get slide Table I - ticks & Rickettsia's

MARCH 26, 2000 SUNDAY

Tick-Borne Disorders

Keynote Willy Burgdorfer, PhD, MD
National Institutes of Health

new species because of PCR and new non pathogenic & pathogenic pathogens

Of the approximately 850 ticks identified so far, about 100 have been shown to maintain and transmit pathogens of animal and human diseases.

Brief historical accounts are presented of the first recognized involvement of ticks in transmitting pathogens. They are credited to T.S. Smith and F.L. Kilborne for demonstrating that the larvae of *Boophilus annulatus* are the vectors of *Babesia bigemina*, the causative agent of Texas Fever, and to H.T. Ricketts for identifying the wood tick, *Dermacentor andersoni* as the vector of Rocky Mountain spotted fever. Ricketts also was the first to show that the causative agent - unknown to him - was passed via eggs to the progeny of infected female ticks. Subsequent quantitative studies including those by your speaker revealed up to 100% of infected filial ticks. However, maintenance of rickettsial strains by transovarial passage for as many as 12 tick generations was found to interfere with the ticks' biological processes.

Transstadial and transovarial transmission occurs in most ticks infected with spotted fever group rickettsiae. For the recently described *Rickettsia peacockii*, transovarial transmission is the only means for survival; it fails to infect salivary glands and is nonpathogenic for its hosts.

The recognition of the East African argasid tick, *Ornithodoros moubata* as the vector of the relapsing fever spirochete *Borrelia duttonii*, led to the detection of additional *argasid* ticks as vectors of spirochetes on several continents. Most of them are efficient in transmitting their spirochetes transstadially as well as transovarially. A detailed account on the development of *Borrellia duttonii* in *Ornithodoros moubata* is presented and compared with the development of the Lyme disease spirochete, *Borrellia burgdorferi* in its tick vector, I*xodes dammini* (=*I. scapularis*). In this tick, ingested spirochetes remain in the midgut until a blood meal initiates their passage via gut epithelium into other tissues including salivary glands. Massive invasion into developing oocytes prevents normal development of infected eggs.

Continuous transovarial transmission of spirochetes without occasional passage though a susceptible host leads to a degradation of the spirochete's pathogenicity - a phenomenon said to be responsible for the gradual disappearance of tick-borne relapsing fever from previously endemic areas in East Africa.

Finally, reference is made to the suspected tick vectors of the recently described agents of ehrlichiosis. Observations similar to those for the development of *Ehrlichia canis* in its tick vector, *Rhipicephalus sanguineus* are not as yet available.

- one-host tick - cow - one for each stage.
1857 - B. detoni (relapsing fever - tick borne)
in 2000 Still don't know natural reservoir of this - most likely only humans.

Bb in
~~Lose~~ midgut ~~after~~. Engorged leave gut + go to various tissues incl salivary glands.

- ~~#~~ Transovarial trans occurs in Bb when host has low # of Spirochetes in. very few larvae + few eggs

TICKS & DISORDERS in NORTH AMERICA - Willy Burgdorfer, PhD, MD (Hon.)

NAME	AGENT	TICK VECTOR(S)	GEOGRAPHIC DISTRIBUTION
Lyme disease	*Borrelia burgdorferi* *B. lonestari*	*Ixodes scapularis (=dammini)* *I. pacificus* *Amblyomma americanum*	Northeastern & midwestern U.S., California, Oregon, southern & southeastern U.S., southern & eastern U.S.
Rocky Mountain spotted fever	*Rickettsia rickettsii*	*Dermacentor andersoni* *D. variablilis* *A. americanum*	Throughout U.S. except Maine, Alaska, Hawaii
Tick-borne relapsing fever	*B. hermsii* *B. turicatae* *B. parkeri*	*Ornithodoros hermsi* *O. turicata* *O. parkeri*	Northwestern U.S., southern Canada, southwestern U.S. Western U.S.
Colorado tick fever	CTF virus	*D. andersoni*	Rocky Mountain region
Powassan encephalitis	*Encephalitis virus*	*D. andersoni* *Ixodes spp.*	U.S., Canada
Tularemia	*Francisella tularensis*	*D. andersoni* *D. variabilis* *A. americanum*	Throughout U.S., especially Missouri, Kansas, Tennesee, Kentucky, Arkansas, Oklahoma, Illinois, Texas, Colorado, Utah
Human babesiosis	*Babesia microti*	*I. scapularis* other tick spp.	Massachusetts, New York, Rhode Island, Wisconsin, Mexico
Tick paralysis	Toxin (unidentified)	*Dermacentor* spp.	Northwestern & eastern U.S. western Canada
Human monocytic ehrlichiosis (HME)	*Ehrlichia chaffeensis*	*A. americanum* *D. variabilis*	Southeastern U.S.
Human granulocytic ehrlichiosis	unnamed	*I. scapularis* *D. variabilis*	Midwestern U.S.
Canine ehrlichiosis	*E. canis*	*Rhipicephalus sanguineus*	Southern & Southwestern U.S.
Equine ehrlichiosis	*E. equi*	*Dermacentor* spp. *I. pacificus* *A. americanum*	California, Illinois, Colorado, Florida
Potomac horse fever	*E. risticii*	??	Throughout U.S.
Anaplasmosis	*Anaplasma marginale*	*Dermacentor* spp.	Western & southern U.S.
Epizootic bovine abortion	*B. coriaceae*	*O. coriaceus*	Western U.S.

