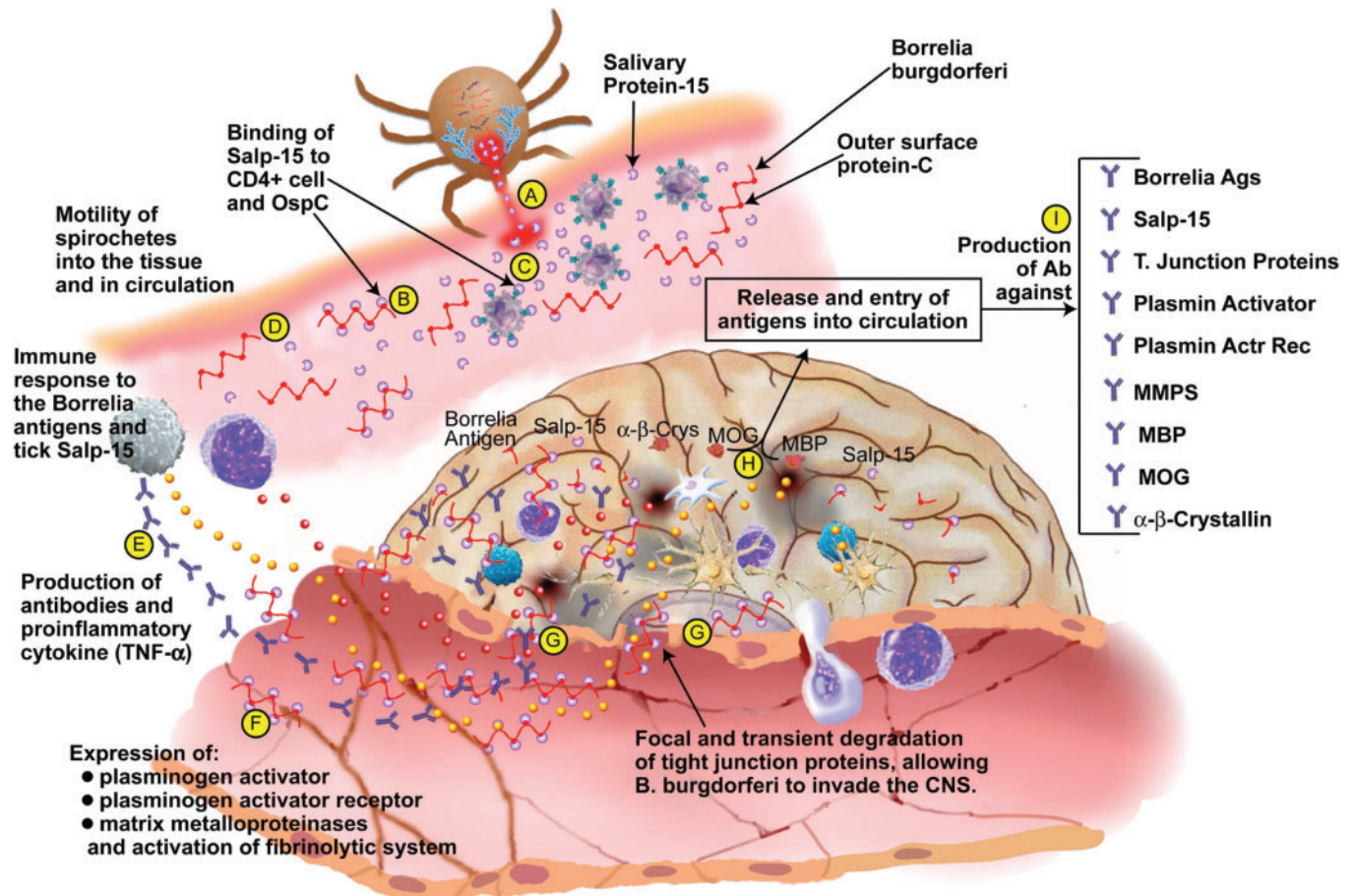
### Mechanisms by which Tick Salivary Protein-15 Facilitates Bacterial Invasion

The causative spirochete of LD not only employs means to change antigenic structures, but also borrows a practical coat from the saliva of its vector, the *Ixodes* tick (91, 92). The coating is present on the many active molecules introduced into the mammalian host during attachment and feeding. The LD bacterium replicates and resides in the mid-gut of an infected tick and moves transiently through the salivary glands during tick feeding. This transition into the salivary glands is accompanied by a major phenotypic switch at the bacterial surface, from expression of OspA to OspC (91, 92).

