Microscopy, Culture or PCR-verified cases
of persistent [seronegative] Lyme borreliosis.
(list far from complete)

European cases - American cases - Animal studies

European cases

Schmidli J, Hunziker T, Moesli P, Schaad UB
Cultivation of Borrelia burgdorferi from joint fluid three months after treatment of facial palsy due to Lyme borreliosis [letter]
J Infect Dis **1988 Oct**; 158(4): 905-6
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=89010012&dopt=Abstract

15.5y girl. **Tickbite Oct. 1986. No EM. No 'flu'. Jan. 1987 left facial palsy.** **Positive Bb serology.** Amoxicillin-clavulanate 625mg x4, 12d - discontinued due to rash.
Betamethasone 1mgx2, 2 weeks. Lumbar puncture, **normal CSF.** Oral doxycycline 100mgx2, 14d, facial palsy resolved.
Two months later arthritis in the right knee. Cloudy joint fluid 60ml, **positive culture for Bb.** Ceftriaxone 4g/d, 3g - 2g/d, 11d.

Cimmino MA, Azzolini A, Tobia F, Pesce CM
Spirochetes in the spleen of a patient with chronic Lyme disease.
Am J Clin Pathol **1989 Jan**; 91(1): 95-7
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=2910019&dopt=Abstract

A 54-year-old man had intermittent evening fever, arthralgia, transient erythematous macular eruption on the skin, and splenomegaly of two year's duration. **Immunofluorescence tests for Borrelia burgdorferi serum antibodies had positive results**, but **G-penicillin treatment was ineffective**. Splenectomy with lymph node biopsy was performed to rule out lymphoproliferative disorders. **Borrelia-like spirochetes were identified histologically in the spleen; this finding was consistent with persistence of B. burgdorferi organisms in inner organs in chronic Lyme disease.**

Pfister HW, Preac Mursic V, Wilske B, Einhaupl KM, Weinberger K
Latent Lyme neuroborreliosis: presence of Borrelia burgdorferi in the cerebrospinal fluid without concurrent inflammatory signs.
Neurology **1989 Aug**; 39(8): 1118-20
http://www.ncbi.nlm.nih.gov/htbin-post/Entrez/query?db=m&form=6&Dopt=r&uid=89344421

17y old M, 20 tickbites Aug-Dec while jogging in forest ..
Dec 87 bilat. **tinnitus** developed during two weeks was the **ONLY symptom**
IgG seropositive for anti-Bb (1:64) with normal IgM titers (indicates 'late'), indicated lumbar puncture.
Bb could be cultured from CSF, without any concurrent signs of inflammation or intrathecal antibodyformation.
CSF: 1 WBC/µl and 21 mg/dl protein.
Excerpts:
Neither oligoclonal IgG bands ..... nor intrathecal production of specific antibodies against B. burgdorferi could be demonstrated .... The CSF/serum ratio for antibodies against B. burgdorferi (RBb) was 0,28% .... **Three months later .... Repeated lumbar puncture revealed 2 white cells/µl and 23 mg/dl protein without demonstration of oligoclonal IgG bands or intrathecal production of antibodies against B burgdorferi.**
The only symptom - tinnitus - didn't respond to usual treatment, so authors conclude this symptom was unrelated to Bb.
Would this young man months or years later have developed symptoms of neuroborreliosis ?

Preac Mursic V, Weber K, Pfister HW, Wilske B, Gross B, Baumann A, Prokop J
Survival of Borrelia burgdorferi in antibiotically treated patients with Lyme borreliosis.
Infection **1989 Nov-Dec**; 17(6): 355-9
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=90129322&dopt=Abstract

1. 5y boy. **July 1985 EM. Aug 1985 Lymphocytic meningitis. Seropositive for IgG and IgM**, no antibodies in CSF. Penicillin V orally 100000 u/kg/d, 14d. Spinal showed fewer cells. September 1985 facial palsy, again pleocytosis in CSF. Doxyc. orally 2mg/kg, 10d. Gradually CFS normalized. **April 1986 relapse, Bb was isolated from CSF after 4 weeks in BSK-medium.** Penicillin 200000 u/kg, 22d. **August 1986 relapse/reinfection with EM and painful meningoradiculitis, Bb antibodies now negative in CFS and serum.** Culture not done!

2. 49y man. **EM. typical signs of LMR-Bannwarth S developed 7 weeks later. Pleocytosis and elevated protein in CSF. Both Borrelia IgM and IgG positive in serum.** Penicillin i.v. 20 MU/d, 10d. Four days after therapy normal examination and no complaints, CSF declining parameters, positive Borrelia-index. **Three months later CSF normal, Borrelia-index now negative, but Bb was cultured from CSF**!