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Characterization of an Immune Evasion System in the Lyme Disease Spirochetes

Richard Marconi, PhD
Medical College of Virginia

The OspE gene family of the Lyme disease spirochetes encodes a polymorphic group of immunogenic lipoproteins. The OspE genes are one of several gene families that are flanked by a highly conserved upstream sequence called the upstream homology box or UHB element. Earlier analyses in our lab demonstrated that OspE related genes are characterized by defined hyper-variable domains (domains 1 and 2) that are predicted to be hydrophilic, surface exposed and antigenic. The flanking of hypervariable domain 1 by DNA repeats may indicate that recombination contributes to *OspE* diversity and thus ultimately to antigenic variation. Using an isogeneic clone of *B. burgdorferi* B31G (designated B31Gc1) we demonstrate that the *OspE* related genes undergo mutation and rearrangement during infection in mice. The mutations that develop during infection resulted in the generation of OspE proteins with altered antigenic characteristics. The data support the hypothesized role of OspE related proteins in immune evasion and suggest that multiple plasmid carried gene families may be involved in the maintenance of chronic infection.

- 2nd talk - Characterize d protein family in serine/theonine phosphorylation — signals Δ in host to change & critically important to genes. look at Relapsing fever & B. Anserina a Borr. to birds found Borrelia in them have this Multi gene family organization in all Borrelia Bacteria do not maintain DNA for no reason - so must be important — RT-PCR - show at { 527 bp, 622 bp, 900 bp } (over)

Assoc w/ Wnd healing, etc.

Investigating How damage occurs!

Matrix Metalloproteinases in Lyme Disease

George Perides, PhD
Beth Israel Deaconess Medical Center (Harvard)

The major clinical features of Lyme borreliosis involve the nervous system and the joints. Very little is known about the proteolytic enzymes involved in the pathogenesis of Lyme disease. We are investigating the ability of *Borrelia burgdorferi* to induce the expression of host proteolytic enzymes and their role in the pathogenesis of Lyme disease.

Regarding the nervous system, we have reported that a matrix metalloproteinase (MMP) with electrophoretic mobility corresponding to 130 kDa, (an MMP-9 complex), is found in the cerebrospinal fluid (CSF) of 78% patients with Lyme disease. Similarly, this MMP was found in the CSF of 62% of patients with post-treatment chronic Lyme disease. This MMP is seen in only 6% of CSF samples from healthy individuals or patients with other neurologic diseases. To determine whether *B. burgdorferi* can induce MMP expression by cells of the nervous system we used primary human and rat neural cultures. *Borrelia burgdorferi* induces the expression of MMP-9 in a dose and time dependent manner. Primary astrocytes express the 130 kDa metalloproteinase when cultured in the presence of the spirochete. The MMPs may play a role in a) the break-down of the blood brain barrier, b) the degradation of the brain extracellular matrix and, c) the digestion of myelin basic protein. They may thus, mimic the presence of MMPs seen in other chronic neuro-inflammatory and neurodegenerative diseases.

Regarding Lyme arthritis, we investigated whether MMPs are present in the synovial fluid from patients with joint involvement due to Lyme disease. The synovial fluid of patients with Lyme arthritis contained MMP-3, MMP-9, and "aggrecanase" (a metalloproteinase that digests the main cartilage proteoglycan, aggrecan). We used a cartilage explant culture system, to determine whether *B. burgdorferi* induces the expression of these enzymes. When bovine or monkey cartilage explants were infected with *B. burgdorferi* they expressed MMP-3, MMP-9 and "aggrecanase". This induction was associated with a dramatic increase in proteoglycan and collagen degradation. This cartilage digestion was inhibited by plasmin- and MMP-inhibitors. We hypothesize that the presence of *B. burgdorferi* in the joint results in the induction and activation of metallopeptidases and fibrinolytic enzymes, that lead to joint destruction and clinical arthritis.

We suggest that *B. burgdorferi* induces the expression of host proteolytic enzymes and these enzymes play a key role in the development of the clinical symptoms associated with Lyme disease.

Role of host enzymes in response to infection
65% of Chronic LD study (Klempner) have this.

