KEYNOTE SPEAKER FRIDAY APRIL 9, 1999

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The Complexity of Arthropod-borne Spirochetes (*Borrelia* spp) with Reference to the Lyme Disease Agent *Borrelia burgdorferi*

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Reference is made to the discoveries of the louse-borne relapsing fever spirochete, *Spirochete obermeieri* by the German physician, Dr. Otto Obermeier in 1868, and about 35 years later, of the tick-borne relapsing fever spirochete, *Borrelia duttonii* by the British investigators Dutton and Todd in Kenya, Ross and Milne in Uganda, and by the German microbiologist Dr. Robert Koch in East Africa.

The development of these spirochetes in their arthropod vectors, the louse *Pediculus humanus humanus*, and the argasid (soft-shelled) tick, *Ornithodoros moubata* has been the subject of intensive research with controversial findings: some investigators suggested spirochetes shortly after ingestion undergo a *negative phase* during with they develop into cysts (blebs, vesicles) which become the sources of new (young) spirochetes. Key references supporting this "granulation theory" are presented. To most other borreliologists, the cysts (blebs, vesicles) are the products of spirochetal degeneration. To them, spirochetes multiply by binary fission only.

The discovery in the fall of 1981 of a new tick-borne spirochete *(Borrelia burgdorferi) sensu stricto*, as the long sought cause of Lyme disease in the United States and in Europe signaled the beginning of a new era in the research of arthropod-borne spirochetes. Successful isolation and culturing of spirochetes from vectors, hosts, and patients, the use of immunochemical stains, and the application of transmission and scanning electron microscopy provided the means to reevaluate certain biologic aspects of spirochetes including their development *in vitro* an *in vivo*. Recent findings on this subject are presented.

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Lyme Disease Research in Context of the National Institutes of Health

Dennis Dixon, Ph.D.

The purpose of this presentation is to place Lyme disease (LD) research in the context of the National Institutes of Health (NIH). The NIH is comprised of Institutes and Centers (www.nih.gov). Institutes are further organized into Intramural and Extramural components. The former consists of staff scientists who conduct basic and scientific studies and the latter consists of scientific programs administered by grants and contracts. The National Institute of Allergy and Infectious Diseases (NIAID) is the lead Institute for coordinating research on LD, and is assisted by the LD Coordinating Committee to ensure communication of research needs and opportunities across Institutes. NIAIDs Intramural components, at the Bethesda campus and at Rocky Mountain Laboratories in Montana, and the Extramural component have robust programs in Lyme disease. The Intramural Program is advised by the Board of Scientific Councilors. The National Advisory Allergy and Infectious Diseases Council is the principal advisory body to the NIAID Extramural program. Other advisory groups include Data and Safety Monitoring Boards for large scale clinical treatment trials, and a Lyme Advisory Panel for the largest treatment trial. General themes of emphasis in the Extramural scientific programs focus on diagnosis, treatment, and prevention. These three areas of focus are evident in the LD program and will be summarized in the presentation.

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Neurologic Lyme Disease Update

Patricia K. Coyle, M.D.

Objective: To review current information on neurolgic Lyme disease.

Methodology: Literature review and Stony Brook experience.

Results: Lyme disease is the major human spirochetal infection in the United States and Europe. It is due to a tick-borne pathogen, *Borrelia burgdorferi*. Neurolgic involvement occurs in up to 40% of symptomatic infections. A number of recent developments are helping to shed light on how neurolgic disease occurs. *B. burgdorferi* shows strain heterogeneity, and there appear to be neurotropic strains. The spirochete can change surface proteins, and there may be differential antigen expression within the intrathecal vs. systemic compartment. Dual tick pathogen infections appear to have implications for more severe infections. Although there are well characterized neurolgic syndromes associated with early disseminated and late stage infection, unusual syndromes such as acute central nervous system demyelinating disease have also been noted. Diagnosis of neurolgic Lyme disease may be aided by second generation antibody tests, to document a unique or preferential intrathecal immune response. Optimal treatment of neurolgic Lyme disease remains to be defined; at the current time intravenous ceftriaxone remains the treatment of choice. Neurolgic disease can occur following oral antibiotic therapy, suggesting that penetration into the CNS is important. The recent experience of 30 early Lyme disease patients seen at Stony Brook during the summer of 1998 will be reviewed, with regard to clinical syndromes, neurolgic symptoms, and laboratory data.

Conclusion: Neurolgic Lyme disease continues to be a major clinical concern. It can lead to morbidity, and better diagnostic and therapeutic options need to be developed.

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Functional Brain Imaging in the Diagnosis of Chronic CNS Lyme Disease

Ronald Van Heertum, M.D.

