Comments: Regarding Lyme disease (LD) treatment recommendations
Issues in choosing a new IDSA Guidelines Committee

I am available for questions and in any advisory capacity by phone or email but I am unfortunately house-bound and unable to travel.

David Volkman, Ph.D., M.D.
Emeritus Professor of Medicine and Pediatrics

Background: Ph.D. and M.D., Emeritus Professor of Medicine and Pediatrics at SUNY, Stony Brook. Board certified in Immunology, Diagnostic Laboratory Immunology, and Internal Medicine, and Board Eligible in Infectious Diseases. Previously, Senior Investigator at the NIAID and Chairman of both the Internal and External Review Boards of the NIAID. Among first to isolate and clone human antigen-specific T lymphocytes (1,2) and active in retroviral investigations (3,4). Involved in both clinical and bench research in LD since coming to Stony Brook in 1985.

Recommendations from Guidelines Committees ought to be evidenced based, unbiased, and valid, not consensus dictums based on the authoritative opinions of "experts" which should be objectively evaluated and often challenged. Rather than avoid controversy and dissenting views to achieve unanimity the Guidelines should accurately reflect confirmed medical principals and both sides of unresolved questions. Below are some of the issues that need to be addressed in an objective manner.

1. Persistent/chronic borrelia infection
2. Serology-seronegative infection
3. Flawed Prophylaxis recommendation
4. Conflicts of interest
5. Optimal diagnostic and therapeutic modalities-undefined presently
6. Meaning of the "surveillance" definition of Lyme disease

Persistent borrelia infection

Borrelia is a bacterial spirochete capable of avoiding host defenses and causing chronic constitutional, CNS and arthritic symptoms and relapsing fever in humans. The spirochete is a fastidious slow-growing bacterium that often requires sustained doses of antibiotics for its eradication (5, 6). There is abundant evidence of persistent borrelia infection in both humans and mice (5-14). Contrary to the claims of the IDSA guidelines and its Committee members, chronic borreliosis patients can be either seropositive or seronegative, i.e., individuals with persistent infection have or lack anti-borrelia antibodies (7,8,10-14). After persistent infection borrelia DNA has been isolated from both CSF and synovium of seronegative individuals by PCR (10-14).

In the face of both animal (5,6,9) and human (7,8,10-14) evidence of persistent borreliosis following inadequately treated LD, it is disappointing that Guidelines members continue to dismiss the possibility of persistent borreliosis with unreferenced assertions that it has been "discredited" by "current thinking." (15,16,17,37). Since mouse models of persistent borrelia infection exist (5,6,9) it should be straightforward to design an antibiotic regimen that eliminates this infection. However instead of urging the development of better diagnostic tools to identify individuals with previous infections who may still be infected (36) with chronic symptoms, Guidelines members merely assert

the unsupported dogma that chronically infected people are all seropositive. This claim is simply untrue (7,8,10-14). Some of these committee members have testified as "expert witnesses" for insurance companies attempting to deny health benefits to chronically symptomatic individuals and written articles disputing its existence (17). IDSA committee members deny the possibility of persistent seronegative Lyme disease (15-19).

In addition to available *in vivo* animal models to investigate optimal therapy, the there are well established *in vitro* borrelia culture media. If borrelia is briefly exposed to one of many antibiotics *in vitro* in BSK II medium, the fastidious slow-growing bacteria will often stop growing reverting to a cystic form. They only resume proliferation weeks after the antibiotics are removed and optimal growing conditions are restored (20). Despite Drs. Burgdorfer and Barbour having pioneered the isolation and growth of *B. burgdorferi* at the CDC, much of the current work on persistent borreliosis is being done in Europe as American work in humans may have been suppressed by prevailing dogma (21).

Instead of fostering research on the optimal antibiotic regimen to eradicate persistent borreliosis in *in vitro*, animal, and human models, committee members have stifled investigation by their obdurate insistence that persistent borreliosis does not exist (15-19,22). It remains unclear what combination of antibiotics and sustained treatment will eliminate a carrier state and minimize morbidity. The IDSA's Committee should be a strong proponent of this sort of research rather than an obstacle.

