Introduction and general session.

VII Annual International Scientific Conference on Lyme borreliosis and other spirochetal and tick-borne diseases was held on April 22, 23, 1994 in Stamford, CT. The Conference was organized by Lyme Disease Foundation and Stamford Department of Health and co-sponsored by Stamford Hospital. The Conference was attended by 350 people, health care professionals mostly, but open to public. The emphasis of the Conference was on neurologic manifestations and neuropsychiatric aspects of Lyme borreliosis.

The general session was chaired by who also had the opening presentation. The importance of clinical observation and publishing data were discussed in general and applied then specifically on situation in Lyme disease. Steps, which would replace numerous controversies with knowledge and data were suggested.

Doctor Ackermann, distinguished neurologist from Germany gave a detailed and comprehensive historical perspective on Lyme neuroborreliosis. His presentation surprised not only with the depth of the presented data, but also with the statement, that chronic Lyme disease predominantly manifests as Lyme arthritis and not neurologic disease, as thought before!

Doctor Edwin Masters, family physician from Missouri had presented data, suggesting Lyme disease is prevalent in Missouri. Missouri, as well as the entire area of south central U. S. is known as nonendemic area for Lyme disease. The isolation of borrelias in this area can represent the missing link between the existence of disease in these areas and patients with clinical symptoms. More attention and research is needed to determine the extent and potential of the infection.

The closing presentation of this session reviewed the classical clinical manifestations of Lyme borreliosis. Dr. Scrimenti and Dr. Lesser reviewed dermatologic, arthritic, cardiac and neurologic symptoms in very illustrative and educational manner.

Clinical Session

Clinical session was started with very interesting case report presented by Dr. Kornelia Keszler. She demonstrated the difficulty, primary care physicians are having in attempt to provide full care to patients, whose clinical manifestation of Lyme disease is not well defined and the course of infection is rather complicated. This case offered more questions, than answers, the trend, which was confirmed by following presentations as well.

The clinical spectrum of disease is not fully recognized even in the most researched areas of Lyme disease as is dermatology. Dr. E. Aberer presented interesting data on borrelial etiology of circumscribed scleroderma. In the diagnostic assessment, the lymphocyte stimulation test, dismissed in the U.S. with skepticism, was the most helpful. Treatment antibiotic trial confirmed the etiology by *Borrelia burgdorferi*.

Question of lactation and Lyme disease, remains to be answered, despite presentation of three cases by Dr. D. DeSilva. *B. burgdorferi* PCR-positive breast milk suggests, but not confirms, that this is the possible way of transmission for the baby. In this respect, transplacental transmission of the infection is much more serious consideration, but clearer answer should be given to lactating women.

Dr. Cameron and Paparone had presentation on characteristics of geriatric Lyme disease and its comparison to other age groups. This is rather unresearched area and presented data cannot be compared to larger population. The conclusion suggests, that clinical picture, treatment response and length of treatment in geriatric group is similar to other age group. Results of Dr. Paparone also suggest, that serology probably correlates with severity of symptoms, but this need to be confirmed by further studies.

Last presentation of the session and Conference was brilliant and provocative overview of Lyme borreliosis treatment given by Dr. Sam Donta. He has shown the most insight and innovative approach, which, if adopted by other, could bring some fast and more definitive answers to the most controversial issue of Lyme borreliosis: the treatment. However, as this point, presentation had to be limited to questions and hypothesis, rather than answers and solutions.

SUMMARY

LYME DISEASE: 1994 STATE OF THE ART CONFERENCE
TRACK B
MICROBIOLOGY & TREATMENT IMPLICATIONS

The session was opened by chairman, Dr. Claude F. Garon with a welcome. He commented that while in the past we have often congratulated ourselves on the conquest of infectious diseases, microorganisms causing disease have continued to do what microorganisms have always done - adapt and survive. Although vaccine and antibiotic development have produced wonders, problems still exist including new disease presentations, old pathogens that are now multiply drug resistant, and old pathogens with new virulence determinants. Progress in finding real solutions to these problems is often hindered by a lack of real understanding of exactly when and how microorganisms cause disease. Dr. Garon described the basic research conducted at the federal Rocky Mountain Laboratories and at other laboratories around the world as a molecular dissection of a pathogen, such as Borrelia burgdorferi, with the aim of explaining, in molecular terms, how disease is produced by infection. An important by-product of this approach is the ability to use well characterized bioproducts for the production of new generation vaccines and development of designer drugs aimed at specific targets. Research leading to the identification of three such targets in Borrelia burgdorferi was discussed: 1) a coordinated DNA replication system; 2) a critical circular DNA coiling/uncoiling enzyme; and, 3) a specific iron-acquisition surface receptor.