Lyme disease, caused by infection with the spirochete *Borrelia burgdorferi*, is often diagnosed by a combination of clinical findings including a history of deer tick exposure, systemic symptoms, appearance of a characteristic rash and positive Lyme titers. Chronic central nervous system (CNS) involvement, which, frequently manifests as nonspecific neurobehavioral symptoms, presents a major diagnostic challenge to the treating physician. Conventional structural imaging methods such as CT and MRI are frequently not helpful. As a result, patients maybe misdiagnosed or even labeled as malingering. Recently, however, functional brain imaging, using radionuclide techniques, have been reported to show promise as a contributory adjunct technique in the diagnosis of chronic CNS Lyme disease. The objectives of this presentation are: 1) review the currently available brain imaging techniques; 2) discuss the relative strengths and weaknesses of the available techniques; 3) describe the findings on brain SPECT in chronic CNS Lyme disease, and 4) review recent data supporting the role of Brain SPECT in differentiating CNS Lyme disease from other disorders such as Alzheimer's disease, mild head trauma and major depression.

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Lymerix™ Alternate Schedule Studies

Dennis L. Parenti, M.D.

Lymerix™, SmithKline Beecham Biologicals' recombinant outer surface protein A (OspA) vaccine, was approved by the FDA for prevention of Lyme disease for those aged 15 to 70 years on a 0, 1, 12 month schedule. Flexibility of dosing schedules is highly desirable to ensure rapid and convenient immunization due to the seasonal aspect in some parts of the country. The mechanism of protection of the OspA based vaccine suggests that high and sustained antibody levels would be required to prevent infection. In the field efficacy trial, it was noted that vaccine failures had post vaccinations titers below those of the controls. Statistical methods were used to identify a serological correlate of protection. An IgG anti-OspA antibody titre of 1200 EL.U/ml prior to the start of the tick season appears to provide high probability of protection. In the field efficacy trial, >90 % of the subjects had antibody titers ≥ 1200 EL.U.

Alternative dosing schedules have been investigated in 2 clinical studies. A total of 750 subjects were vaccinated in one study which compared the reactogenicity and immunogenicity of Lymerix™, either on a 0, 1, 6 month schedule or on a 0, 1, 12 month schedule. The results demonstrated that equivalent antibody titers were elicited with the two immunization schedules.
Another clinical study evaluated the safety and immunogenicity of three doses of Lymerix™ on a 0, 1, 2 month schedule. This study demonstrated that the 0, 1, 2 immunization schedule provides similar antibody responses to the licensed schedule.
In both clinical trials discussed above, at least 93% of the vaccines had IgG titres >1200 EL.U/ml one month after the 3rd dose.

In conclusion, Lymerix™ elicits comparable protective immune responses following three doses on a 0, 1, 2 or 0, 1, 6 or 12 month schedule which would allow maximal 0, 1, 2 to 12 month flexibility.

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Safety and Efficacy of a Commercial Recombinant OspA Vaccine for Dogs

Andrew K. Eschner, DVM

The first commercially available, non-adjuvanted, monovalent recombinant OspA vaccine for the prevention of Lyme disease in dogs was launched to the veterinary medical community in 1996. Research has shown that one of the major outer surface proteins of *Borrelia burgdorferi*, designated OspA, is a potent immunogen and provides protection against spirochete infection in a variety of animals. The primary goal of the research presented herein was to establish that a purified recombinant OspA protein would be both safe and efficacious in dogs.

The safety and efficacy of the recombinant OspA canine Lyme disease vaccine was determined in both laboratory (efficacy and safety) and field trials (safety) utilizing both commercially acquired specific-pathogen-free laboratory dogs and dogs in the population at large (multi-center field safety trial). Efficacy trials were conducted using 9-10 week old purpose-bred Beagle dogs which were randomly assigned to treatment and control groups. All aspects of animal housing, care and research methods complied with Animal Welfare Guidelines as set forth in the 9CFR (Code of Federal Regulations) and met Institutional Animal Care and Use Committee (IACUC) requirements. A natural tick challenge model utilizing live-caught, nymphal *Ixodes scapularis* ticks known to be infected with *Borrelia burgdorferi* was employed. In total, three efficacy trials were conducted prior to licensure by the United States Department of Agriculture: a 3 week short-term, a 5 month medium-term and a 1 year long-term trial.

Vaccine efficacy was determined principally by spirochete re-isolation techniques on skin punch biopsies harvested at various times post-challenge. Clinical signs and antibody titers were also assessed. Vaccine safety was determined by assessment of local and systemic reactions at various times after immunization and also by a USDA mandated field safety trial involving over 1,400 dogs of various ages which were followed for a four month time period. The non-adjuvanted, recombinant OspA canine Lyme disease vaccine was proven to be highly effective in preventing both spirochete dissemination and clinical signs of canine Lyme borreliosis in short and long term efficacy (DOI) trials. No adverse effects were noted in the laboratory setting and the vaccine was markedly reaction-free in a field safety trial.