Binding of OspC to salivary protein-15 (Salp15) in tick saliva facilitates bacterial invasion of, and binding to, salivary glands. This suggests that one function of OspC is to serve as a ligand for a salivary gland receptor. The function of Salp15 is to inhibit IgG antibody response to foreign antigens by halting CD4+ T-Cell activation, and inducing immune suppression, by binding to a receptor on the CD4+ T-Cell (91, 92). A direct connection occurs between CD4+ and C-terminal amino acid residues of Salp15, allowing for Salp15 to act at the very beginning of the signaling cascade initiated by T-Cell receptor binding (92). This Salp15-mediated immunosuppressive mechanism, along with the activation of the fibrinolytic system and proinflammatory cytokine production, allows for facilitated invasion of the host's immune and CNSs, as is shown in Fig. 1. This figure shows that after a tick bite, in addition to *B. burgdorferi*, tick salivary proteins enter the host and exert an immunosuppressive effect (A).

**Table 2.** CAM approach for comprehensive measurement of antibodies against *Borrelia*-specific antigens that are expressed *in vitro* and *in vivo*, their cross-reactive microorganisms and target tissue antigens or enzymes

<i>Borrelia</i> specific antigens	Cross-reactive microorganisms	Target tissue antigens or enzymes
<i>B. Burgdorferi</i> lysate	<i>Treponema pallidum</i>	Thyroid stimulating hormone receptor
Outer surface protein-A	<i>B. microti</i>	Cytokeratin-10
Outer surface protein-C	<i>Ehrlichia</i> ( <i>Anaplasma phagocytophilia</i> )	Plasmin
Outer surface protein-E	<i>Bartonella henselae</i>	Plasminogen
Leukocyte function associated antigen	<i>Y. enterocolitica</i>	Collagenase MMPS 1,2,3
VMP immunodominant (C2 peptide)	<i>B. hermsii</i>	Gelatinase MMPS-9
VMP immunodominant (C6 peptide)	<i>Salmonella</i>	Urokinase
Variable major protein like sequence	<i>Shigella</i>	Myosin
Complement regulator-acquiring surface Protein-1	Mycoplasma	Collagen
Decorin binding protein of <i>B. burgdorferi sensu stricto</i>	<i>Brucella</i>	Arthritis related protein (Glutathione S-transferase)
Decorin binding protein of <i>B. burgdorferi garinii</i>	Mycobacteria	Myelin basic protein
Decorin binding protein of <i>B. burgdorferi afzelii</i>	<i>Leptospira</i>	Myelin oligodendrocyte glycoprotein
	<i>Chlamydia Pneumonia</i>	Ganglioside
	Bacterial heat shock protein (HSP-60)	$\alpha$ - $\beta$ -Crystallin
	Rota virus	Sulfatide
	Klebsiella	Human heat shock protein-60 (H-HSP-60)
	Streptococcal M protein	

**Figure 1.** Entry of *Borrelia* in circulation and different tissue, induction of immuno-suppression with tick salivary protien, activation of the inflammatory and fibrinolytic systmes, and breaking the blood brain abrier, which allows invasion of the CNS, resulting in neuroborreliosis.



This is done by tick Salp15 binding to OspC of *B. burgdorferi* and protecting it from immune attack (B). Salp15 could also bind to CD4 on helper cells, inhibiting TCR ligation-induced-T-cell signaling and immunosuppression (C). This way the spirochete can move freely in the circulation and possibly in different tissues (D). Eventually, immune response to *Borrelia* and Salp15 occurs, resulting in antibody as well as proinflammatory cytokine production (E). Simultaneously, a few spirochetes may make contact with the endothelial cells of the BBB, stimulating expression of plasminogen activators, plasminogen activator receptors and MMP, all of which contribute to activation of the fibrinolytic system (F). This in turn results in focal and transient degradation of tight junction proteins, allowing *B. burgdorferi* to invade the CNS (G). This invasion of the CNS may result in the destruction of neuronal cells, the release of neural cell antigens and (H) the production of antibodies against MBP, MOG,  $\alpha$ -B-crystallin and other neural cell antigens (I). Another possibility for the production of these antibodies against neural cell antigens is through molecular mimicry between outer surface protein A (OspA) and neural tissue (93). DNA sequences of Bb OspA compared with a data base of DNA sequences of human neural tissue yielded three sequences that were identical. The three corresponding Bb peptides were synthesized, and antibodies were induced against them. The antibodies cross-reacted with human neural tissues, including neurons in the human brain, spinal cord and dorsal root ganglia.

These findings imply that antibodies developed by LD patients against OspA will also bind to their own neural tissue, representing a form of autoimmune disease in which a person's immune system attacks his own tissues (93).