Dr. Garon introduced his own presentation by showing a high resolution transmission electron micrograph of the causative agent of Lyme disease - Borrelia burgdorferi and a schematic drawing showing the total genetic capacity of the microorganism. Although made up of both unique linear and circular molecules, the relative copy number of these molecules appears to be tightly controlled. In contrast to some autonomously replicated, microbial extrachromosomal elements which are represented hundreds, if not thousands, of times in each cell, the number of individual DNA molecules seen in Borrelia burgdorferi, whether linear or circular, is low and seems not to vary significantly over many generations of culture. Structures which appeared to show the intimate association of DNA molecules into networks within spirochetes were presented. Since important genes have been mapped to the linear chromosome, and to both linear and circular plasmids, this linkage control mechanism seems to be important and may be exploited using new biochemical agents which target DNA replication. Of the replication inhibitors tested, agents which cross-link DNA strands appear to be the most effective at inhibiting the growth of Borrelia burgdorferi in the laboratory in culture.

Dr. Scott Samuels presented data on the kinetics of killing by the antimicrobial agent coumermycin. Coumermycin is a non-clinically useful antibiotic, but one which is valuable in the laboratory to examine the role of DNA gyrase in the growth of Borrelia burgdorferi. DNA gyrase is a critically important enzyme which controls the coiling and uncoiling of DNA molecules within a cell. The effect of various concentrations of coumermycin over time on the survival and growth were established during laboratory culture. Concentrations of coumermycin

down to 0.003 μg/ml showed some effect, although 1000-fold more drug was required to completely inhibit growth. Cell killing required at least 2 μg/ml coumermycin. One hundred per cent killing was achieved with either 50 μg/ml coumermycin for at least 12 days or 2 μg/ml for 30 days. Resistance to the drug, which was shown to require but a single point mutation in the DNA gyrase B gene, spontaneously occurred in several static populations exposed to less than 50 μg/ml coumermycin.

Dr. David Dorward presented data on the disruption of cell surface binding by human transferrin to Borrelia burgdorferi by antibodies targeted at an iron stress-induced outer sheath protein. Acquisition of ferric iron is required for persistent systemic infection by Borrelia burgdorferi. In order to understand the molecular mechanism of iron acquisition by these spirochetes, studies were undertaken to monitor the effects of iron deprivation on spirochetal growth and metabolism, and to examine in detail the interaction between spirochetes and physiological iron sources. Initial experiments demonstrated that Borrelia burgdorferi could utilize human holotransferrin to support growth in iron restricted medium. Furthermore, 41 and 45 kilodalton proteins were preferentially expressed under iron stress. Murine antibodies raised against the 41 kilodalton protein bind to cell surfaces and appear to block binding of holotransferrin. By western blot analysis, the antibodies recognized a protein which was affinity purified from spirochetal cultures using holotransferrin coated magnetic beads. This membrane protein was also recognized by anti-Borrelia burgdorferi rabbit serum. These results show that iron acquisition from holotransferrin involves a surface-exposed transferrin receptor. Since antibodies to an iron stress-inducible protein appear to block binding of holotransferrin to its receptor, specific antibodies may also inhibit critical iron acquisition by the spirochetes.

An open question and answer session followed involving the session presenters and the audience.

NORTH AMERICAN LABORATORY GROUP
A Boston Biomedica Company

DATE: April 29, 1994

TO:

Lyme Disease Foundation, Inc.

FAX NO: 203/525-8425

FROM: Dr. Richard C. Tilton

COMPANY: NALG FAX NO: (203) 223-6279

OF PAGES (including this sheet): 3

Re: Track C-Diagnostic Imaging
Dr. Richard C. Tilton

Dr. Tilton convened the session which was devoted primarily to the diagnostic aspects of Lyme Disease.

Dr. Buschmich described her very interesting work on dairy cattle. She tested cattle from both endemic and non-endemic areas and found both populations to have antibodies against the OspA and OspB antigen of B. burgdorferi. These findings suggest either infection with another Borrelia or cross reactivity due to a rumen spirochete.

Both Dr's. Magnarelli and Aberer discussed diagnostic tests using urine as a sample source. Dr. Magnarelli described a mouse model in which he was able to detect B. burgdorferi antigen in mouse urine using an inhibition ELISA. Of the 87 urine samples tested, approximately two-thirds had antigen, over 60% were antibody positive, and over half were culture positive.

Dr. Aberer studied the shedding of B. burgdorferi in human urine as detected by PCR. The majority of patients with ECM were PCR positive prior to antibiotic therapy. She also observed excretion of DNA up to 10 months after the initiation of disease. There was speculation that excretion of B. burgdorferi DNA can serve as an important indicator of disease activity.