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OspA Induces Lyme Arthritis In Hamsters

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Recently, we presented evidence that adverse effects, particularly severe destructive Lyme arthritis (SDLA), can develop in vaccinated hamsters after challenge with *Borrelia burgdorferi* sensu lato isolates. Hamsters were vaccinated with whole-cell preparations of inactivated *B. burgdorferi* sensu stricto isolates in alum. SDLA was readily evoked in vaccinated hamsters after challenge with homologous or other *B. burgdorferi* isolates. Arthritis was evoked before high levels of protective borreliacidal antibody developed or after the levels of protective antibody declined. We now show that vaccination with recombinant Osp A, the vaccine against Lyme disease, can also induce SDLA. Hamsters were vaccinated with 30, 60, or 120 mg of recombinant Osp A or an Osp A vaccine for dogs. Eleven days after vaccination with the recombinant Osp A, vaccinated hamsters were challenged in the hind paws with 10^6 *B. burgdorferi* isolates 297 or C-1-11. Swelling was detected 7 days after infection, peaked on day 11 and gradually decreased. In addition, histologic evidence of erosive and destructive arthritis was demonstrated in the hind paws of Osp A vaccinated hamsters challenged with *B. burgdorferi*. These findings demonstrate that vaccination with Osp A can induce adverse effects. Vaccination of humans with Osp A should not be recommended until the vaccine has been shown to be incapable of inducing SDLA.

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Borreliacidal Antibodies Against OspC: Implications For Vaccine Development And Serodiagnosis

Steven M. Callister, Ph.D.

Borreliacidal or killing antibodies are produced after infection with *Borrelia burgdorferi.* These highly specific antibodies are produced in response to stimulation of the immune system by *B. burgdorferi* outer surface proteins (Osp), including OspA, OspB, OspC, and others. We recently confirmed the ability of OspC and other proteins to induce borreliacidal antibodies *in vivo* shortly after infection, while anti-OspA and anti-OspB borreliacidal antibodies are produced primarily during later stages of the illness. Detection of anti-OspC borreliacidal antibodies has implications for vaccine development and serodiagnosis.

Despite the presence of high concentrations of borreliacidal antibodies, Lyme disease spirochetes are often not eliminated. However, borreliacidal antibodies can provide protection from infection if they are present at high levels prior to challenge with *B. burgdorferi.* Anti-OspA borreliacidal antibodies are responsible for providing protection after vaccination with OspA. Vaccination with OspC has also been shown to provide protection against Lyme disease. However, anti-OspC borreliacidal antibodies have not previously been detected, leading to speculation that protection was due to other mechanisms. The ability of OspC to induce borreliacidal antibodies provides a possible explanation for the ability of OspC vaccination with to provide protection. In addition, serodiagnostic tests which detect borreliacidal antibodies can be sensitive and highly specific.

In a recent investigation, blinded CDC serum samples were analyzed for the presence of borreliacidal antibodies. High concentrations of borreliacidal antibodies were detected in 9 (70%) of 13 culture-defined early Lyme disease sera using an OspAB- *B. burgdorferi* isolate expressing high levels of OspC. In contrast, little or no borreliacidal antibodies were detected in 24 (96%) of 25 potential cross-reactive sera. In addition, detection of borreliacidal antibodies using this isolate will not be hindered by vaccination with the current OspA vaccine.

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Potential Role of the UHB Gene Family of the Lyme Disease Spirochetes in Immune Evasion

Richard T. Marconi, Ph.D. & Shian Ying Sung (Ph.D. candidate)

Evidence suggests that the Lyme disease spirochetes evade destruction by the immune system in part through antigenic variation in outer surface proteins. While the *vls* gene family has been demonstrated to contribute to antigenic variation, the overall process is likely multi-factorial. We have conducted extensive analyses on a series of lipoprotein encoding genes that form a super gene family. All members of this gene family exhibit homology with each other, particularly in the N terminal region of their deduced amino acid sequences and are flanked at their 5' end by a conserved upstream, promoter carrying sequence element called the upstream homology box (UHB).

Hence we have designated this gene family as the UHB gene family. We have demonstrated that this gene family contains 3 distinct sub-families (*ospE*, *ospF* and *pG* groups). Members of these subfamilies exhibit significant variation. PCR analyses revealed the presence of highly polymorphic domains in the coding sequences of both the *ospE* and *ospF* sub-families. In the *ospE* related genes these variable domains are flanked by direct repeat elements (up to 38 bp) and computer analyses predict this domain to be hydrophilic, surface exposed, and antigenic. To determine if immune pressure drives rearrangement or sequence changes in the UHB flanked genes we have analyzed the stability of these genes over an infection period in mice of 2 months.

While plasmid composition changed over the course of infection, gross gene rearrangements in the *ospE* and *ospF* related genes were not detected through PCR analyses. We are currently sequencing the amplicons to determine if point mutations have arisen during the course of infection. In addition to the analyses outlined above we are also assessing the transcriptional expression of UHB flanked genes under different environmental conditions and are characterizing the humoral immune response to these proteins. The characterization of the UHB gene family among Bbsl species will prove important in attempts to decipher its role in the biology and pathogenesis of the Lyme disease spirochetes.

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