Seronegative Borreliosis (SNB)

Removing the bulk of a bacterial inoculum before a mature immune response can develop may leave an infected individual without enough bacterial antigens for T-B cell cognate recognition. Cognate T-B cell recognition requires B cells to bind available borrelia, digest, and re-express them on their surface in the context of MHC II self-molecules. T cells then recognize the antigen-MHC complex, activate, and deliver potent maturation and growth cytokines. Antibiotics impede the rapid expansion of a bacterial inoculum and leave insufficient antigen to bind to B cells and promote a humeral antibody responses (38).

In our original report (7) we described a group of 17 patients who all suffered from either neurological or arthritic signs frequently attributed to chronic borrelia infection. These individuals lived in areas endemic for Lyme disease, all had had a pathognomonic erythema migrans (EM) rash, all had a course of antibiotics (tetracycline, erythromycin, or an abbreviated course of another antibiotic) early in their illness, all had T cell blastogenic responses consistent with exposure to borrelia, and curiously, **all lacked detectable antibodies against borrelia**. Although early antibiotic treatment abrogated antibody responses, it did not eradicate infection. When retreated, most of these chronic patients markedly improved within a month of completing a course of intravenous ceftriaxone, consistent with their problems being due to persistent, ongoing occult infection; although borrelia was not isolated in most cases (PCR was not yet widely available). SNB was subsequently confirmed in other laboratories which detected borrelia DNA by PCR in the cerebral spinal fluid (CSF) and synovial tissue or fluid of seronegative patients with chronic neurologic or arthritic signs and/or symptoms (10-14). These and similar observations led to recommendations by some authorities for antibiotic retreatment of patients with documented persistent ongoing neurological symptoms (24). Along similar lines, established borrelia infections in mice seem to concentrate in

collagen rich tissue and are difficult to eradication (9) even with repeated parenteral antibiotics.

In addition to the initial data documenting T-B cell dissociation (7), SNB was confirmed by many investigators both here and abroad who isolated *B. burgdorferi* (Bb) by culture or by PCR from seronegative individuals (10-14,25). Steere's lab also confirmed that about 5% of chronic Lyme arthritis patients with PCR+ Bb DNA in their synovium were seronegative (14). Thus, there are many peer-reviewed, published "scientific" reports of SNB.

SNB was also demonstrated by investigators who showed that a single dose of oral doxycycline, a therapy that results in 80% of mice thus treated having persistent infection (5), left 87% of their human subjects with borrelia infected tick exposure both seronegative and without an EM (26). Follow-up in these patients was limited to 6 weeks so no long-term symptoms or disability was observed as seen in a similar azithromycin study (8). Although some individuals had fever and/or flu-like symptoms, PCR or culture was not used to isolate Bb in treated subjects. The investigators erroneously equated the blocking of EM with eradication of infecting borrelia. In two other studies Steere's group confirmed SNB (14,27). Seronegative patients who had chronic Lyme arthritis or neuroborreliosis and/or PCR+ joint effusions sometimes had positive T cell blastogenesis (in about 5% of symptomatic seronegative patients) confirming our previous findings (7).

SNB was also observed in volunteers infected with *B. persica* causing Rat Bite Relapsing Fever (RBRF) (28). PCR confirmed borrelia DNA in their blood. These individuals remained seronegative if they received antibiotics within 5 days of infection. The sole individual who was antibody positive did not get antibiotics until day 6. Similarly, individuals receiving azithromycin for *B. burgdorferi* induced EM remained seronegative despite half developing persistent signs and symptoms of chronic borreliosis (8). The erroneous insistence that widely disseminated borrelia infection cannot occur in the absence of anti-borrelia antibodies (16,17). This view, reiterated by the IDSA, leaves seronegative persistently infected symptomatic sufferers without the proper diagnosis, treatment, or credibility to pursue appropriate treatment. The conceit that a yet discovered serological test will detect SNB is wrong headed as in some cases a humeral response is simply blocked. The newer serological tests are no more sensitive and only slightly more specific (29,30) than using sonicated antigen, but have less background nonspecific binding.