### **CAM Diagnosis and Treatment for LD: Examples of Patients' Results**

LD is a complex and devastating disorder requiring efficient means of diagnosis in order to begin treatment at the onset of infection. We have outlined the method of transmission and the mechanisms of infection and maintenance, both of which provide critical information for developing new diagnostic techniques. During the various life cycles of *Borrelia* in the body, the expression of different antigens is tightly regulated such that the spirochete may disseminate and survive for long periods of time. We have shown that the variety of antigens, identified through IVIAT (43), is integral to accurately identify infection and indicate the stage of infection. Information gauged from clinical diagnostic methods assessing these two components, most easily implemented into an ELISA method, provides an infection profile from which an appropriate medical treatment may be

determined. In addition to IVIAT and its use in multi-peptide ELISA, work is being done to develop a method to detect immune response to *Ixodes* proteins (32–34, 93, 94). Using this novel technology for the diagnosis of LD, examples of four patients with signs and symptoms of LD are depicted in Fig. 2. Each specimen was tested for IgG and IgM antibodies simultaneously against *B. burgdorferi* lysate and peptides from outer surface protein-A (OspA) and OspC, OspE, LFA, immunodominant peptides (C2 + C6), VMP, *B. b. sensu stricto*, *B. garinii*, *B. afzelii*, *Babesia*, *Ehrlichia*, *Bartonella*, *Treponema pallidum*, as well as myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG) and collagen as tissue antigens.

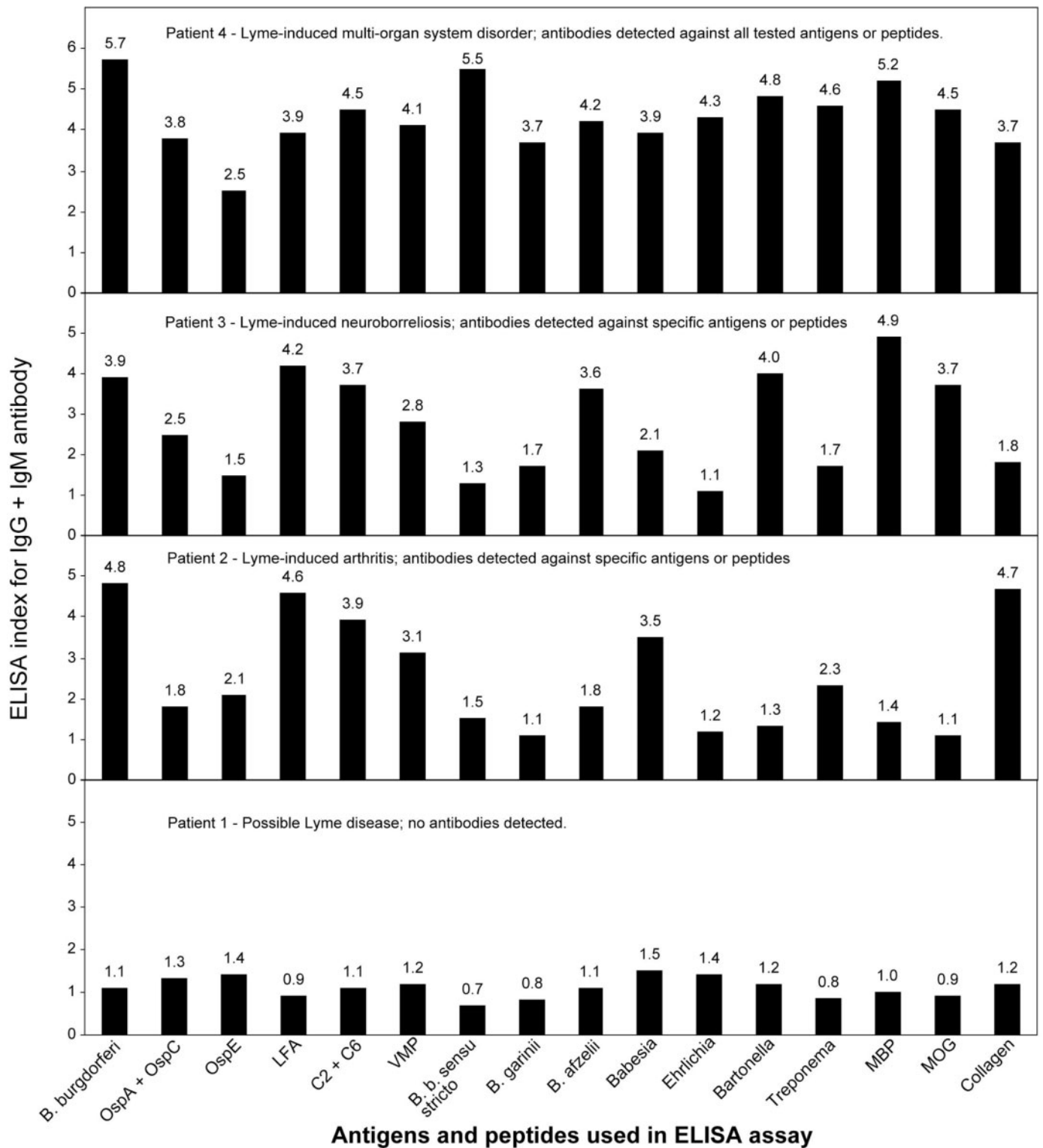
Note that in Patient 1, despite presentation of clinical symptomatology of LD, antibodies were not detected against *B. burgdorferi* three tested subspecies, antigens and peptides, *Babesia*, *Ehrlichia*, *Bartonella*, *Treponema* or tested target tissue antigens. Since symptoms of hundreds of other diseases can overlap with those of LD, the classification of disease should not be based solely on clinical findings but in conjunction with the most sensitive laboratory examinations available. For instance, Patient 2 was classified with Lyme-induced arthritis, but in this case the diagnosis is confirmed by the pattern of detected antibodies, particularly against *B. burgdorferi* lysate and leukocyte function associated antigen, as well as very high levels of antibodies against collagen. In Patient 3, who was classified with neuroborreliosis, the diagnosis was also confirmed by the pattern of detected antibodies against *Borrelia* antigens and neuroantigens (MBP and MOG).

