Presentations April 6, 2002 Saturday

Keynote: Politics in Healing

Dan Haley
Former Legislator, Author

Back to Basics – Back to a Hippocratic Policy for Our Medical System – Do No Harm

"Back to Basics" – A "DO NO HARM" policy for our medical system is my key message – if a doctor does no harm, leave them alone! If a medicine does no harm – leave it alone!

The Food and Drug Administration (FDA) was set up to make sure consumers don't get poisoned by drugs or food. It is doing a terrible job. The fourth cause of death in the US is from FDA-approved pharmaceutical drugs that are used as directed!

Legislation needs to be passed removing from the FDA any regulatory authority over anything non-toxic. If that had been the law, my book, "Politics in Healing" would not have been written, since everything described in it pertained to products that were non-toxic. Most of these products are no longer available – not because they didn't work, but because of political (regulatory) problems!

Starvation stress response of *Borrelia burgdorferi*

David Nelson, PhD
University of Rhode Island

Borrelia burgdorferi, the causative agent of Lyme disease, is unable to synthesize fatty acids *de novo* and serum is thought to provide a source of fatty acids and lipids. When *B. burgdorferi* cells were serum-starved in a defined medium for 48 h, over 90% of the cells converted from motile helical vegetative forms into non-motile spherical starvation-stress forms. Formation of the spherical starvation-stress cells was inhibited by tetracycline. Spherical starvation-stress form opening and recovery into helical vegetative cells was rapidly induced by the addition of rabbit serum or BSK-H. The percentage of viable cells recovered after starvation ranged from 2.9% to 52.5% with viability inversely proportional to the time of starvation. Protein synthesis by *B. burgdorferi* during starvation was examined by labeling cells with [^{35}S]methionine and [^{35}S]cysteine and analyzing the labeled proteins by two-dimensional gel electrophoresis and fluorography. Serum starvation induced the synthesis of over 20 proteins. Western blots of proteins from vegetative cells and starved cells probed with sera from either *B. burgdorferi*-infected humans or monkeys revealed that several starvation-induced proteins were antigenic. These data suggest that although *B. burgdorferi* cells posses a small genome and extremely limited biosynthetic capabilities, they are able to rapidly respond to conditions of serum starvation by inducing changes in protein synthesis and cell morphology. To determine whether the starvation response of *B. burgdorferi* is controlled by the stringent response, the putative *spoT* gene of *B. burgdorferi* was cloned. The cloned gene complemented *Escherichia coli* CF1693 (D*relA* D*spoT*) to allow growth on M-9 minimal media. The *spoT* gene of *E. coli* encodes a bifunctional enzyme capable of synthesizing and degrading (p)ppGpp, which mediates the stringent response during carbon source starvation. Thin layer chromatography was used to detect (p)ppGpp extracted from [^{32}P]H PO -labeled *B. burgdorferi* cells starved for serum. *B. burgdorferi spoT* gene expression characterized by northern analysis during serum starvation revealed detectable *spoT* message by 2.5 min of starvation. Expression of *spoT* increased ~6-fold during 30 min of starvation. Further, *spoT* expression decreased when serum was added to serum-starved cells. Expression of *spoT* was not increased by either amino acid starvation or glucose starvation. Additionally, *spoT* mRNA was detected in cells incubated in tick saliva for 15 min. The data demonstrate that starvation for serum increases the expression of *spoT* in *B. burgdorferi* cells and that changes in *spoT* expression and, therefore, (p)ppGpp concentration, control the starvation response of *B. burgdorferi*.

Borrelia burgdorferi response to limiting oxygen: Implications for the vector mammal Cycle

Jon Skare
Texas A&M University System Health Science Center

Borrelia burgdorferi encounters different environmental signals as it cycles between the arthropod vector and the mammalian host. Several studies have shown that there is altered gene expression in *B. burgdorferi* in response to variations in temperature, pH and conditions mimicking the mammalian host during in vitro cultivation. Another potentially significant environmental signal is the level of dissolved oxygen that *B. burgdorferi* would encounter during different stages of its life cycle. In order to determine the role of dissolved oxygen in modulating gene expression, infectious isolates of *B. burgdorferi* strains B31 and N40 were grown in BSK-II media depleted of oxygen.