SNB (7,23) has been dismissed by members of the Guidelines Committee (16). They have misquoted published data to support single dose prophylaxis and used a single 1991 unreliable report which found 8/12 normal controls Bb blastogenesis positive to dismiss T cell evidence of seronegative infection (confirmed in Steere's own lab (27)). Even their admissions that there are no reliable serological tests that detect early Lyme disease or SNB (29,30) are couched in language that obfuscates the lack of certitude in claims that an infected patient is always seropositive (29). A critical letter to Clinical Infectious Diseases, the official organ of the IDSA, regarding this article was rejected less than a day after its submission. The rejected letter summarizing Steere's errors follows.

To the Editor:

The recent article by Steere (1) and the accompanying editorial by Weinstein (2) reiterate the current status of Lyme disease tests, i.e., there is no serological test that will reliably detect early borrelia infection. There is plentiful evidence from humans and mice that while early but inadequate antibiotic treatment may block seroconversion, it can leave the recipient persistently infected (3,4). Despite published, confirmatory, data, Steere continues to ignore seronegative Lyme disease and claims that seronegative disease has been "discredited" and "that all patients with objective neurologic, cardiac, or joint abnormalities associated with Lyme disease have serologic responses to *B. burgdorferi*." (1) In his article these claims are unsupported and unreferenced. The claims are however consistent with Steere's recent article (5) and the erroneous recommendations of the IDSA sponsored Guidelines Committee (6). The IDSA's Committeee has been cited by the Attorney General of Connecticut for its conflicts of interest, its commercial and financial links to purveyors of serological tests, and its links to insurance companies denying antibiotic treatment (7). Seronegative Lyme disease was first reported in 1988 (3), recognized in clinical trials (8), and confirmed by isolating borrelia DNA by PCR in seronegative patients (4,9). Wormser, the Chairman of the Guidelines Committee, advocated a single dose oral doxycycline treatment for acute tick bites in a Lyme disease endemic environment (10), and this recommendation was codified in the Guideline recommendations (6). A similar single dose oral doxycycline treatment at the time of infection leaves 80% of mice persistently infected with borrelia (11) and persistent infection in mice is often refractory to even parenteral antibiotic treatment (12). The IDSA has neither retracted nor modified its dangerous recommendation. Yet despite these scientifically published observations, Steere et al obdurately continue to ignore and deny the existence of seronegative Lyme disease and the potential for persistent seronegative infection after a single oral dose of doxycycline, publishing incorrect statements like "there is no scientific evidence the there can be infection without anti-borrelia antibodies" (5) and false and inaccurate claims about murine studies (13). In a settlement the IDSA has agreed to a reassessment of the Guidelines recommendations using an independent arbiter, but as Steere's article highlights, Committee members have continued to deny seronegative disease and perpetuate misinformation.

Although acute Lyme disease with a pathognomonic rash (erythema migrans) if recognized can be effectively treated almost all the time, the proper treatment of seronegative Lyme disease detected at a chronic stage remains undefined. Early inadequate doxycycline treatment that blocks seroconversion but leaves patients with persistent and difficult to diagnose borreliosis should be eschewed, not recommended by the IDSA. Individuals with possible persistent borreliosis should be carefully evaluated, not dismissed as hypochondriacs.

1. Steere AC, McHugh G, Damle N, Sikand VK. Prospective study of serologic tests for lyme disease. Clin Infect Dis. **2008**;47:188-95.

2. Weinstein A. Editorial commentary: laboratory testing for lyme disease: time for a change? Clin Infect Dis. **2008**;47:196-7.

3. Dattwyler, R.J., Volkman, D.J., Luft, B.J., Halperin, J.J., Thomas, J., and Golightly,M.G. Seronegative late Lyme borreliosis: Dissociation of *Borrelia burgdorferi* specific T and B lymphocyte responses following early antibiotic therapy. *N Engl J Med*, **1988**; 319: 1441-1446.

4. Oksi J, Uksila J, Marjamaki M, Nikoskelainen J, Viljanen MK. Antibodies against whole sonicated Borrelia burgdorferi spirochetes, 41-kilodalton flagellin, and P39 protein in patients with PCR- or culture-proven late Lyme borreliosis. J Clin Microbiol **1995**; 33(9):2260-4.

5. Feder HM Jr, Johnson BJB, O'Connell S, Shapiro ED, Steere AC, Wormser GP. A Critical Appraisal of "Chronic Lyme Disease" N Engl J Med 357:1422, 2007. and response to letters, **2008;**358:428-31..