3. 26y woman. **Headache, radicular pain. Normal neurological exam. Multiple horseflie bites. CSF pleocytosis and elevated protein. Negative Borrelia-ELISA in CSF and serum.** Ceftriaxone i.v. 2g/d , 10d. Improved. **7.5 month later recurrent episodes of radicular pain, headache, arthralgia, fever.** Normal neurological exam. **Negative serology. Normal CSF. Bb cultured from CFS after 6 weeks in MKP-medium.** Cefotaxime 3 x 2g/d i.v., 14d.

4. 44y man. **EM june 88, no other complaints. Seropositive. Bb isolated from skin biopsy from border of EM.** Phenoxymethylpenicillin 1 MU x3/d, 12d. Three months later normalized serology, but **Bb was again isolated from skin biopsy adjacent to the scar of the first biopsy.** No other manifestations of Borreliosis. Ceftriaxone 2g, 21d. Later skin-culture negative.

5. 40y man. **EM, fatigue, headache.** Penicillin G 10 MUx1, 10d, starting 5 weeks after the tick bite. **Serum Borrelia IgG and IgM negative. Two months after treatment headache and fatique, low Borrelia-titre positive. At bite area, but no sign of EM, was culture positive for Bb, 2.2 mo. after treatment.**

6. 60y woman. **Oct. 87 EM 32x20 cm of duration 6 months.** Methylprednisolone 4 mg daily for asthma for years. Sep. 87 she had doxycycline 200 mg daily, 10d, by family physician for cold. Palpitations, dizziness, but had had angina pectoris for years. **IgM and IgG against Bb negative. Bb isolated from edge of EM oct 20, 87.** Pt. refused to take more antibiotic.

Pfister HW, Preac Mursic V, Wilske B, Schielke E, Sorgel F, Einhaupl KM
Randomized comparison of ceftriaxone and cefotaxime in Lyme neuroborreliosis.
J Infect Dis **1991 Feb**; 163(2): 311-8
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=1988514&dopt=Abstract

In this prospective, randomized, open trial, 33 patients with Lyme neuroborreliosis were assigned to a 10-day treatment with either ceftriaxone, 2 g intravenously (iv) every 24 h (n = 17), or cefotaxime, 2 g iv every 8 h (n = 16). Of the 33 patients, 30 were eligible for analysis of therapeutic efficacy. Neurologic symptoms improved or even subsided in 14 patients of the cefotaxime group and in 12 patients of the ceftriaxone group during the treatment period. At follow-up examinations after a mean of 8.1 months, 17 of 27 patients examined were clinically asymptomatic [i.e 10/27 = 37% were symptomatic]. **In one patient Borrelia burgdorferi was isolated from the cerebrospinal fluid (CSF) 7.5 months after ceftriaxone therapy.** CSF antibiotic concentrations were above the MIC 90 level for B. burgdorferi in nearly all patients examined. Patients with Lyme neuroborreliosis may benefit from a 10-day treatment with ceftriaxone or cefotaxime. **However, as 10 patients were symptomatic at follow-up and borreliae persisted in the CSF of one %patient, a prolongation of therapy may be necessary.**

Peter O, Bretz AG, Zenhausern R, Roten H, Roulet E
[Isolation of Borrelia burgdorferi in the cerebrospinal fluid of 3 children with neurological involvement]
Schweiz Med Wochenschr **1993 Jan** 13; 123(1-2): 14-9

Isolation of Borrelia burgdorferi from the CSF is relatively rare. The present report describes the first three isolations in Switzerland. Clinically, our first observation confirmed the frequent association of B. burgdorferi with peripheral facial paresis in children. The other two cases illustrate the variety of symptoms in neuro-borreliosis.

1. In the first case the **culture was positive after 6 weeks. The results of serologic tests (indirect immunofluorescence and ELISA) for detection of antibodies against B. burgdorferi were negative or non-significant in this child's serum. On the other hand, specific antibodies (IgG) were detected in the serum by western blot.**

2. **Culture of the second CSF already showed Borrelia growth after 10 days. Immunofluorescence revealed high antibody titers (1/256) against B. burgdorferi in this patient's serum. IgG showed a weakly positive reaction in western blot.** The reliability of this result was confirmed by isolation of Borrelia.

In neither of the two CSF could intrathecal synthesis of specific antibodies be demonstrated.