Analysis of protein profiles between organisms grown with and without oxygen limitation showed differences in total protein and antigenic profiles. The level of NapA (BB0690), a general stress response protein often up-regulated during conditions of oxidative stress, was found to be significantly lower in organisms subjected to oxygen limitation by immunoblotting with anti-NapA mono-specific serum. Several immunoreactive proteins were also found to be up or down regulated by immunoblotting of protein samples from oxygen depleted samples relative to untreated organisms when probed with sera generated from either infected mice or convalescent human Lyme disease serum.

The identity of some of these differentially expressed proteins was subsequently determined by using mono-specific sera to specific antigens. While levels of OspC, DbpA, DbpB, were upregulated in the oxygen limited samples, there was altered reactivity with anti-VraA and anti-VlsE sera in oxygen depleted samples. Mono-specific sera directed against the full length and N-terminal half of VraA reacted with a protein smaller than the observed size for VraA (70 kDa) in the oxygen limited organisms. For VlsE, a known antigenic variant of *B. burgdorferi,* two additional immunoreactive bands were observed in oxygen limited organisms suggesting that new variants of VlsE were being formed. We are currently in the process of determining whether these antigenic species represent new variants since VlsE antigenic variation is normally limited to in vivo analyses. These results, taken together, imply that the redox status of *B. burgdorferi* is a signal used by these spirochetes to modulate gene expression and, in the case of *vlsE,* recombination.

Activation of synovial sites in post-Lyme patients

Paul Fawcett, PhD
Alfred A. duPont Hospital for Children

Some individuals infected with *Borrelia burgdorferi* develop chronic disease symptoms which do not resolve following treatment with antibiotics. Hypotheses put forth to explain this phenomena include persistent infection, autoimmune mechanisms and psychosomatic conditions. To date there has been no conclusive evidence to prove or disprove the various hypotheses concerning chronic Lyme disease suggesting that other or multiple mechanisms are involved in this condition.

This study was performed to examine synovial cells obtained from pediatric patients diagnosed with Lyme arthritis whose symptoms persisted despite what is generally accepted to be adequate treatment with antibiotics. Synovial cells from patients were used to establish in-vitro cultures. The cells were then assessed for growth characteristics, activational status, responsiveness to stimulating agents and molecular and cellular markers.

Results obtained indicate that the synovial tissues of pediatric patients with treatment resistant chronic Lyme arthritis include a population of rapidly dividing cells of mesenchymal origin which remain activated even in the absence of lymphocytes. Similar cells have recently been described in the joint tissue of patients with rheumatoid arthritis and in the circulation of normal individuals. The presence of these cells which show pluripotential characteristics and spontaneous cytokine secretion in culture may account for at least some instances of chronic antibiotic resistant Lyme disease. Further work will be required to determine if similar populations of cells exist and possibly play a role in other non-articular manifestations of chronic Lyme disease.

Pathogenesis of Lyme neuroborreliosis

George Perides, PhD
Harvard Medical School

Lyme disease is caused by the spirochete *B. burgdorferi*. From the skin inoculation site the organism disseminates to the joints, the heart and the nervous system. Neurologic manifestations of Lyme disease include encephalopathy and central and peripheral radiculopathy. The spirochete has been cultured from the cerebrospinal fluid (CSF) and *B. burgdorferi* DNA is frequently identified in the CSF by polymerase chain reaction.

The mechanism by which the spirochete invades the central nervous system is not elucidated yet. We use human brain microvascular endothelial cells (BMEC) to study how the spirochete crosses the blood-brain barrier. BMEC plated in a transwell system form a monolayer exhibitng high electric resistance and block inulin diffusion modeling thus a blood-brain barrier. *B. burgdorferi* can cross this blood-brain barrier model only after the addition of human plasminogen. This trasversal is inhibited by the presence of the plasmin inhibitors a2-antiplasmin and e-aminocaproic acid.