Finally, Patient 4, who was classified as having multi-system organ disorder, reacted strongly with all 16 antigens tested in the assay. While patients of this type with multi-reactive autoantibodies are called universal reactors, it is plausible that Ig-secreting plasma cells and memory B cells activated by antigens have undergone somatic hypermutation and/or class switch recombination, thereby producing these pathogenic autoantibodies (95). Indeed, defects in B cell check points have been shown to be associated with the development of multi-reactive autoantibodies in patients with systemic lupus erythematosus or rheumatoid arthritis (96). These four sample patients' test results with four different patterns of reactivity detected only by our novel comprehensive approach to laboratory examinations justifies CAM intervention, as summarized in Table 1 with pertinent references (6–29).

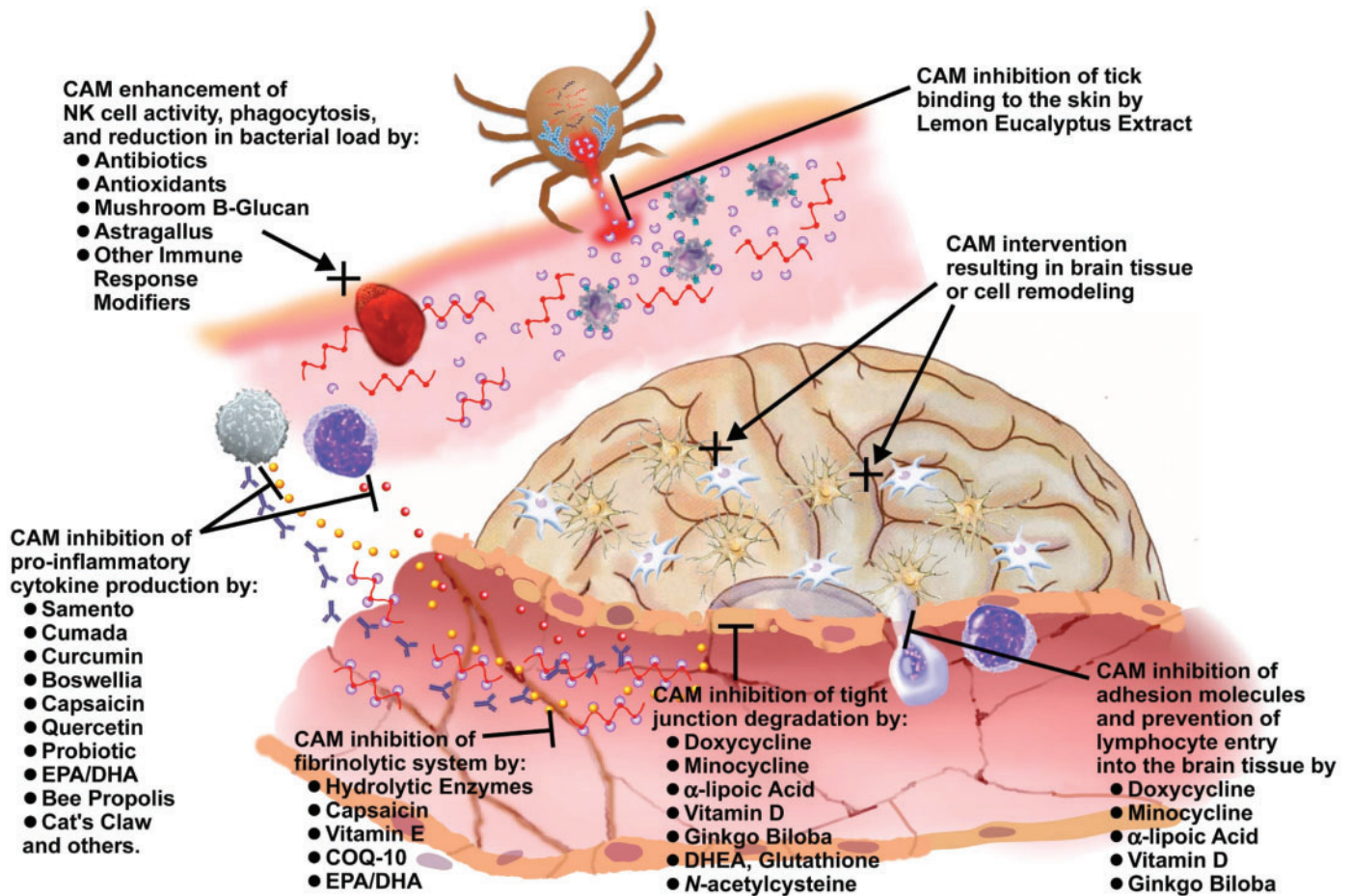
### **Conclusion**

Researchers and practitioners of CAM understand the necessity for an all-inclusive diagnostic approach and treatment (as shown in Tables 1 and 2) and can bring





**Figure 2.** IgG + IgM measured against 16 different peptides and antigens from *Borrelia*, cross-reactive microorganisms (*Babesia*, *Ehrlichia*, *Bartonella*, *T. palidum*) and target tissue antigens such as myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG) and collagen. Index was calculated based on ELISA optical densities of each specimen obtained at 405 nm divided by the OD of normal human serum tested against the same antigen or peptide simultaneously. Note that each patient has a different pattern of antibody reactivity against the sixteen different tested antigens or peptides.



**Figure 3.** Using CAM treatment could prevent different processes ranging from the attachment of the tick to the inflammatory process, CNS invasion, and the induction of neuroborreliosis. CAM can act through the enhancement of natural killer cell activity, macrophage function, inhibition of pro-inflammatory cytokine production, inactivation of the fibrinolytic system, and repair of blood brain barriers.

new perspectives and ideas to this developmental endeavor. Antibodies against *Borrelia*-specific antigens and their cross-reactive microorganisms and target tissue antigens shown in Table 1 can be detected in blood long before people show symptoms of various autoimmune disorders. We conclude, therefore, that tests that can detect these predictive autoantibodies could warn of the need to take preventive action by CAM practitioners.