6. Wormser GP, Nadelman RB, Dattwyler RJ, Dennis DT, Shapiro ED, Steere AC, Rush TJ, Rahn DW, Coyle PK, Persing DH, Fish D, Luft BJ. Practice guidelines for the treatment of Lyme disease. The Infectious Diseases Society of America. Clin Infect Dis. **2000** Suppl 1:1-14.

7. News from Attorney General Blumenthal, May 1, 2008.
http://www.ct.gov/ag/cwp/view.asp?a=2795&q=414284

8. Luft BJ, et al. Azithromycin compared with amoxicillin in the treatment of erythema migrans. A double-blind, randomized, controlled trial. *Ann Intern Med* **1996** 124:785-91.

9. Keller TL, Halperin JJ, Whitman M. PCR detection of Borrelia burgdorferi DNA in cerebrospinal fluid of Lyme neuroborreliosis patients. Neurology **1992**;42(1):32-42.

10. Nadelman RB, Nowakowski J, Fish D, Falco RC, Freeman K, McKenna D, Welch P, Marcus R, Aguero-Rosenfeld ME, Dennis DT, Wormser GP. Prophylaxis with Single-Dose Doxycycline for the Prevention of Lyme Disease after an Ixodes scapularis Tick Bite. *N Engl J Med* **2001**;345:79.

12. Zeidner NS, Massung RF, Dolan MC, Dadey E, Gabitzsch E, Dietrich G, Levin ML. A sustained-release formulation of doxycycline hyclate (Atridox) prevents simultaneous infection of Anaplasma phagocytophilum and Borrelia burgdorferi transmitted by tick bite. *J Med Microbiol.* **2008**;57:463-8.

12. Hodzic E, Feng S, Holden K, Freet KJ, Barthold SW. Persistence of Borrelia burgdorferi following antibiotic treatment in mice. Antimicrob Agents Chemother. **2008**;52:1728-36.

13. Wormser GP, Dattwyler RJ, Shapiro ED, Dumler JS, O'Connell S, Radolf JD, Nadelman RB. Single-dose prophylaxis against Lyme disease. *Lancet Infect Dis.* **2007**;7:371-3. FAX 631 862 6561, volkmans@optonline.net

In addition to attacking T cell blastogenic assays which documented previous borrelia exposure in SNB and despite confirmation of SNB by Steere's group and others (13,27), Committee members have assailed the PCR evidence from several labs that detected

borrelia DNA in the CSF or joint effusions of seronegative patients (11,13,14). Feder (17) cited problems with nested primers in disputing PCR evidence. However, as shown below the PCR data was reliable and reproducible.

From Dr. Tracy Keller:

Contamination is indeed a potential problem in the diagnostic application of PCR (though Feder's citation on this point is a review which in turn cites only an anecdotal report of a single case example of false positive due to contamination). While meticulous care must be taken to avoid false positives, you have cited examples where this was successfully achieved. In the intervening years since our study, PCR has become well established in many settings as a powerful tool for molecular diagnostics of infectious disease. It is general practice in science to evaluate a study and its conclusions on its own merits. Feder seems to be negating our conclusions by association with other problematic studies, or flatly accusing us of failing to meet high scientific standards. If Feder has a specific criticism that our study falls short of high scientific standards, he needs to back that up with data.
The general concerns Feder raises regarding false positives and contamination in clinical Lyme diagnostics using PCR are real, but that fails to negate the published literature regarding PCR positive, seronegative disease. Three studies (Keller, Oksi, Pachner (2, 4, 5)) show PCR positive CSF in seronegative patients with clinical findings consistent with LB (only a single example in the Pachner paper, multiple examples in Keller, Oksi). An additional study (Lebech) demonstrated PCR positive skin samples in seronegative erythema migrans patients. All four papers have extensive controls for contamination, in Keller et al amplicons were sequence verified and PCR and samples were analyzed blind and were prepared, aliquotted and coded at a separate institution from the one where PCR was done.