3. In the third case, however, **immunofluorescence showed IgG antibody titers of 1/128 in the CSF and 1/512 in serum. Intrathecal synthesis of specific antibodies was demonstrated with an index of 13.4 (norm < 2). Western blot confirmed the specificity of the reactions observed with the serum and CSF IgG. Culture of CSF produced significant growth of Borrelia within 7 days.** Protein profile and reactions with poly- and monoclonal antibodies confirmed that the three strains belonged to B. burgdorferi.(ABSTRACT TRUNCATED AT 250 WORDS)

Oksi J, Viljanen MK, Kalimo H, Peltonen R, Marttia R, Salomaa P, Nikoskelainen J, Budka H, Halonen P
Fatal encephalitis caused by concomitant infection with tick-borne encephalitis virus and Borrelia burgdorferi.
Clin Infect Dis **1993 Mar**; 16(3): 392-6
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=8452951&dopt=Abstract

We describe a 38-year-old farmer from the southwestern archipelago of Finland where both tick-borne encephalitis (TBE) virus and Borrelia burgdorferi are endemic. He presented with fever and headache, developed severe meningoencephalitis in

3 days, and, after 1 month, died without regaining consciousness. **High titers of IgG and IgM antibodies to TBE virus were present in both serum and CSF. Serology for Borrelia was negative.** Autopsy revealed necrotizing encephalitis and myelitis with involvement of the dorsal root ganglion. **With use of polymerase chain reaction tests, segments of two separate genes of B. burgdorferi were amplified from the patient's CSF.** This case demonstrates that the possibility of dual infection should be considered for patients residing in geographic areas where Ixodes ticks may carry both the TBE virus and B. burgdorferi. We believe that the most severe damage in this case was caused by TBE virus rather than by B. burgdorferi. Nevertheless, the coinfection might have contributed to the fatal outcome that has not been previously observed in Finnish patients with TBE.

[Pt. was initially treated with penicillin plus gentamicin, next day changed to cefotaxime, later erythromycin was added. ... plus **dexamethasone**. All discontinued when TBE virus infection was confirmed. Later highly febrile from pneumonia (40ºC), imipenem, rifampicin and vancomycin and amphotericin B. Imipenem-sensitive E.coli was isolated from the trachea. Later Clostridum difficile was isolated from a stool sample obtained while the patient had diarhea. .... Died.]
"**With use of ELISA, significantly raised antibodies to sonicated whole B. burgdorferi and to purified endoflagellar antigen were not detected in either serum or CSF (table 1).** A CSF specimen for polymerase chain reaction (PCR) was taken." ....."**With use of the PCR method, a B. burgdorferi-specific segment of a gene coding for 41-kD endoflagellin [7] and a Borrelia-specific segment of 16S rDNA [8] were amplified from the CSF (table 1).**"

Preac Mursic V, Pfister HW, Spiegel H, Burk R, Wilske B, Reinhardt S, Bohmer R
First isolation of Borrelia burgdorferi from an iris biopsy.
J Clin Neuroophthalmol **1993 Sep**; 13(3): 155-61; discussion 162
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=8106639&dopt=Abstract

The persistence of Borrelia burgdorferi in six patients is described. **Borrelia burgdorferi has been cultivated from iris biopsy, skin biopsy, and cerebrospinal fluid also after antibiotic therapy for Lyme borreliosis. Lyme Serology: IgG antibodies to B. burgdorferi were positive, IgM negative in four patients; in two patients both IgM and IgG were negative. Antibiotic therapy may abrogate the antibody response to the infection as shown by our results. Patients may have subclinical or clinical disease without diagnostic antibody titers. Persistence of B. burgdorferi cannot be excluded when the serum is negative for antibodies against it.**

Demonstrates 6 cases with persistent culture positive Bb, isolated from 1) iris, 2) skin and 3-6) 4 x CSF,
1) **pos. iris culture** despite previous tx doxy 200mg/dg 4 weeks. x 2, **seropositive**;
2) **EM +culture,** tx doxy 200mg/dg 10 days. **Seronegative**. 1 yr iritis, not responsive to steroid, but to ceftriaxon.
3) **Weak seropos. CSF normal, neg. index,** but **culturepos. CSF**
4) **Weak seropos. CSF normal, neg. index,** but **culturepos. CSF**
5) tickbite, penicillin orally x 2 for 12 days. **4 month later CSF elev. prot. 6/3 cells, seronegative + CSF-seronegative**, but **CSF culturepos. for Bb.**
6) **Weak seropositive in serum, CSF negative, Bb was cultured from CSF.**

Haupl T, Hahn G, Rittig M, Krause A, Schoerner C, Schonherr U, Kalden JR, Burmester GR
Persistence of Borrelia burgdorferi in ligamentous tissue from a patient with chronic Lyme borreliosis.
Arthritis Rheum **1993 Nov**; 36(11): 1621-6
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=94059203&dopt=Abstract

Excerpts:

CASE REPORT
The patient, a 48-year old woman, presented with progressive disturbance of the central vision in her right eye. Ophthalmoscopy demonstrated **multifocal choroiditis,** with 1 focus involving the macula lutea. The patient reported that **2 months previously, a tick had bitten her left lower leg, near the ankle, and she had experienced occipital headaches and a macular skin lesion.** Serologic tests performed at our university revealed a **positive IgG antibody titer against B burgdorferi.** Other infectious diseases, particularly toxoplasmosis, were excluded by laboratory evaluations. The patient was treated with 200 mg/day of doxycycline (orally) for 6 weeks (Figure 1). The visual disturbance was ameliorated, the inflammatory foci of the choroid diminished, and scar tissue formed.
Four weeks after the end of antibiotic therapy, the patient began to experience brief episodes of an asymmetric arthritis, primarily involving the metacarpophalangeal (MCP) and proximal interphalangeal joints. Routine electrocardiography (EKG) revealed negative P waves, with an ectopic atrial pacemaker that had not been present on an EKG performed at gall bladder surgery 1 year previously. On ophthalmoscopic examination of the eye fundus, there was no evidence of a recurrence of the choroiditis.
Tests for antinuclear antibodies, immune complexes (by Clq binding assay and polyethylene glycol precipitation), rheumatoid factor (RF), immunoglobulins, serum complement levels, and C-reactive protein levels all gave normal or negative results. HLA phenotyping revealed HLA-A24/26:B7/27;Bw4/6;Cw2/7;DRl5(DR2 split);DQ1. Despite the presence of the HLA-B27 antigen, there was no evidence of any typical manifestation of a seronegative spondylarthropathy.

Therapy was started with 2 gm of ceftriaxone, intravenously, for 14 days (Figure 1). Within the next 4 weeks, the ectopic atrial rhythm converted to a normal sinus rhythm, and the arthritis disappeared. **After 2 months free of clinical symptoms, the visual disturbances recurred. Ophthalmoscopy showed reactivation of the initial foci of choroiditis.** Analysis of CSF demonstrated normal levels of albumin and IgG, no disturbance of the blood-CSF barrier, negative findings on immunofluorescence (IF) analysis for B burgdorferi-specific antibodies, and a normal cell count; thus, there was no evidence of an inflammatory process involving the central nervous system.
Antibiotic therapy with a combination of 300 mg of roxithromycin, 1,600 mg of sulfamethoxazole, and 320 mg of trimethoprim per day, which has been described as effective in several cases of advanced Lyme borreliosis (6,7), was initiated. **However, tenosynovitis and mild arthralgia of the patient's hands occurred. Despite concurrent antibiotic therapy, "trigger thumb" developed within 2 weeks, accompanied by pronounced pain of the MCP joint, and surgical splitting of the flexor retinaculum was performed (Figure 1).**
After exsanguination of the patient's right arm, surgery was performed in a bloodless field. The macroscopic appearance was typical of "trigger finger." A specimen of the altered ligament was obtained, with particular attention to avoiding surface contamination of the tissue sample. The specimen was rinsed several times in saline and medium, and was placed in culture with modified BSK medium. The patient's postoperative course was without complications, and normal functioning of the operated thumb was achieved. Six weeks after the course of antibiotics, the choroid foci were scarring. Unfortunately, the patient had an irreversible, 70% reduction of vision in the right eye. Approximately 3 weeks later, the arthralgia also disappeared. Currently, after approximately 2 ½ years of followup, there has been no evidence of reactivation of the Lyme borreliosis.