- Zinc & calcium dependent proteolytic enzyme

Groups - Gelatinases
Collagenases
Stromelysins
Membrane-type metalloproteases

secreted as zymogens - are activated by other enzymes

Expression of Interleukin-10 During Acute Lyme Arthritis in Dogs

R. K. Straubinger[1]*, P. Florian[2], I. Braumiller[3]
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Objective: In previous work we had shown that the chemokine Interleukin (IL)-8 is up-regulated in the synovia of dogs with acute Lyme arthritis. IL-8 is a proinflammatory chemokine, which probably is responsible for the accumulation of polymorphonuclear neutrophils in affected joints. The detrimental effect of proinflammatory cytokines and chemokines and therefore the extent of the inflammation are controlled by the host with inhibitory factors such as IL-10. Since Lyme arthritis in dogs is episodic and self-limiting, we investigated whether there is an up-regulation of IL-10 mRNA in synovial membranes of dogs showing clinical signs of acute Lyme arthritis.

Design: Tissues had been collected previously from *Borrelia burgdorferi*-infected dogs at the time when the first clinical signs of arthritis were apparent or during the following three days. Messenger RNA was recovered from frozen tissues and the amount of IL-10-specific mRNA was measured with a real-time PCR technique using the ABI7700 Sequence Detection System. Quantities of IL-10 mRNA in various tissues were compared to those of tissues with no signs of inflammation.

Results: Tissues evaluated for IL-10 mRNA content consisted of the skin from both sides of the chest; six lymph nodes (axillary, superficial cervical, and popliteal lymph nodes); six joints (shoulder, elbow, and knee joints); four muscle and four fascia tissue samples representing each limb; and samples from the peritoneum, pericardium and meninges. Our data show that IL-10 was up-regulated very early during joint inflammation (first day of lameness), predominately in synovia and lymph nodes of infected dogs. Over the following days, IL-10 seemed to be down-regulated and was only detectable in small quantities in all tissues.

Conclusions: The cytokine IL-10 seems to be involved in the pathogenesis of acute Lyme arthritis. Its early up-regulation during arthritis probably limits the detrimental effects of inflammatory factors such as IL-8, IL-1α, IL-1β, and TNF-α. Further studies should help to define the role of IL-10 and possibly other inhibitory factors in Lyme borreliosis.

JID - later data - *.

PI - Intramural Clin. Resrch

T-Cell Response: Identification of *Borrelia burgdorferi* Epitopes and Molecular Mimics in Lyme Disease

Adriana Marques, MD
National Institutes of Health

The study of the immune response to infectious agents is a prerequisite for understanding disease pathogenesis. In chronic infectious diseases such as Lyme disease, immune-mediated damage may add to the effects of direct infection by means of molecular mimicry to tissue autoantigens. The use of a novel, unbiased methodology to effectively identify both microbial epitopes and candidate autoantigens will be described. This approach combines analysis of T cell clone response to positional scanning peptide combinatorial libraries and the analysis of the results by generation of scoring matrices followed by large-scale database searches. Using this new method, the peptide specificities of one T cell clone derived from the CSF was determined. These studies documented for the first time that this novel strategy can be applied to identify target epitopes for clones that have been expanded from an organ compartment in an infectious disease.

Furthermore, it was shown that the T cell clone can recognize with high affinity multiple epitopes from one infectious organism as well as multiple other peptides derived from viral proteins and autoantigens that are potentially relevant for the disease process. Despite this degenerate T cell recognition, the T cell clone recognized *B. burgdorferi* epitopes with highest affinity, and bioinformatic data analysis revealed that the clone discriminates between *B. burgdorferi* and a closely related spirochete, *Treponema pallidum*. These observations have profound implications for our understanding of how T cell specificity is encoded and will be important for future studies.

Basic Res. ~~Results~~ — Looking for cross reacting based peptide

Infectious agent incites host antibodies to respond but Infectious agent ~~has~~ similar to host cells, so immune response gets confused & attacks host cells,

fact of autoimmune

Identify protein piece (eg Osp A) & sequence either run computer search to identify human cell candidate — ~~The test~~

to test —

grow - T cell clones

have shown can have cross reacting w/ out single similar sequence — Because it may not be individual sequence but w/ some amino acids in similar places/location.