The confirmation of positive PCR with the infectious disease "gold standard" of culture in some seronegative patients makes dismissal of these results on the basis of vague, anecdotal counterexamples, completely unwarranted. Along these lines, Preac-Mursic (6) and Oksi have cultured organism from seronegative patients, including an instance of successful borrelia culture following a multi-week ceftriaxone treatement. This proves the point that, in some instances, organism can survive the most aggressive treatment regimens currently in use and confirms the existence of seronegative Lyme Disease.

The Lyme PCR and culture studies referred to above use highly pre-selected patient populations and relatively small sample sizes, and wide variation in the incidence of seronegative, PCR positive patients. It is therefore difficult to extrapolate from these studies the frequency of seronegative, PCR-positive patients in the general population. This work also does not address the question of the extent to which PCR-positivity predicts responsiveness to antibiotic therapy. These studies do establish, however, that the phenomenon of Borrelia DNA in seronegative patients does exist. What proportion of these patients may respond positively to antibiotic therapy, and what type or duration of therapy is optimal, are unanswered questions. Both further research and standardization of practices to optimize the sensitivity and specificity of LD PCR diagnostics are important to examine as thoroughly as possible the significance of bacterial DNA persistence for the design of treatment studies. Feder's dismissal of the phenomenon of bacterial persistence in seronegative patients, in spite of multiple diverse lines of confirmatory evidence that it is indeed real, seems designed to discourage rather than promote further investigation in this area.

Additional points:

Steere, an author on the Feder review, confirmed the phenomenon of T-cell proliferation positive, seronegative LD (1). While he does not support its use as a clinical diagnostic tool, his work does validate the phenomenon that LD patients can have a T-cell proliferative response while remaining seronegative.

Klempner et al (3), cited multiple times to support conclusions regarding treatment in the Feder et al review, defines 52 seronegative people with "proven Lyme disease" in his study. While Klempner concludes that the antibiotic protocol he used did not help these people, he explicitly acknowledges that a significant patient population with clinically-evident Lyme disease are seronegative.

1. Dressler, F., N. H. Yoshinari, and A. C. Steere. 1991. The T-cell proliferative assay in the diagnosis of Lyme disease. Ann Intern Med 115:533-9.
2. Keller, T. L., J. J. Halperin, and M. Whitman. 1992. PCR detection of Borrelia burgdorferi DNA in cerebrospinal fluid of Lyme neuroborreliosis patients. Neurology 42:32-42.
3. Klempner, M. S., L. T. Hu, J. Evans, C. H. Schmid, G. M. Johnson, R. P. Trevino, D. Norton, L. Levy, D. Wall, J. McCall, M. Kosinski, and A. Weinstein. 2001. Two controlled trials of antibiotic treatment in patients with persistent symptoms and a history of Lyme disease. N Engl J Med 345:85-92.
4. Oksi, J., J. Uksila, M. Marjamaki, J. Nikoskelainen, and M. K. Viljanen. 1995. Antibodies against whole sonicated Borrelia burgdorferi spirochetes, 41-kilodalton flagellin, and P39 protein in patients with PCR- or culture-proven late Lyme borreliosis. J Clin Microbiol 33:2260-4.

5. Pachner, A. R., and E. Delaney. 1993. The polymerase chain reaction in the diagnosis of Lyme neuroborreliosis. Ann Neurol 34:544-50.
6. Preac-Mursic, V., K. Weber, H. W. Pfister, B. Wilske, B. Gross, A. Baumann, and J. Prokop. 1989. Survival of Borrelia burgdorferi in antibiotically treated patients with Lyme borreliosis. Infection 17:355-9.

Tracy L. Keller, Ph.D.
Department of Developmental Biology
Department of Cell Biology
Harvard Medical School

Prophylaxis Guidelines Recommendation

Based on a flawed tick bite prophylaxis article (26) in which the authors showed that a single oral dose of doxycycline blocked EM and seroconversion in 87% of newly infected patients. They ignored fever, flu-like symptoms, and limited their follow-up to 6 weeks; the investigators erroneously equated the blocking of EM with eradication of the infecting borrelia and declared their prophylaxis 87% effective. The Guidelines Committee wrongly recommended this unproven single oral doxycycline dose for tick bite prophylaxis. As noted below, an identical treatment was ineffective in mice. The IDSA Guidelines Committee codified this ineffective therapy in its recommendations. Once established, chronic borrelia infections have proved difficult to cure in mice even with repeated parenteral doses of antibiotics (9). Patients with chronic post infection arthritis or neurological symptoms are labeled as "antibiotic unresponsive." This diagnosis fails to entertain the possibility that some of these patients are persistently infected as in the murine model.