METHODS AND RESULTS

Immunofluorescence assay and findings. The IF assay was performed as described earlier, using the German isolate of B burgdorferi, PKo 2-85 (3,8). Serum samples were preabsorbed with Treponema phagedenis lyophilysate (Behringwerke, Marburg, Germany). For the detection of specific IgM antibodies, a second absorption step with RE absorbent (Behringwerke) was performed. Using this technique, titers in control sera were <1:16. In parallel, the serum samples were analyzed using a commercial B burgdorferi enzyme-linked immunosorbent assay (ELISA) kit (Viramed, Munich, Germany), with a protein preparation of B31 Borrelia,, as antigen. A positive, a negative, and a borderline control specimen served as internal standards. The ratio between the optical density of the serum samples versus that of the borderline specimen was used to quantitate the results. Ratios >1.0 were considered positive; those <1.0 were negative.
IF analysis revealed positive titers of IgG, but not IgM, antibody directed against B burgdorferi only during the early stage of infection when the patient presented with the first episode of choroiditis. **Analysis by ELISA revealed comparable results, with specific IgG ratios of 1.15 at the onset of disease and 0.85 in the later stage. The IgG titer rapidly decreased within a few weeks after the first antibiotic therapy, and remained negative in both the IF and ELISA evaluations, despite progression of the disease.**
Immunoblot analysis and findings. For immunoblot analysis, we used a method previously described (9), in which lysed specimens of whole B burgdorferi strain PKo 2-85 and LW2, the isolate from the patient (see below) (protein concentration 100 µg/ml), were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (5 µg of protein per lane) in a 10% gel. Proteins were transferred to nitrocellulose and incubated with the sera at a dilution of 1:100, which we had previously found yielded optimum results. Bound immunoglobulins were visualized by application of peroxidase-conjugated reagents. Polyclonal antisera from patients with Lyme disease and monoclonal antibodies against outer surface protein A (OspA) and flagellin (the latter kindly provided by M. D. Kramer, Institute of Immunology and Serology, University of Heidelberg, Germany) were used to compare the isolated spirochete LW2 and the B burgdorferi strain PKo 2-85 by immunoblot.
All serum samples from the patient were tested against protein preparations of both the LW2 and the PKo 2-85 strain, on immunoblots. There were no significant differences in reactivity to the isolates. Upon repeated analysis of several consecutive serum samples, only nonspecific faint bands (75 kd, 41 kd, and 15 kd) were revealed, demonstrating a pattern different from that found in typical patients with stage III disease (10).
Preparation of mononuclear cells and results of lymphocyte proliferation assay. Peripheral blood mononuclear cell (PBMC) separation and antigen stimulation were performed as described previously (8.9) Freshly isolated cells (10^5/well) were stimulated in triplicate with either 10^5 or 10^6 PKo 2-85 B burgdorferi per well, 10 µg/ml of recombinant OspA, 10 µg/ml recombinant flagellin (41-kd protein) from B burgdorferi (both expressed and purified as described elsewhere [9]), or 7 µg/well of T phagedenis lyophilysate. Previous studies had shown these to be optimal concentrations (8,9). Control wells received either medium alone or tetanus toxoid (10 µg/ml; Behringwerke). Samples from normal blood donors were run in each assay to exclude any nonspecific response. It has been clearly documented by appropriate separation experiments (9) that reactivity to Borrelia antigens under these conditions is T cell derived.
Stimulation of the patient's PBMC with the 2 strains of B burgdorferi as well as with OspA resulted in significantly elevated 3H-thymidine uptake at all times tested (compared with normal PBMC) (Figure 1). Tetanus toxoid induced high levels of 3H-thymidine uptake, between 143,000 and 181,000 Δcounts per minute (stimulation values in the presence of antigen minus those in the absence of antigen). In the earlier disease stages and prior to ceftriaxone therapy, proliferation to Borrelia antigens corresponded well to the clinical course, despite negative findings on IF and ELISA testing, reaching peak values at peak disease activity, as manifested by intermittent arthritis and cardiac involvement (Figure 1). After the ceftriaxone therapy and a 2-month symptom-free period, PBMC proliferation de-

creased, but during a period of minor inflammatory reactivation of the disease, was still elevated. The patient's symptoms at that time were choroiditis, mild arthralgia, and the development of "trigger finger." B burgdorferi was isolated from ligament samples.

Isolation and characterization of B burgdorferi strain LW2. Tissue samples from the flexor retinaculum of the patient's right thumb were taken during surgery for the "trigger finger." Samples were cultured at 370C under microaerophilic conditions, in modified BSK medium. **Cultures were evaluated weekly for spirochetes in the supernatant, as detected by darkfield microscopy. After 3 weeks, viable spirochetes were seen.** This particular spirochete, designated LW2, was used as antigen for immunoblot. B burgdorferi-specific immune sera and monoclonal antibodies against OspA and flagellin stained protein bands in a pattern comparable to that of the PKo 2-85 bacterial antigen (data not shown). An aliquot of this supernatant was examined for B burgdorferi-specific gene sequences by polymerase chain reaction (PCR) amplification and Southern blot.

PCR and Southern blot techniques. Cultured spirochetes from the patient's tissue specimen were investigated by standard PCR procedures (11). We used primers which amplified a 276-basepair segment (kb-ladder; Gibco BRL Life Technologies. Munich, Germany) of the 41-kd flagellin protein of B burgdorferi (primer 5'-TTCAGGGTCTCAAGCGTCTTGGACT-3'; reverse primer 5'-GCATTTTCAATTTTAGCAAGTGATG-3') (12,13). The amplification protocol consisted of 40 cycles: 1-minute denaturation at 94^0C, 30-second annealing at 50^0C, and -minute extension at 67^0C.

An amplification product, which could be hybridized by Southern blot technique with the 32P-γ-ATP-labeled probe 5'-CTCTGGTGAGGGAGCTCAAACTGCTCAGGCTGCACCGGTTCAAGAGGGT-3' (13) using Hybond-N nylon-blotting membranes (Amersham, Amersham, UK), was obtained. The amplimer, which was characterized by Picken (13), is derived from the central, nonhomologous region of the flagellin gene. It has been shown to be both specific for B burgdorferi and discriminatory for 3 groups of this spirochete, based on the nucleic acid sequence. Water, synovial tissue from a patient with rheumatoid arthritis, and B burgdorferi strain PKo 2-85 were used as negative and positive controls for the studies.