Protection against Tick-Transmitted Disease in Dogs Vaccinated with a Multiantigenic Vaccine

Alan B. Frey*, T. Dharma Rao, Eugene Davidson, and Reinhard Straubinger;
Department of Cell Biology, *New York University School of Medicine*,
Department of Biochemistry, *Georgetown University School of Medicine*, and
James A. Baker Institute of Veterinary Medicine *Cornell University*

In an effort to develop a safe and effective vaccine for prevention of Lyme disease, dogs were vaccinated with a multiantigenic preparation of sonicated *Borrelia burgdorferi*, strain N-40. 20 days later animals were boosted, rested for 50 or 198 days, and then challenged by feeding of field-isolated ticks. Control nonvaccinated dogs, dogs vaccinated with sonicate, or dogs vaccinated with a commercial rOspA formulation were monitored for development of infection by: histopathologic assessment of tissues at necropsy; measurement of serum anti-*B. burgdorferi* antibodies using ELISA, immunoblot analysis, and spirochete growth inhibition in vitro; growth of spirochete in vitro from skin punch biopsy; and PCR analysis of tissues. Sonicate-vaccinated dogs were completely protected from infection by all criteria utilized, developed high-titer anti-spirochete serum antibodies and growth inhibitory activity which persisted for over 200 days, and did not demonstrate any untoward consequence of vaccination. These data demonstrate that a multiantigenic vaccine is effective in preventing Lyme disease via the natural vector.

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Update on the SKB OspA Vaccine

Dennis Parenti, MD
SmithKline Beecham Biologicals

Clinical and Serological Characteristics of Microbiologically Confirmed Erthema Migrans (EM)

SmithKline Beecham conducted a double-blind, phase III vaccine efficacy study for the prevention of Lyme disease, in 31 centers, in endemic areas. Approximately 11,000 volunteers (ages 15-70) received LYMErix((Lyme Disease vaccine, recombinant OspA), or placebo on a 0,1,12 month schedule. Subjects with suspected LD had acute and convalescent serologic testing and culture/PCR of skin lesions as well as photographs of the lesions.

The clinical and serologic characteristics of the 119 microbiologically confirmed (culture and PCR) cases will be discussed. EM rashes were seen a median of 3 days post onset (range 1-30 days). In this study setting, subjects presented to the physician early in the course of disease; in fact earlier than in other published reports. The data from this group must be interpreted in light of that fact. 7½% had multiple EM's – (may be lower because early)) reinfection & organisms were different strains.
88% were culture positive and 60% were both culture and PCR positive. Two blinded reviewers examined all the photographs and characterized the rashes as being homogeneous (50-60%), having central erythema (31-31%) or having central clearing (5-11%). Punctum were present approximately 30% of the time and vesicular lesions were identified in 6-8% of cases. Other signs and symptoms associated with EM were present in 57% of subjects with myalgias, arthralgias, fever and headache being the most common.

Serologically, subjects were WB negative for both IgM and IgG at the time of initial presentation 66% of the time. Only 26% were IgM positive at the time of initial presentation. In routine clinical practice, skin biopsies are not an option, EM is a clinical diagnosis.

* EM data – very early appearance.

24-48 hrs – data to show short term –

EM w/ pos. skin biopsy –

- size & location –

– 5 – 10 cm – 10-15

Location – 10 – most lower extremity –

mostly early March to May mostly and cases in Nov.

Swiss agent caused EM's that are smaller.

Rickettsia helvetica = Swiss agent found in a variety of Ixodes & Dermacentor ticks.

1997 - Disc. in France
2010 - disc. to cause meningitis also found in Croatia.

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Atypical EM and Acute Lyme Disease

Edwin Masters, MD
Regional Primary Care Physicians

Typical and atypical erythema migrans lesions and their possible significance are presented and discussed, with particular emphasis on erythema migrans in the southern United States. Explanations and possible etiologies are presented, along with comparisons to culture proven erythema migrans lesions.

Possible explanations for *Borrelia* associated erythema migrans lesions in the Southern United States include:

1. Tick to tick transmission of co-feeding ticks on an infected or uninfected animal (if one of the ticks is already infected). (Mather, et al. 1996 International Lyme Conference, San Francisco; Ogden, et al. 12/97 *J Parasit*; and Partican, et al. 11/97 *Am J Trop Med Hyg*).

2. Possible 'bridge vectors' analogous to California epidemiology, involving ticks feeding on an infected animal reservoir host (Oliver, et al. *J Clin Microbiol*, Jan 1998).

3. Demonstrated, albeit limited, transtaadial (= molting to next life stage.) *Amblyomma americanun (Aa)* transmission of *Bb* (Ryder, et al. *J Med Entomol* 1992;29:525-530).

4. Adaptation of related, but possibly atypical *B.b.* variants to different ticks, (e.g. *Amblyomma amoricanum* or lone star ticks), not unlike HGE *Ixodes* vectored vs. HME lone star tick vectored ehrlichiosis due to *E. chaffeensis* (Masters, *Arch Intern Med*, 1998; 158:2162-2164). Already documented is marked southern *Bb* sensu stricto and even one *Aa* Missouri *Bb* isolate.

5. Human infection with pathogenic *Bb* sensu lato.

~~Phiny the elder~~ Vol 4 No 3-93-
- Ixodes v Amblyomma -
~~Lone Star Ticks in NY~~
1970 - 2 counties
Now - 42 counties
Lone star acct for some of puzzling serology LD in NY & NJ.