To deteremine whether BMEC can activate plasminogen in response to the interaction with *B. burgdorferi* we tested the supernatant from BMEC cultures. Supernatant from control BMEC can activate plasminogen at a very low rate. However, when BMEC are co-cultured with *B. burgdorferi* the rate of plasminogen activation is increased by 6-7 fold.

In addition BMEC show no ability to activate plasminogen on their surface. In the presence of *B. burgdorferi* however, BMEC bind and activate plasminogen. We propose a model by which *B. burgdorferi* induces the expression of plasminogen activator(s) and plasminogen activator receptor(s). Thus plasmin activity is localized to the site of spirochete-endothelial cell interaction leading to the transient breakdown of the blood-brain barrier and facilitating the invasion of *B. burgdorferi* in the central nervous system.

Neurologic manifestations of Lyme disease

Carolyn Britton, MD
College of Physicians and Surgeons of Columbia University

Neurologic complications occur in 15 – 40 % of patients with Lyme disease and are recognized in the earliest descriptions of the illness. In Europe, neurologic symptoms predominate in early reports of tick-related illnesses and include meningitis, radiculitis, cranial and peripheral neuritis. In the United States, early reports of Lyme disease note the association with arthritis and soon thereafter, early and late neurologic manifestations of Lyme are recognized.

Borrelia burgdorferi, the spirochetal cause of Lyme, disseminates widely after bloodstream inoculation and may cause meningitis, cranial neuritis (typically Bell's palsy), and sensory or motor neuritis/radiculitis, alone or in combination. Encephalitis, encephalomyelitis and myelitis occur but are less common. Early Lyme-related symptoms are well-known and typically promptly diagnosed and treated. This is not the case for neurologic involvement due to late Lyme, illness that occurs months to years after known or inapparent tick bite. Clinical syndromes include encephalopathy +/- psychiatric features, psychiatric disorders, encephalomyelitis, spinal cord syndromes, cranial or peripheral neuropathy, painful radiculitis. Less common manifestations include stroke, leukoencephalopathy with a multiple sclerosis-like clinical picture, movement disorders such as chorea and cerebellar ataxia and mass lesions. More speculative is the relationship of Lyme to degenerative disorders like Parkinson disease, amyotrophic lateral sclerosis, progressive dementia and seizures. Ocular syndromes include optic neuritis, uveitis and episcleritis.

The diagnosis of neuro-Lyme for acute syndromes is usually not problematic because abnormal cerebrospinal fluid parameters, diagnostic or suggestive serology in blood and/or spinal fluid, epidemiologic risk for Lyme and/or lack of alternative treatable diagnosis make treatment prudent. This is not the cause for chronic neuro-Lyme, if it occurs post-treatment for acute Lyme, if initial infection is inapparent or if serologic and cerebrospinal studies are non-diagnostic. The mechanism of late and post-treatment neuro-Lyme is debated. On-going infection +/- auto-immune or inflammatory mechanisms are possible. Treatment for confirmed acute or chronic neuro-Lyme with intravenous antibiotics is standard but the duration of necessary treatment is debated. The appropriate treatment of "post-Lyme" syndromes and neurologic syndromes of unclear origin, possible Lyme, is a matter of intense debate and research. In some cases, for ill and disabled patients, empirical antibiotic therapy should be considered.

A rational treatment approach to chronic Lyme disease

Amiram Katz, MD
Yale University School of Medicine

Chronic Lyme disease is a condition that usually implies persistent borreliosis, residual damage following Lyme disease, post infectious auto immune process. Other inflammatory / infectious processes, or other conditions, can present with a similar clinical picture.
The diagnostic process should involve a careful workup to document borrelial infection and exclude other conditions.