Electron microscopy techniques and results. Transmission electron microscopy was performed on the ligament tissues. The specimen was removed from the culture medium and prepared and analyzed as described previously (14). Semithin sections were cut from the plastic block for the light microscopic evaluation. Thin sections were cut from selected areas and placed onto copper grids (Polysciences, St. Goar, Germany). After counterstaining with 10% uranyl acetate, followed by 2.8% lead citrate (both from Merck, Darmstadt, Germany), sections were studied using Siemens EM 101 (Munich, Germany) and Zeiss EM 902 (Wetzlar, Germany) electron microscopes.

The ligament tissue was found to be heavily infiltrated by spirochetes. Some of the organisms lay between unaltered collagen fibers (Figure 2A); others were closely attached to the cell surface of the fibroblasts (Figure 2B). There were numerous fibroblasts deeply invaginated by the spirochetes, thereby creating membrane-bound cavities. These cavities appeared as vacuoles in transverse tissue sections.

DISCUSSION

The patient whose case is presented herein had relapsing Lyme borreliosis, with choroiditis, arthritis, carditis, and tendinitis. The humoral immune response correlated with neither the cellular reactivity in vitro nor the clinical activity of the disease manifestations. Repeated antibiotic treatment was necessary to stop the progression of disease, but obviously did not completely eliminate B burgdorferi from all sites of infection. This was confirmed by the culture of viable B burgdorferi from a ligament sample obtained surgically. This organism characterized by molecular biology studies by our group, was subsequently evaluated in a genomic comparison study of other isolates. In that study, Wallich et al identified the genogroup AAA (flagellin type at, heat shock protein [HSP] 60 type A, and HSP 70 type A), and OspA genotype I (15). Electron microscopy of the ligament revealed spirochetes situated between collagen fibers or associated with fibroblasts, deeply invaginating these cells. This is the first time that B burgdorferi was isolated from human ligamentous material.

These data indicate that vital B burgdorferi persisted (a) despite several courses of antibiotic therapy, (b) even when clinical symptoms subsided, and (c) even when no humoral immune response was detectable by ELISA or by IF. Therefore. the hypothesis may he raised that an inadequate immune response as well as an evasion into immunologically privileged sites may be the mechanisms of microbial persistence in patients with chronic Lyme borreliosis.

The specific humoral and cellular immune responses to B burgdorferi, which were elevated during early disease manifestations, apparently were not sufficient to eliminate the pathogen. In the later stage, these specific immune responses became discordant, with negative humoral and positive cellular immunity, as has been described in another cohort with chronic disease (16) [Dattwyler RJ et al. Seronegative Lyme disease: dissociation of specific T- and B-lymphocyte response to Borrelia burgdorferi, NEJM 1988; 319:1441-46].

Interestingly, the cellular immune responses were also directed against the surface protein OspA during each recurrence of clinical symptoms, even though anti-OspA antibodies were not detectable by immunoblot. Interpretation of this dissociation of the humoral and cellular immune responses is difficult and requires further investigation. Initial experiments with T cell clones in patients with chronic Lyme disease (17) [Yssel H et al. Borrelia burgdorferi activates a T helper type 1-like T cell subset in Lyme arthritis. J Exp Med 1991 Sep 1; 174(3): 593-601] suggest that selective activation of a T cell subset may occur, producing a restricted pattern of cytokines which are incompetent to activate B cells.

Even in the presence of an ineffective immune response, antibiotic therapy should have eradicated the spirochetes and stopped the disease progression in our patient**. However, several of the treatment regimens recommended in the then-**

current literature, including combination therapies which have been described as effective in several refractory cases of advanced-stage disease (6,7), did not eliminate the pathogen. Of interest, our patient showed the DR15 (split of DR2) HLA type (possibly even homozygous), which has been shown to be associated with a poor response to antibiotic therapy in chronic B burgdorferi infection (4) [Steere Ac et al, Association of chronic Lyme arthritis with HLA-DR4 and HLA-DR2 alleles, NEJM 1990; 323:219-23]. Possible explanations for the persistence of Borrelia are that spirochetes either develop resistance to the antibiotics (though not experimentally documented so far) or escape into sites at which drug levels are ineffective. The detection of spirochetes between collagen fibers of bradytrophic dense connective tissue supports the second hypothesis. Moreover, the motility of spirochetes has been shown to be enhanced in fluids as viscous as the extracellular matrix (18) [Kimsey RB et al Motility of Lyme disease spirochetes in fluids as viscous as the extracellular matrix. JID 1990; 162: 1205-8]. **The hypothesis of evasion supports the use of more aggressive therapy as described in recent reports (19), in which 3-4 weeks of intravenous antibiotics was suggested as first-line treatment when systemic manifestations develop, such as the choroiditis in our patient.**
Although our electron microscopic studies were of a subcultured ligament specimen, and therefore in vitro effects cannot be excluded, it is of great interest **that spirochetes penetrated into the extracellular matrix without causing apparent destruction. Spirochetes had also deeply invaginated into fibroblasts, thereby suggesting transcellular passage.** Penetration of cell monolayers by B burgdorferi has been demonstrated (20) [Comstock LE et al: Penetration of endothelial cell monolayers by Borrelia burgdorferi. Infect Immun 1989; 57:1626-28]. In conclusion, an inappropriate immune response as well as the evasion of B burgdorferi into specific sites that are only slightly accessible to antibiotics and immunologic attack, may be mechanisms that lead to chronic infection with B burgdorferi.