Ixodes Scap. in SouthEast, Mo. -

Cape Girardeau over one of the largest spiny branches of Ixodes flycatcher.

Tick vectored Trial - Bb, HGE, Babesiosis feels similar analyses thread vectored by lone star.

Neurologic Lyme Disease in Adults

Patricia Coyle, MD
SUNY at Stony Brook School of Medicine

Lyme disease (LD) is a systemic spirochetal infection includes the nervous system as one of its main target organs. Characteristic neurologic syndromes are seen with early disseminated and late stage infection. Early disseminated LD can be associated with aseptic meningitis, cranial nerve palsy (most commonly involving the facial nerve), and acute painful radiculoneuritis. Late stage infection can be associated with mild encephalopathy, chronic polyradiculoneuropathy, or parenchymal encephalomyelitis.

The goal of this study was to examine neurologic symptoms in early vs. late/chronic LD, compared to seronegative controls from the same endemic area.

We evaluated 38 patients with early LD (ill < 3 months), 30 patients with late/chronic LD (ill > 3 months), and 35 seronegative healthy controls. All LD patients met the CDC surveillance case definition for LD. All subjects filled out a symptom checklist, which rated a series of symptoms on severity, from 0 (absent) to 3 (severe). They also underwent neurocognitive testing. In addition, LD patients underwent lumbar puncture. The early LD group consisted of 23 men and 15 women, ranges in age from 21 to 67 years. Lyme disease syndromes were single erythema migrans (EM) (N=22), multiple EM (N=4), meningitis (N=4) and acute radiculoneuritis (N=4). The late/chronic LD group consisted of 16 men and 14 women ranging in age from 23 to 60 years. Their original Lyme disease syndrome was single EM (N=14), arthritis (N=12), meningitis (N=2), and cranial neuropathy (N=1)--patients had been treated for Lyme disease, but remained symptomatic more than 3 months after completion of their antibiotic therapy.

The most common symptom in both LD groups was fatigue (> 85%, vs. 39% in controls). With regard to pain complaints, early LD patients were more likely to complain of headache and stiff neck, while late / chronic LD patients were more likely to complain of joint and muscle pain. Over 70% of early and late/ chronic LD patients had sleep disturbance. Cognitive, vision, hearing, and vertigo problems were more common in the late/chronic patients. LD had a negative impact on social/vocational activities in 60 to 77% of early and late/chronic patients. With the exception of headache, most symptoms were rated as more severe in late/chronic patients. With regard to cognitive impairment, moderate/severe abnormalities were noted in 15% of early LD patients compared to 80% of late/chronic LD patients and 5% of healthy controls. CSF abnormalities were more common in early LD patients. CSF abnormalities were noted in 6/26 (23%) EM/multifocal EM patients, and 9/12 (75%) early neurologic patients.

In summary, neurologic symptoms are common in both early and late patients. They are more severe in late patients. In contrast, CSF abnormalities are more marked in early patients. In this study, cognitive problems were more significant in early LD patients. Adults with LD often experience neurologic morbidity, even at the early infection (EM) stage. Almost 25% of EM/multifocal EM patients show objective CSF abnormalities suggestive of CNS invasion.

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Neurologic Manifestation of Lyme Disease in Children

Dorothy M. Pietrucha, MD, FAAP
Jersey Shore Medical Center, Medidan Health System

The central and peripheral nervous systems may both be involved in Lyme. Patients may present with meningitis, encephalitis, encephalomyelitis, radiculitis, cranial neuritis, and neuropathy.

Cases have been reported with pseudo-tumor cerebri. Rarely, there have been reports of stroke. Patients have had seizures.

Frequently, there may be an encephalopathy with memory deficits, short attention span, difficulty learning new material. This causes significant difficulties in school.

The skeletal muscles may be involved with elevated muscle enzymes and muscle pain and weakness.

In addition to treating the underlying Lyme disease with therapy medications may have to be used to control the symptoms of these neurologic manifestations such as diuretics for the increased intracranial pressure, anticonvulsant medications for seizures and analgesics for the pain, physical therapy for the weakness and appropriate educational intervention and support for those who are having academic difficulties.

Dr. Pietrucha leave the card after 3 things in mind)
① pseudotumor cerebri
② encephalopathy
③ frustrate

Kids -- Headache – Migraines
- tired fatigued
- aches/pains
- Dizzy
- Chest pain
- abdom pain
- sore throats
- radicular pain
- sleep disturbances
- Blurry vision
- Memory loss
- Difficulty Concentrating

to evaluate LD: MRI not Cat Scan, EEG might help –

Lyme – CNS
Meningitis
Encephalitis
Myelitis
Polyneuropathy

Meningitis – Pseudotumor Cerebri – spinal clear

	cells	Sugar	Protein	culture
early –	Lymph	NL	Normal or incr	rarely pos
Late –	few to none	NL	Incr. or NL.	Neg typ.