Dr. Tilton reported on over 500 patients from practices in New York, New Jersey, and Connecticut. PCR on plasma samples from all patients were negative, even in those patients who were seropositive and demonstrated classical symptoms of Lyme disease. The conclusion of this study was that blood is not a satisfactory specimen for detection of B. burgdorferi by PCR unless methods can be improved to detect as few as 1-5 spirochetes per ml. of blood.

Dr. Hulinska described the work done on Lyme borreliosis in Prague, Czech Republic. During 1993, over 33,000 cases were seen and the best clinical marker was ECM and neurologic symptoms. She showed some definitive electron micrographs of spirochetes in a variety of tissue samples such as skin, synovium, and muscle. It was postulated that Borrelia survive in their intracellular locale and may be protected from antibodies.

In summary, there were several caveats:

- Serologic tests for Lyme disease performed on cattle from both endemic and non-endemic areas reflect either infection with another Borrelia or cross reactions due to rumen treponemes.
- Urine appears to be a desirable specimen for detection of B. burgdorferi antigens and B. burgdorferi DNA, particularly in acute disease.
- With the present sensitivity of PCR, plasma does not appear to be an adequate specimen in late disseminated Lyme disease.
- Borrelia survive intracellularly and are protected from some antibiotics and maybe host factors.

Sincerely,

Richard C. Tilton, Ph.D.
Sr. Vice President and
Chief Scientific Officer

RCT/hlm

J. RAWLINGS

Four papers were presented in the Epidemiology and Public Health Issues section of the program. The first was an overview of various tick-borne diseases by Julie Rawlings of the Texas Department of Health. It is important to remember that although Lyme borreliosis is the most prevalent tick-borne disease, there are others such as Rocky Mountain spotted fever, Colorado tick fever, tularemia, human ehrlichiosis, babesiosis and tick-borne relapsing fever to be aware of. Dr. S.N. Banerjee presented four cases of tick-borne relapsing fever diagnosed at the University of British Columbia in Vancouver, Canada in 1993. Three patients had vacationed in Idaho prior . onset of their illness; the other had visited India. Laboratory diagnoses were based on clinical manifestations, the observation of spirochetes in blood and/or serologic test results. Dr. Matthew Cartter, with the State Department of Public Health and Addiction Services, discussed the epidemiology of Lyme disease in the 12-town area around Lyme, Ct, an area which continues to have one of the highest rates of Lyme disease in the world. The incidence of reported cases has increased nearly 9-fold since 1977. In addition, David Wolfe summarized the epidemiology of Lyme disease in Delaware, where the number of reported cases increased 246% between 1989 and 1992. Further, he found that in 1992, 94% of Delaware physicians treated patients with acute Lyme disease for at least three weeks; whereas only 52% did so in 1991.

The New York State Psychiatric Institute
722 West 168th Street #13
New York, New York 10032

5/8/94

Lyme Disease Foundation
1 Financial Plaza
Hartford, CT. 06103

Att: Steven Schutzer, MD
Martina Ziska, MD

Dear Steve and Martina:

I've asked each of the speakers to summarize their talks for me. Unfortunately, I haven't yet received anything. I know you are under a deadline pressure. So, I'll summarize the talks myself.

Neuropsychiatry Section

1. Lyme Disease: A Review of its Neuropsychiatric Features

Dr. Brian Fallon reviewed the literature regarding the psychiatric features associated with Lyme borreliosis. The literature consists primarily of case reports, although a few controlled series have been reported. Case reports have linked Lyme borreliosis with paranoia, thought disorders, delusions, auditory and/or olfactory hallucinations, stereotypies, anorexia nervosa, obsessive compulsive disorder, major depression, disorientation, confusion, violent outbursts, mood lability, panic attacks, mania, personality changes, catatonia, and dementia. Nine reports of larger series of patients with Lyme disease have been reported. Irritability, mood lability, and'or depression were reported in 7 of the 9 studies with a frequency ranging from 26% to 66% of the sample. One study of children with neurologic Lyme disease found that behavioral or mood disturbances were the second most common symptom. Although each of the reports had methodologic limitations, the presence of mood disturbance was a consistent finding. The psychiatric aspects of this disorder need further study and greater recognition.