Addition: Intracellular location protect Bb from antibiotics that do not cross cell-membranes ex. penicillin, cephalosporin [Georgilis K et al. Fibroblasts protect the Lyme disease spirochete, Borrelia burgdorferi, from ceftriaxone in vitro. J Infect Dis 1992 Aug; 166(2): 440-4]

Hulinska D, Krausova M, Janovska D, Rohacova H, Hancil J, Mailer H
Electron microscopy and the polymerase chain reaction of spirochetes from the blood of patients with Lyme disease.
Cent Eur J Public Health **1993 Dec**; 1(2): 81-5
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=94272413&dopt=Abstract

95 patients, only 3/30 attempts were culture positive, but by Immune electron microscopy IEM 9 of 30 blood samples demonstrated borreliae.
The decisive diagnostic method was:

1. serology and clinical symptoms in 65 and
2. in 30 patients by **visualization of spirochetes** by IEM in
   - heparinised blood (9)
   - CSF (13) or
   - skin from EM (8).

8/30 IEM positive had EM, 21 could be classified as early Lyme borreliosis with neurological symptoms, 6 as late stage Lyme borreliosis (encephalomyelitis).
14 had antibodies only in blood, 6 both in blood and CSF, the remaining 10 non-reactive by 2 different tests.

The **one blood culture positive patient** had prior history of EM followed by meningoradiculitis and **positive IgM and IgG in serum** the **second [with positive culture]** had facial palsy with complaints of headache, weakness and pain in the arms that lasted 8 days, **while the serology being negative.** The other **(third) with positive culture** had a **long history of Lyme borreliosis with arthralgias and complaints fulfilling the criteria for neuroborreliosis. He had an erythema and often removed ticks from his dog. During his illness he had all symptoms of Lyme borreliosis and also positive serology. He was in good condition after having taken antibiotics for 3 months before the blood culture was done.**
One patient with positive IEM and subsequent (positive) culture went through all phases of Lyme borreliosis and two years of persisting complaints! … after repeated treatment [drug? dose? duration?]
Our culture of spirochetes from the patient with meningoradiculitis followed after skin Erythema migrans confirmed the presence of B. garinii, identified by PCR, which caused the neurological disorder - even in the presence of antibodies and antibiotic treatment. **One patient remained symptomatic for a period for two years after repeated treatment, but B. burgdorferi was cultured when the patient was relatively well.**

Vartiovaara I
Living with Lyme [see comments]

Lancet **1995 Apr** 1; 345(8953): 842-4
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=95206022&dopt=Abstract

The patient - a physician, psychiatrist, who at the time was editor of the Finnish Medical Journal, tells his own story - a must read for all physicians, since he describes not only the tecnical details of his disease, but also about how difficult it is to handle the situation, when a physician is becoming a patient.
He contracted Lyme disease when attending a meeting in Vancouver, where he got bitten by a tick in bed at night and three weeks later he was sick. **A history of antibiotic-responsive Lyme borreliosis, but relapsing when treatment was discontinued. Seronegative**.
PCR positive for borrelia in CFS+blood after three antibiotic treatments.

Oksi J, Mertsola J, Reunanen M, Marjamaki M, Viljanen MK
Subacute multiple-site osteomyelitis caused by Borrelia burgdorferi.
Clin Infect Dis **1994 Nov**; 19(5): 891-6
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=7893875&dopt=Abstract

In a pediatric case of **severe multiple-site osteomyelitis caused by Borrelia burgdorferi, the presence of spirochetes in a bone lesion was documented both by culture and by the polymerase chain reaction (PCR).** Positive PCR results were also obtained with culture fluid yielding spirochetal growth and with acute-phase serum. **Although the disease evidently was a late manifestation of Lyme borreliosis, antibodies to B. burgdorferi were low in titer and were restricted to the IgM class.** The distribution of osteomyelitic lesions in multiple bones and the positive PCR results obtained with serum argue for hematogenous spread of the spirochetes. **Before the specific diagnosis was established, the patient received several potent antimicrobial drugs, without a favorable outcome.** In contrast, therapy with ceftriaxone led to a rapid cure that persisted thereafter. We conclude that infection due to B. burgdorferi must be considered a possible cause of subacute pediatric osteomyelitis.