Prophylactic treatment for tick bites has poor scientific underpinnings and will block seroconversion. The indications for prophylactic treatment proposed by decision analysis (31), i.e., at least 3.6% of ticks infected with borrelia and several other criteria, are so restrictive that few even in endemic areas qualify (32). The implementation of the inadequate Guidelines prophylaxis treatment recommendation will do more harm than good by leaving patients symptomatic and difficult to diagnose. In murine studies the investigators were so certain that the infected mice would be seropositive as stated by the Guidelines Committee they discarded their sera without testing it. In mice a single dose oral doxycycline dose similar to that recommended for humans results in 57-80% of newly infected mice having persistent infection (5,6). When prophylaxis is indicated, a proven effective sustained dose of an appropriate antibiotic should be given rather than the inadequate single oral dose of doxycycline recommended by Wormser and his Guidelines Committee (15,26).

CDC Surveillance Definition

In the 1980s as the Ixodes ricinus tick vector spread beyond its usual habitat, human borreliosis followed. In order to reliably track the geographically expanding incidence of LD, the CDC tried to derive a case definition that would include only definitive cases and exclude possible ambiguous ones that might or might not be true LD. Dr. Steere and I were members of the "Committee to Develop a Surveillance Case Definition for Lyme disease" and traveled to Atlanta to write the surveillance definition. We identified a number of Western Blot bands most highly associated with definitive cases of LD and established a minimal number of these that would pick up true cases of Lyme disease but, more importantly, exclude conditions whose etiology was uncertain. The CDC explicitly cautioned against using this restrictive case definition for clinical diagnosis and reiterated this proscription with every re-issuing of its "Surveillance Definition." It has been a

source of frustration and confusion that some in the medical community wrongly insist that a Lyme patient must satisfy CDC criteria (see memo below).

> Yes, CDC has always warned against using the surveillance case definition for clinical diagnosis. However, we can not obviously regulate inclusion criteria for Lyme disease studies conducted by other investigators.
> You will also find that the new 2008 Lyme disease case definition has expanded ability to detect other clinical presentations of Lyme disease, and thus the use of "CDC criteria" may not be as frequent in the future.

Sincerely,
Kiersten Kugeler
Centers for Disease Control and Prevention
Division of Vector-Borne Infectious Diseases
Bacterial Diseases Branch
Fort Collins, Colorado

Conflicts

Members of the current IDSA Guidelines Committee for the Treatment of Lyme have been cited by the Connecticut Attorney General (33) for receiving payments from insurance companies as expert witnesses testifying against patient claims for treating chronic Lyme disease. In addition, members received payments for consulting to LD testing companies regarding their accuracy in detecting LD serologically. The Guidelines Committee has denied the existence of chronic borreliosis and has insisted that all LD patients are seropositive. The Committee's conflicts of interest violate recommendations for guidelines committees (34). The Chairman also lists ownership of Diaspex, a company that mysteriously states it offers no products or services (35) (I think he may have since sold his company). New committee members should be free of conflicts that may color their treatment recommendations.

In conclusion, current treatment guidelines ignore persistent borreliosis, SNB, and recommend an ineffective prophylaxis regimen. Moreover, members of the Committee should declare their conflicts of interest and clarify the meaning of the "surveillance definition." Finally, encouraging improved diagnostic (36) and therapeutic tools should be a major priority of a new Committee. Recommendations should be evidence-based not unsupported opinions of Committee members. Controversies need to be delineated not ignored in the interest of consensus. More credible infectious diseases participation should be incorporated; individuals need not have expertise in borrelia.

References

1. Sredni, B., Volkman, D., Schwartz, R.H., and Fauci, A.S.: Antigen-specific human T-cell clones: Development of clones requiring HLA-DR-compatible presenting cells for stimulation in presence of
antigen. Proc. Natl. Acad. Sci.78:1858-1862, 1981.