When active CNS borreliosis is documented - intra venous antibiotics should be prescribed up to symptomatic improvement and stabilization. A combination of antibiotics with both bacteriostatic and bacteriocidal properties should be considered during the treatment. The role of sporicidal agents will be discussed.

Residual damage should be treated with nutritional supplements and physical therapy. Myofascial syndrome with persistent H/A is extremely common and should be treated by myofascial release, muscle relaxants and trigger point injections. The role of agents used in chronic pain will be discussed.

Sensory overload should be treated with the most tolerated antiepileptic drug (AED), cognitive difficulties with cognitive remediation and psychiatric manifestations - with psychotherapy and appropriate pharmacologic agents. When there is evidence **of post infectious autoimmune disease**, anti inflamatory drugs and immune modifying agents should be considered.
Other condition with similar manifestations should be actively and repeatedly looked for (e.g.; demyelinating diseases, collagen vascular diseases, other infections, intoxications, etc.), and treated, or referred to the appropriate specialists.

The role of some of the less conventional treatment approaches (Teasel, Questran, etc.) will be discussed.

NIH-funded double-blind, placebo-controlled trial of IV treatment for post-Lyme fatigue

Lauren Krupp, MD
Suny Stonybrook School of Medicine

Information not available for publication at this time.

Neuropsychiatric LD, encephalopathy & clinical NIH-funded treatment trial

Brian Fallon, MD
College of Physicians and Surgeons of Columbia University

This talk will address two issues: 1) recent findings in neuropsychiatric Lyme Disease; 2) baseline characteristics of patients from our chronic Lyme Encephalopathy Study.

Case reports indicate that Lyme Disease may induce a wide array of psychiatric disorders, such as mania, mood lability and irritability, psychotic states, attention-deficit-like disorders, and anxiety. While anxiety, depression, and inattention may well arise as a secondary factor due to pain, fatigue, or other physical or cognitive disability, the more severe psychiatric disorders, such as mania or psychoses, when linked to Bb infection would not be expected to be a secondary psychological reaction. In a recent study in Prague (Am J Psychiatry 159: 2002), Hajek et al compared the prevalence of Bb antibodies among 449 psychiatric patients and 449 gender and age matched healthy controls. Among the matched pairs, 33% of the psychiatric patients and 19% of the healthy comparison subjects were seropositive in at least one of 4 assays. This study further supports an association between Bb infection and psychiatric morbidity and raises the issue of screening for Lyme disease in psychiatric settings in Lyme endemic areas.

A report by Morgen et al (Neurology 57, 2001) on 27 patients with "Post-Treatment Lyme Disease" revealed that 4 patients (14.8%) had a MS-like picture (focal neurologic deficits, relapsing-remitting disease, lesions in a distribution typical of MS), 12 patients (44.4%) had normal MRIs, 10 (37.0%) had primarily punctuate and subcortical lesions, and 1 had multiple periventricular lesions. The primarily subcortical arteriolar watershed location of the white matter hyperintensities supports the small vessel vasculitis hypothesis but is non-specific diagnostically.

At Columbia we are conducting a brain imaging and treatment study of Lyme Encephalopathy. Several findings from our screening o patients are of note thus far. Of 538 screened patients who had had IV therapy for Lyme Disease, the mean amount of prior IV therapy was 4.6 months (median 2 months) and the mean amount of prior oral therapy was 14.5 months (median 7 months). Approximately one-quarter of the patients screened are currently disabled and about one-quarter are working full-time. Among the patients with a history of well-documented Lyme disease and a recently positive IgG Western blot (73 patients), 40% recalled tick bite and 58% recalled EM rash. Prevalence of symptoms in the sample of 73 patients include: 82% joint swelling; 97% arthralgias; 89% radicular pains; 84% severe headaches; 79% paresthesias; 96% cognitive problems; 88% mood problems; 10-20% facial nerve palsy; 75% balance or coordination problems; and 67% had sensory hyperacuses. 28% of the patients reported having had an abnormal MRI and 65% reported having had an abnormal SPECT. 32% reported having had an abnormal CSF, but only 56% of the sample had had a lumbar puncture. Among these 73 patients, patients had been symptomatic a mean of 8 years and it took about 3 years for the diagnosis to be made. The significance of these findings will be discussed.