2. Central Nervous System Cognitive & Emotional Effects of Chronic Lyme Disease.

Dr. Martin Schinedling reported his results on a study of 46 patients with Lyme disease who had received a battery of neuropsychological tsts, 16 of whom had been sick less than 4 years and 20 who had been sick greater than nine years. He noted that those sick longer had a higher degree of unemployment, greater difficulty on a test of abstract concept formation, and greater difficulty with shifting cognitive set. The most common symptoms included memory problems, abnormal sensations, fatigue, pain, phono/photophobia, concentration problems and speech/language difficulties. On the MMPI, the Lyme patients scored high on

depression, hysteria, and schizophrenia. Dr. Schinedling noted that these high scores need to be interpreted in the context of patients who have a physical illness, as is done in studies of patients with brain trauma. High scores on the depression subscale seemed to reflect a truly depressed mood, while the high scores on the hysteria and schizophrenia sub-scales were misleading. The high hysteria sub-scale scores resulted from the report of multiple somatic symptoms and the high scores on the schizophrenia sub-scale resulted from the cognitive difficulties and unusual sensory experiences of these patients. Given incorrect conclusions that can easily be drawn from a casual use of the MMPI, Dr. Schinedling urged that the MMPI be interpreted cautiously among patients with Lyme disease, with particular attention being paid to the individual items within the subscales.

3. Depression and Psychopharmacology of Lyme

Dr. Catherine Saunders reviewed the key symptoms of depression which include two weeks of five of the following features: depressed or irritable mood; anhedonia; weight loss or gain; insomnia or hypersomnia; psychomotor agitation or retardation; fatigue or loss of energy; feelings of worthlessness or inappropriate guilt; poor concentration or indecisiveness; or recurrent thoughts of death. Depression due to organic factors, such as a CNS infection, can be effectively treated with standard anti-depressant therapies, as long as the underlying infection has also been sufficiently treated. Because tricyclic antidepressants have significant anti-cholinergic side-effects, many psychiatrists prefer to treat patients with an organic affective disorder with the selective serotonergic reuptake inhibitors, such as fluoxetine (Prozac), sertraline (Zoloft), or paroxetine (Paxil). Failure to respond to these interventions may warrant switching to an anti-depressant of another class or the addition of adjunctive medications, such as Lithium, T3, or the psychostimulants.

4. The Cognitive Profile of Lyme Disease

Dr. Marian Rissenberg reported on 37 patients with putative Lyme Disease (60% seropositive, 27% seronegative, 13% equivocal) who had been given an extensive battery of neuropsychological tests. The average duration of illness was 43 months with more than 90% reporting a fluctuating symptom pattern. Dr. Rissenberg noted that a fluctuation in mental acuity ("better" one day, "worse" the next) seemed characteristic of Lyme disease and caused considerable vocational and family problems. Typical symptoms included memory loss (100%), irritability (89%), confusion (76%), decreased initiative (70%), depression (70%), anxiety (49%), and personality change (43%). The performance on the neuropsychological battery did not reveal a specific typical pattern. Rather, deficits for the group were noted across all measures - short term memory, mental tracking, abstract reasoning, planning and sequencing, expressive and receptive language, and visuospatial processing. These deficits occurred independently of degree of depression. Neuropsychological evaluation can be

helpful in the documentation of objective deficits, in the assessment of treatment efficacy, and in the planning of strategies of cognitive remediation.

****

I hope this is what you wanted. If you want something briefer, just let me know.

Brian

Brian Fallon, MD

James H. Katzel, M.D.

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THE POSTER SESSION

The poster session, chaired by Dr. James Katzel, drew much attention from the conference attendants. The poster area became a location of intense discussions sparked by the varied topics presented in poster form. Abstract were accepted from around the world. Dr. Katzel presented an interactive tour of Lyme disease risk behaviors, clinical signs and symptoms, and treatment results. Medical education was the primary focus. On the topic of laboratory diagnosis, Dr. Tilton described the importance of a quality assurance panel for antibody test kit validation.

Clinical manifestations of Lyme disease were represented in a number of interesting displays. Dr. Schwartzberg introduced us to a woman patient hospitalized with a polymyalgia rheumatica like syndrome, and a positive lyme elisa and western blot. Dr. Marshall questioned a possible link between MS and Lyme disease. Dr. Cleveland showed yet another presentation of Lyme disease, that of a chest wall mass which developed months after antibiotic treatment. An intriguing case of frontal type dementia with Lyme disease was discussed by Dr. Wanick. Two beautiful posters came to us from Australia; Drs. Barry and Hudson showed the laboratory detection and the epidemiology of the disease down under. A rheumatologist in training, Dr. Powell, showed the association of borrelia specific immune complexes in patients with chronic fatigue syndrome. Dr. Feaga called for prompt research to study humans who voluntarily exposed themselves to the veterinary Lyme disease vaccine. And finally, a high school student stressed the occurrence of behavioral and cognitive deficiencies in adolescents with Lyme disease.

The complete abstract for each of these poster presentations is available in the conference compendium published by the Lyme Disease Foundation.

James Katzel, M.D.
Poster Session Chair

Jim H. Katzel

(Photo of part of the poster area enclosed)