Pseudotumor non LD – more in females in puberty –

Neuropsychological Evaluation of Children with Lyme Disease: Implications for Education and Treatment

Marian Rissenberg, PhD, and Dana Leonardi, MA
Columbia Presbyterian College of Physicians and Surgeons

Patients with Lyme disease (LD) experience varying degrees of cognitive dysfunction, from mild concentration difficulties to more severe impairment that interferes with daily functioning and job performance. Children with LD also experience cognitive difficulties, though few studies have addressed the issue. These may be expressed as a decline in school performance or a slowing of academic progress. Frequent absences, fatigue, and discomfort make it difficult to keep up with instruction and assignments, while specific processing deficits may interfere directly with learning. Thus, the appropriate and effective education of children with LD, especially when the illness is chronic, presents a distinct challenge to school districts, which have been uninformed and ill-equipped to deal with the physical, emotional and cognitive impact of LD on their students.

Neuropsychological evaluation can identify cognitive deficits and learning difficulties in children with LD, and provide information essential to the development of appropriate educational programs and services. The current study demonstrates that children with Lyme disease may have significant cognitive deficits that interfere with their academic progress, and further, that aggressive antibiotic treatment can ameliorate at least some of these deficits.

Nine children with LD, referred by their treating physicians, underwent repeated neuropsychological evaluation, once soon after their initial diagnosis and again between twelve and thirty-four months later, having been treated with antibiotics continuously during the interim. At the time of the initial evaluation, all children demonstrated significant variability in their cognitive profile, reflecting cognitive dysfunction, and as a group, a significant discrepancy between Verbal and Performance IQ, the latter being lower. On repeat testing, tests sensitive to speed of mental and motor processing showed the greatest increases. Mental tracking and reasoning also improved following treatment. On academic tests, reading comprehension improved significantly on two separate measures. On one, scores increased significantly, relative to each child's grade expectation, in eight out of nine children.

Neuropsychological evaluation can reveal cognitive deficits in children with LD that have direct and important implications for education. Extended antibiotic treatment can result in significant gains in both cognitive and academic performance. More studies are needed of the impact of LD on learning, its long-term effects on overall development and the effectiveness of various types of treatment. Particularly in areas where the incidence of Lyme disease is high, school districts will need to establish programs for the identification and remediation of Lyme-related academic problems.

- Symptoms checklist by parents - Conflated, ~~&~~ fatigue, joint pain, irritable, academic difficulty, & sleep disturbs,
- Testing showed LD kids were behind ~~one~~ (1) 1½ yrs reading, math & spelling.
  - Number operations most behind.

Neuroimaging in Neuropsychiatric Lyme Disease: Uses, Abuses, and the Future

Brian A. Fallon, MD
Columbia University College of Physicians & Surgeons

Structural Brain Imaging

MRI scans among patients with neurologic Lyme disease may demonstrate punctate white matter lesions on T2 weighted images, similar to those seen in demyelinating or inflammatory disorders, such as multiple sclerosis, systemic lupus erythematosus or cerebrovascular disease. In early neurologic Lyme disease, hyperintensities may be seen in as many as 50% of patients with evidence of meningitis or encephalitis. Comparable to meningo and cerebrovascular syphilis, European authors suggest that CNS micro- and macrovasculitis may cause both clinical symptoms and MRI changes in patients with CNS borreliosis.

The usefulness of MRI scans in American chronic Lyme encephalopathy is less clear, with abnormalities seen 15% to 41% of the time. After treatment, roughly half of the patients may show resolution of the signal hyperintensity. In late stage encephalomyelitis, MRI scanning often demonstrates focal areas of inflammation, most commonly in the white matter and occasionally in the cortical and subcortical gray matter of the brain.

Combined MRI and PET studies can help to examine the pathophysiology of these hyperintense areas (perfusion, reactivity to hypercapnia, metabolism) and whether they have prognostic significance. Do these hyperintensities represent demyelination or perivascular inflammation? Is the disease process underlying the hyperintensities primarily neuronal metabolic or vascular? FLAIR sequence and magnestization transfer techniques can be used to maximize the yield on identifying white matter hyperintensities.

Functional Brain Imaging

Single photon emission computerized tomography (SPECT) and positron emission tomography (PET), provide a dynamic picture of the brain's functioning: metabolism, blood flow, and chemistry. In comparison to SPECT scans, PET scanning is able to provide better spatial resolution images (4-6 mm vs 6-9 mm) and can be used to provide an absolute quantitative assessment of regional perfusion or metabolic abnormalities. SPECT studies of patients with Lyme Disease reveal multifocal areas of decreased perfusion in both the cortex and the subcortical white matter. Logigian reported that patients with definite Lyme encephalopathy had significantly more perfusion deficits that patients with possible LE who in turn had significantly more deficits than normal controls. After treatment with one month of IV ceftriaxone, a partial reversal in brain perfusion deficits was observed, raising the question of whether longer antibiotic therapy may have resulted in even fewer perfusion deficits.