Early Lyme disease: Human tick-bite and experimental animal treatment studies

Charles Pavia, PhD
New York Medical College School of Medicine
New York College of Osteopathic Medicine

Although effective antibiotic treatment regimens for Lyme Disease (LD) have been reasonably well established, it is still unclear what the minimally effective dosage is for the antibiotics recommended for use in curing this disease. Toward that end, it was shown recently that a single 200-mg dose of doxycycline given within 72 hours of an *Ixodes scapularis* tick bite can prevent the development of early Lyme disease in the vast majority of the studied tick-bite victims.

As a follow-up to these intriguing findings, in this study we evaluated the efficacy of short course treatment with ceftriaxone (CTX – a cephalosporin-type antibiotic frequently used for treating disseminated LD) in vivo in a mouse infectivity model for LD. Separate groups of C3H mice were injected intradermally with 100,000 culture-grown, low-passage strain BL206 of *Borrelia burgdorferi* (Bb). It should be noted that strain BL206 was isolated from the blood of a patient having early disseminated LD. Two to 3 weeks later, the mice were given a single daily dose of either saline, or CTX (50 mg/kg/bw), for 5 consecutive days, or 5 doses of CTX (50 mg/kg) given once every 6 hours over a 24-hr period. One week after treatment, cultures of the urinary bladders and of ear samples were established in BSK media.

The results showed that short-term treatment (5 equally spaced doses over a 24-hr period) was as effective as the 5-day treatment regimen with CTX in eliminating viable Bb from these infected mice. In another set of experiments, it was found that concomitant administration of the immunosuppressive drug cortisone acetate did not impair antibiotic efficacy in Bb-infected mice. Based on these findings, consideration should be given for determining more precisely the appropriate duration of antibiotic therapy in patients with Lyme disease, especially those with early infection following a tick bite.

PRESENTATIONS APRIL 6, 2002 SATURDAY

Discussions and Questions

New clinical assays: C-6 Peptide, Borreliacidal assay

Lony Lim, PhD
Specialty Laboratories

Two new laboratory tests for detection of *Borrelia burgdorferi* specific antibodies will be discussed. The Lyme C6 Peptide Antibody Assay and The Borreliacidal Antibody Test. Results of clinically and laboratory defined samples will be reviewed.

Correlation of borreliacidal antibody response with *borrelia burgdorferi* Infection

Steve Callister, PhD
Gundersen Lutheran Medical Center

Borreliacidal antibodies against several *B. burgdorferi* proteins can be detected in sera from patients with Lyme disease, but there have been few studies to determine the ability of this response to predict the severity of infection or eliminate the spirochetes from the host. We needle-challenged hamsters in the footpad with *B. burgdorferi* 297, monitored the severity of infection for 40 weeks, and determined whether the borreliacidal antibody response correlated with the infection. *B. burgdorferi* were recovered from spleens, kidneys, and bladders within 3 weeks and the tissues remained heavily infected for 14 weeks. Thereafter, spirochetes were not detected in spleens or kidneys and urinary bladders were sterile after 40 weeks. *B. burgdorferi* were also not detected in ear tissues until 14 weeks and spirochetes were still recovered after 40 weeks. The anti-OspA and anti-OspB borreliacidal antibody titer correlated directly with the appearance and clearance of spirochetes from the spleens, kidneys, and bladders. Additional experiments showed that passively administering 3-week immune serum from the infected animals to *B. burgdorferi*-infected irradiated hamsters also eliminated the spirochetes, but the animals remained infected if the anti-OspA and anti-OspB antibodies were removed. In contrast, the appearance of spirochetes in the ear tissues correlated directly with the development of borreliacidal antibodies against *B. burgdorferi* 50772, which does not express OspA or OspB. Challenging additional hamsters with the infected ear tissues also induced only anti-50772 borreliacidal antibodies but passive administration of serum from these animals did not prevent or eliminate infection with *B. burgdorferi* when the hamsters were challenged with infected ear tissue. Thus, the borreliacidal antibody response could accurately predict the severity and location of infection and could eliminate some spirochetes. When the *B. burgdorferi* were sequestered in ear tissue, however, the antigenicity changed significantly and the borreliacidal antibodies could not prevent or eliminate infection. Additional studies to determine mechanisms responsible for the inability of the borreliacidal antibodies to eliminate the skin-adapted spirochetes are ongoing.