2. Volkman, D.J., Matis, L.A., and Fauci, A.S.: Human antigen-specific T cells: Development of exogenous interleukin-2-independent, Ia-restricted, lymphokine-producing helper/inducer clones. Cell Immunol. 88:323-335, 1984.

3. Popovic, M., Flomenberg, N., Volkman, D.J., Mann, D., Fauci,.S., Dupont, B., and Gallo, R.C. Alteration of T-cell function by infection with HTLV-I or HTLV-II. Science 226:459-462, 1984.

4. Volkman, D.J., Popovic, M., Gallo, R.C., and Fauci, A.S. Human T-cell leukemia/lymphoma virus-infected antigen-specific T cell clones: indiscriminant helper function and lymphokine production. J. Immunol., 134: 4237-4243, 1985.

5. Zeidner NS, Massung RF, Dolan MC, Dadey E, Gabitzsch E, Dietrich G, Levin ML. A sustained-release formulation of doxycycline hyclate (Atridox) prevents simultaneous infection of Anaplasma phagocytophilum and Borrelia burgdorferi transmitted by tick bite. J Med Microbiol. 2008;57:463-8.

6. Zeidner NS, et al. Sustained-release formulation of doxycycline hyclate for prophylaxis of tick bite infection in a murine model of Lyme borreliosis. Antimicrob Agents Chemother. 2004 48:2697-9.

7. Dattwyler RJ, Volkman DJ, Luft BJ, Halperin JJ, Thomas J, Golightly MG. Seronegative late Lyme borreliosis: dissociation of Borrelia burgdorferi specific T and B lymphocyte responses following early antibiotic therapy. N Engl J Med 1988;319:1441–6.

8. Luft BJ, Dattwyler RJ, Johnson RC, Luger SW, Bosler EM, Rahn DW, Masters EJ, Grunwaldt E, Gadgil SD. Azithromycin compared with amoxicillin in the treatment of erythema migrans. A double-blind, randomized, controlled trial. Ann Intern Med. 1996;124:785-91

9. Hodzic E, Feng S, Holden K, Freet KJ, Barthold SW. Persistence of Borrelia burgdorferi following antibiotic treatment in mice. Antimicrob Agents Chemother. 2008;52:1728-36.

10. Holl-Wieden A, Suerbaum S, Girschick HJ. Seronegative Lyme arthritis. Rheumatol Int. 2007 ;27:1091-3.

11. Keller TL, Halperin JJ, Whitman M. PCR detection of Borrelia burgdorferi DNA in cerebrospinal fluid of Lyme neuroborreliosis patients. Neurology 1992;42(1):32-42.

12. Chmielewski T, Fiett J, Gniadkowski M, Tylewska-Wierzbanowska S. Improvement in the laboratory recognition of Lyme borreliosis with the combination of culture and PCR methods. Mol Diagn. 2003;7(3-4):155-62.

13. Oksi, J., J. Uksila, M. Marjamaki, J. Nikoskelainen, and M. K. Viljanen. 1995. Antibodies against whole sonicated Borrelia burgdorferi spirochetes, 41-kilodalton flagellin, and P39 protein in patients with PCR- or culture-proven late Lyme borreliosis. J Clin Microbiol 33:2260-4.

14. Nocton JJ, Dressler F, Rutledge BJ, Rys PN, Persing DH, Steere AC. Detection of Borrelia burgdorferi DNA by polymerase chain reaction in synovial fluid from patients with Lyme arthritis. N Engl J Med. 1994;330:229-34.

15. Wormser GP, Dattwyler RJ, Shapiro ED, Dumler JS, O'Connell S, Radolf JD, Nadelman RB. Single-dose prophylaxis against Lyme disease. Lancet Infect Dis. 2007;7:371-3.

16. Kannian P, McHugh G, Johnson BJ, Bacon RM, Glickstein LJ, Steere AC. Antibody responses to Borrelia burgdorferi in patients with antibiotic-refractory, antibiotic-responsive, or non–antibiotictreated Llyme arthritis. Arthritis Rheum 2007;56:4216–25.