Hypoperfusion defects visualized on SPECT scans may result from any process that alters the radiotracer distribution, including vascular delivery to neurons, transport of the tracer into the cells, and retention of the radioactive tracer in the cells. Problems may arise secondary to direct infection of neurons, from cellular dysfunction due to the indirect effects of neurotoxic immunomodulators such as cytokines, or from decreased perfusion through arterioles secondary to vasculitis. In other words, areas of hypoperfusion may result from a cellular-metabolic and/or a vascular problem.

How may clinical SPECT scans be useful? First, a scan with diffuse abnormalities may confirm that an objective abnormality is present in a patient considered to have a factitious disorder. Second, a normal scan in a patient with prominent neuropsychiatric symptoms may suggest that

a psychiatric disorder is the primary cause of a patient's distress and not Lyme disease. Third, improvement after treatment provides objective evidence of physiologic change.

How may clinical SPECTs be abused? One cannot conclude from a SPECT scan that a patient has Lyme disease, as similar patterns of abnormality may be seen with other diseases as well. Other disease processes that demonstrate a heterogeneous tracer uptake include vascular dementia, chronic fatigue syndrome, CNS Lupus, HIV encephalopathy and chronic or acute stimulant abuse.

Future Studies

Studies combining MRI and PET technology, MR Spectroscopy and functional MRI will each contribute significantly to our understanding of the pathophysiology of chronic neurologic Lyme disease.

212-543-5367

Information for Doctors Referring Patients
to the
Columbia Persistent Lyme Encephalopathy Study

Who is Eligible?

Age:	18-60
Lyme History:	Well-documented history of either: a) erythema migrans; or b) at least one of the CDC clinical features of Lyme disease and a reactive IgG or IgM Western blot historically
Current Lyme Status:	Reactive IgG Western blot or a positive PCR Current cognitive problems
Prior Lyme Treatment:	Patients must have completed a total of at least 8 weeks of IV antibiotics previously (e.g., at least two 4-week trials or one 8 week trial or more).

What is the Study?

Sixty patients with previously treated persistent Lyme encephalopathy will be randomly assigned to receive 10 weeks of IV antibiotic or 10 weeks of IV placebo. While 2 of 3 patients will get IV antibiotic during the double-blind phase, 1 of 3 patients will be given IV placebo. Patients given IV placebo during the double-blind phase will have the option of getting 6 weeks of IV antibiotic at no cost at the end of the study. Patients will be evaluated in several ways at three major points (baseline, week 12, and week 24): brain imaging (MRI and PET using 3 different types); neuropsychological testing; clinical exam; blood and CSF tests. From week 10-24, patients will be off antibiotics so that we can determine whether they improve further, stay the same, or relapse. After week 24, patients are free to pursue any other treatment they wish.

What are the three goals of the study?

1) **Brain Imaging**. To understand better the pathophysiologic basis of Lyme encephalopathy. In particular, is LE caused by a Lyme-induced small vessel vasculitis or a nerve cell metabolism problem? Do the brain imaging deficits correlate with cognitive deficits or CSF abnormalities? Do the brain imaging deficits improve with time or remain static?
2) **Treatment.** To determine whether 10 weeks of IV antibiotics are beneficial for patients with persistent symptoms who have already received abundant treatment.
3) **Prognostic Factors**. To determine whether there are features at baseline that might be associated with response (e.g, amount of prior treatment; CSF abnormalities; level of CSF matrix metalloproteinase; presence of immune complexes; ability of the brain vasculature to dilate normally to a carbon-dioxide challange;....)

What does the study mean to the patient?

1) The patient will get state-of-the-art brain testing using MRI, PET, and neuropsychological testing. A report will be generated at the end of the patient's participation in the study summarizing some of the key findings. The testing and the treatment costs alone for each patient amount to over $25,000.
2) The patient will need to come to Columbia for the testing and imaging. The treatments however will be done at the patient's home via a home infusion company.

What does the study mean to the referring doctor?

1) We ask you to see your patient at various intervals during the study in order to provide a hands-on assessment and to be available for any emergencies should they arise. While in the study, you cannot provide any antibiotics as that would confuse the results of our study and make your patient ineligible to continue in the study. Our grant has funds to compensate you for patient visits. You agree to write a brief note summarizing every patient visit and fax it back to us. You therefore become an integral part of the patient's treatment team during the study.

How do I refer a patient to the study? Ask the patient to call Dr. Fallon at 212-543-5487.