Pivotal Role of PCR in the detection of coinfections

Eli Mordechai, PhD
Medical Diagnostic Laboratories

Eli Mordechai, Ph.D.1,2, Martin Adelson, Ph.D.1, Kimberly Cabets, B.S.1, Eugene Eskow, MD2,3, Lesley Fein, MD4, and Jim Occi5.

1 Medical Diagnostic Laboratories LLC, Mt. Laurel, NJ; 2 Vector-Borne Disease Research Institute LLC and 3 Hunterdon Medical Center, Flemington, NJ; 4 Bloomfield, NJ; 5 Cranford, NJ.

Mounting evidence indicates that persistent neurologic symptoms associated with chronic Lyme disease may be associated with a second tick-borne pathogen. Recently, we have published a case report based study in which a subset of patients with on-going symptoms attributed to chronic Lyme disease and with residence in a Lyme endemic region of New Jersey, were co-infected with Bartonella henselae (Arch. Neurol. (2001) 58:1357-63). In this study, we examined the prevalence of Borrelia burgdorferi, B. henselae, Babesia microti, Ehrlichia, and Mycoplasma spp. by PCR in Ixodes scapularis ticks collected from February through July in northern New Jersey. I. scapularis ticks (n=123) were determined to be infected with B. burgdorferi (30.9%), B. microti (8.1%), Ehrlichia (1.6%), B. henselae (39.0%) and Mycoplasma spp. (14.6%). None of these microorganisms were detectable in 34.1% of the black-legged ticks examined. The most prevalent co-infections were with B. henselae and B. burgdorferi (10.6%) and B. henselae and Mycoplasma spp. (8.1%). In addition, we examined the prevalence of these pathogens as a function of seasonal effect by stratification of the samples at two-month intervals. Our data suggests a statistically significant two-fold increase in the frequency of B. burgdorferi infections in April/May while B. henselae infection rates increase three-fold in ticks collected during June/July. The presence of Mycoplasma spp. in a subset of the ticks examined may partly explain the high prevalence of Mycoplasma infection among chronic Lyme disease patients (approximately 25%). The results presented herein provide evidence for co-infection and could contribute to the variable manifestations and clinical responses that have been noted in some patients with tick-borne diseases.

Improvements in serologic testing for Lyme disease and ehrlichiosis

Louis Magnarelli, PhD
Connecticut Agricultural Experiment Station

Serologic testing for antibodies is relied on heavily to confirm Lyme borreliosis and granulocytic ehrlichiosis infections. Indirect fluorescent antibody staining methods, enzyme-linked immunosorbent assays (ELISA), and Western blot analysis have been helpful aids for laboratory diagnosis. However, these conventional methods, which incorporate whole-cell antigens, are sometimes limited by false positive reactions, added costs associated with performing ELISA and blot analyses, and difficulty in interpreting results of complex blot banding patterns. Highly specific recombinant antigens (i.e., fusion proteins) of *Borrelia burgdorferi* and *Anaplasma (Ehrlichia) phagocytophila* were tested by ELISA. Results for select antigens, such as VlsE and outer surface proteins C and F for *B. burgdorferi* and protein 44 for granulocytic ehrlichiosis, show increased sensitivity and specificity.