17. Feder HM Jr, Johnson BJB, O'Connell S, Shapiro ED, Steere AC, Wormser GP. A Critical Appraisal of "Chronic Lyme Disease" N Engl J Med 357:1422, 2007. and response to letters, 358:428-31, 2008.

18. Steere AC. Reply to letter by Volkman commenting on the possible onset of seronegative disease in Lyme arthritis. Arthritis Rheum. 2009 ;60:310.

19. Wormser GP, Dattwyler RJ, Shapiro ED, Halperin JJ, Steere AC, Klempner MS, et al. The clinical assessment, treatment, and prevention of Lyme disease, human granulocytic anaplasmosis, and babesiosis: clinical practice guidelines by the Infectious Diseases Society of America. Clin Infect Dis 2006;43:1089–134.

20. Murgia R, Piazzetta C, Cinco M. Cystic forms of Borrelia burgdorferi sensu lato: induction, development, and the role of RpoS. Wien Klin Wochenschr. 2002 31;114:574-

21. Volkman D. Seronegative Lyme disease: denial and dogma. Perspect Biol Med. (in press).

22. Steere AC, McHugh G, Damle N, Sikand VK. Prospective study of serologic tests for Lyme disease. Clin Infect Dis. 2008;47:188-95.

23. Volkman D. Prophylaxis after tick bites. Lancet Infect Dis. 6:370-1. 2007.
24. ACP. 2004. Lyme disease initiative.
htp://www.acponline.org/clinical_information/resources/lyme_disease.

25. Preac-Mursic, V., K. Weber, H. W. Pfister, B. Wilske, B. Gross, A. Baumann, and J. Prokop. 1989. Survival of Borrelia burgdorferi in antibiotically treated patients with Lyme borreliosis. Infection 17:355-9.

26. Nadelman RB, Nowakowski J, Fish D, Falco RC, Freeman K, McKenna D, Welch P, Marcus R, Aguero-Rosenfeld ME, Dennis DT, Wormser GP. Prophylaxis with Single-Dose Doxycycline for the Prevention of Lyme Disease after an Ixodes scapularis Tick Bite. N Engl J Med 345:79, 2001.

27. Dressler F, Yoshinari NH, Steere AC. The T-cell proliferative assay in the diagnosis of Lyme disease.Ann Intern Med. 1991;115:533-9.

28. Hasin T, Davidovitch N, Cohen R, Dagan T, Romem A, Orr N, Klement E, Lubezky N, Kayouf R, Sela T, Keller N, Derazne E, Halperin T, Yavzori M, Grotto I, Cohen D. 2006. Postexposure treatment with doxycycline for the prevention of tick-borne relapsing fever. N Engl J Med 355:148–55.

29. Steere AC, McHugh G, Damle N, Sikand VK. Prospective serologic tests for Lyme disease. CID 2008;47: 188-95.

30. Weinstein A. Editorial commentary: laboratory testing for Lyme disease: time for a change? Clin Infect Dis. 2008;47:196-7.

31. Magid D, Schwartz B, Craft J, Schwartz JS. Prevention of Lyme disease after tick bites -- a cost-effectiveness analysis. N Engl J Med 1992;327:534-541.

32. Volkman D.J., Kaell A.T., Bosler E.M., and Benach J.L. Prevention of Lyme disease after tick bites. N Engl J Med 328: 138, 1993.

33. Blumenthal R. 2008. News from Attorney General Blumenthal, May 1, 2008. http://www.ct.gov/ag/cwp/view.asp?a=2795&q=414284.

34. Steinbrook R. Guidance for guidelines. N Engl J Med. 2007; 356; 331-4.

35. Wormser GP. Early Lyme disease. N Engl J Med. 2006; 354:2799.

36. Exner MM, Lewinski MA. Isolation and detection of Borrelia burgdorferi DNA from cerebral spinal fluid, synovial fluid, blood, urine, and ticks using the Roche MagNA Pure system and real-time PCR. Diagn Microbiol Infect Dis. 2003;46:235-40.

37. Volkman D. Seronegative disease after inadequate therapy in Lyme arthritis: Comment on the article by Kannian et al. Arthritis Rheum 58:2212, 2008.

38. Delves PJ, Roitt IM. The immune system: First of two parts. *N Engl J Med.* 2000; 343:37-49.