Pharmacologic Properties of Antibiotics and their Relevance to Lyme Disease

Sam T. Donta, MD
Boston University Medical Center

This presentation will review the pharmacologic properties of several different types of antibiotics that have potential utility in the treatment of Lyme disease, and try to address the pros and cons of the various antibiotics in treating Lyme disease from its onset to its chronic phase. Available clinical information will also be reviewed to help guide selection of appropriate antibiotics.

ANTIBIOTIC CLASSES AND MECHANISMS OF ACTION

1. Pharmacokinetic Principles: Absorption, Protein Binding, Distribution, Elimination, Toxicity.
2. The Penicillins, Cephalosporins, and other beta-lactam antibiotics
3. The Tetracyclines
4. The Macrolides
5. The Sulfonamides
6. Vancomycin
7. The Aminoglycosides
8. The Quinolones
9. Clindamycin
10. Metronidazole

In vitro, *B. burgdorferi* is sensitive to various antibiotics, including the penicillins, tetracyclines, and macrolides. The most active antibiotics are ceftriaxone, cefotaxime, azithromycin, clarithromycin, erythromycin, and amoxicillin. The significance and in vivo relevance of these in vitro results are uncertain because of several mitigating circumstances. First, the standards for in vitro antibiotic sensitivities, MIC (Minimal Inhibitory Concentration) and MBC (Minimal Bactericidal Concentration), are for organisms grown over a 24-48 hour period of time. For organisms requiring longer growth times, the results of time/kill studies (i.e., the change in numbers of organisms over sequential periods of time) may be more relevant. Nonetheless, it should be expected that antibiotics that are active in vitro should be active in vivo against these organisms. If, however, the spirochetes are in tissues or cells inaccessible to the antibiotic, or they are not metabolically active at the time the antibiotic is present, the organisms will not be killed or damaged by the antibiotic. In this regard, the studies of Klempner et al. that demonstrate that ceftriaxone has little or no activity against *B. burgdorferi* replicating or residing in fibroblasts in tissue culture may be particularly relevant; ceftriaxone and other beta-lactams are highly active in vivo against extracellular organisms, but are not very active against intracellular organisms. If the Lyme spirochetes take up intracellular residence, then beta-lactam antibiotics may not be very effective, and antibiotics with good intracellular penetration may be the most effective agents.

The results of studies related to the treatment of the earliest phase of Lyme disease, i.e. erythema migrans, demonstrate that most antibiotics are effective in relieving patients of symptoms and preventing later complications. Treatment with tetracyclines or beta-lactams for 10-20 days has been shown to be associated with a high degree of success. In contrast, treatment with erythromycin or azithromycin has been less successful. Despite the reported high rates of successful treatment of early Lyme disease, there are many patients whose symptoms were incompletely relieved or whose symptoms developed in the subsequent few months following a 10-20 day treatment course. These typically nonspecific symptoms include arthralgias, fatigue, and paresthesias, among a number of other complaints. Such patients have been given diagnoses of fibromyalgia, chronic fatigue syndrome, or post-Lyme syndrome. Similar diagnoses are often also given to patients who are treated for later symptoms and signs of Lyme disease with a 2-4 week course of antibiotics who subsequently have relapsing or persistent symptoms. In such

patients, no controlled studies have been conducted to evaluate whether a longer course of treatment would result in a better outcome, or whether one class of antibiotic is any better than any other. Anecdotally, many patients and physicians report improvement when antibiotics are continued for several months.

TREATMENT ISSUES

1. Is the organism extracellular or intracellular?
2. What is the role of the blood-brain barrier?
3. What is the proper duration of therapy?
4. Does antibiotic resistance develop with time and therapy?
5. Would a combination of antibiotics be more effective?

CONCLUSIONS

There are many patients with late symptoms of Lyme disease who appear to benefit by antibiotic therapy. Controlled clinical trials will be needed to adequately address questions such as duration of therapy, efficacy of one class of antibiotics vs another (eg, tetracyclines vs macrolides vs beta-lactams), efficacy of a combination of antibiotics, and use of adjunctive agents. Such trials will likely also be helpful in gaining a better understanding of the pathophysiology of Lyme disease.

March 26, 2000 Sunday

Treatment Roundtable

Joseph Burrascano, MD *Southampton Hospital*
Sam Donta, MD *Boston University School of Medicine*
Lesley Fein, MD *Morristown Memorial Hospital*
Kenneth Liegner, MD *Westchester Medical Center*
Dorothy Pietrucha, MD *Jersey Shore Medical Center, Medidan Health System*

1. What clinical criteria do you use to diagnose Lyme disease? Differential diagnoses
2. What lab tests do you use? Why?
3. Do you do routine lumbar puncture exams? How often are they positive?
4. Do you do MRIs? Brain SPECT scans? Other tests?
5. Do you test for Babesia? Ehrlichia? What clinical criteria support the diagnosis?
6. What antibiotics do you use for Lyme disease? Why? What is the success rate?
7. Do you treat coinfections? What is the outcome?