CAM treatment of chronic LD, in addition to a combination of antibiotics, includes a variety of herbal and nutritional supplements. These herbal and nutritional supplements provide different intervention strategies for CAM practitioners (Table 2). These actions of CAM (summarized in Fig. 3) show that different herbal and nutritional remedies are available to prevent the binding of tick to skin, to reduce bacterial load by enhancement of NK cell activity and phagocytosis, to down-regulate proinflammatory cytokines and inhibit the fibrinolytic system, and repair the BBB. All of these

improvements can result in elimination of *Borrelia* from the tissue and decline in cross-reactive antibodies in the blood.

This approach, which was recently covered in an article published in Scientific American, concluded that one day y-shaped molecules called autoantibodies in a patient's blood may tell doctors whether a patient is 'brewing' certain diseases and indicate when the onset of symptoms will occur in the individual (97). Applying this approach, CAM can make a difference in patients with active or chronic LD who have been given little hope by conventional medical doctors. This tailored treatment for LD by CAM practitioners should be designed specifically for each patient, based on clinical findings and laboratory examination for neuroautoimmunity. In this regard the emphasis should be on immunoregulation and enhancement of cell-mediated immunity (macrophage function, NK cell function) and not enhancement of humoral immunity and antibody production, since induction of antibody production may actually worsen

the autoimmune reaction or autoimmune disease exhibited in many patients with LD.

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## Conflict of Interest

Aristo Vodjani is co-owner and CEO of Immunosciences Lab, Inc. He declares no conflict of interest.

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