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 Sep**; 33(9): 2260-4
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=96025135&dopt=Abstract

41 pt.; only PCR or Culture proven LATE Lyme Borreliosis !;
19 had only weekly positive or borderline antibody levels and **7 were seronegative.**

Preac Mursic V, Marget W, Busch U, Pleterski Rigler D, Hagl S
Kill kinetics of Borrelia burgdorferi and bacterial findings in relation to the treatment of Lyme borreliosis
Infection **1996 Jan-Feb**; 24(1): 9-16
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=97005145&dopt=Abstract

[published erratum appears in Infection 1996 Mar-Apr;24(2):169]: On page 12, because of technical reasons it was unfortunately not mentioned that information regarding Case I was provided by Dr. D. Hassler.

In vitro investigation. **Amoxicillin, doxycycline, cefotaxime, ceftriaxone, azithromycin and penicillin G**. Killing effekt investigated during a 72h exposure in MPK-medium and human serum with negative Lyme borreliosis serological tests.. Twenty clinical isolates were used ...

Exerpts:
.. the results show that the kill kinetics of the borreliae differs from antibiotic to antibiotic. The killing rate of a given antibiotic for borreliae is less dependent on the concentration of the antibiotic than on the reaction time. Furthermore, the data show that the killing effect of isolates of B. garinii differs from that in B. afzelii species. **Very interesting and unexpected is the different effect of antibiotics on isolates within one species. Also the different reaction of one strain to tested antibiotics is surprising**.....
In summary, the result of killing kinetics suggest that:

1. The strains of B. afzelii and b. garinii spp. react differently against antibiotics used in the treatment of Lyme disease.

2. The different reactions of strains to antibiotics also exists within one species.

3. There exist different effects of one antibiotic against strains tested as well as different reactions of the strain to antibiotics tested.

4. The killing rate of a given antibiotic is dependent on reaction time of antibiotics.

5. B. garinii strains seem to be more sensitive to antibiotic tested than B. afzelii strains.

6. **The antibiotics take a long time to become effective**.

7. The different killing kinetics of B. burgdorferi sensu lato strains can be of importance in a treatment regimen.

Furthermore, the persistense of B. burgdorferi s.l. and clinical recurrences in patients despite seemingly adequate antibiotic treatment is described. The patients had clinical disease with or without diagnostic antibody titre to B. burgdorferi. **Includes five case stories showing culture confirmed relapses after 12-14 days treatment courses.**

Case 1: 51y man **plexus neuritis**. **Positive serology for Borreliae IgG, WB**. Cefotaxime 3x2g a day 12 days. **Antibodies to Borreliae disapperead in months. Five years later a new attack with headache and pseudoradicular pain located in the region of the right arm plexus. Negative Bb and Western Blot.** Half a year later progressive cardiac pain and dyspnoe on exertion. Angiography and echocardiography revealed 3rd degre mitral insufficiency. The patient had a history of 7 years of cardiomyopathy. Mitral valve replacement was carried out. **Borreliae was cultured after prolonged incubation (9 weeks) from the excised mitral valve. Bb antibodies negative (ELISA, WB, IFT).**

Case 2: 13y old boy with right **gonarthritis**. **Positive IgG and IgM serology for Borrelia.** Treated with ceftriaxone 2g/day for 14 days. Joint swelling diminished, but later recurred. **Six months later synovectomy grew Borrelia afzelii from synovia as well as from the effusion.**

Case 3: **painfull knees, treated with corticosteroid. Lyme-IFGT-IgG borderline.** Treated with ceftriaxone 2g/day for 14 days. **Recurrent arthritis. About half a year later IgG in serum and and synovial fluid was positive. B. afzelii was isolated was isolated from the effusion.**

Case 4: 35y man. **One year history of headache, intensive back pain, skin eruption (lymphocytoma benignum) and arthralgia. Serum Borrelia negative. Borreliae were isolated from skin biopsy (B. garinii).** Cefriaxone 2g/day 14 days. **The back pain diminished, other symptoms persisted.** Doxycycline dose? 10 days. **Persistent arthralgias. Antibody titers against Bb s.l. negative**, but **Borreliae was isolated from a subsequent biopsy.** Oral penicillin dose? for 14 days. The antibiotic treatment resulted in reduction of arthralgias [comment: but not symptom free, not cured?]

Case 5: 28y woman. **Arthralgia multiple joints.** Corticosteroids and doxycycline dose? duration?. **After a 2-year history of pain and an increase in inflammation in the knee and hands synovectomy was performed**. **Borrelia IgM and IgG was negative,** but nevertheless **B. afzelii was isolated from hand synovia** and the patient was treated with ceftriaxone.

Oksi J, Kalimo H, Marttila RJ, Marjamaki M, Sonninen P, Nikoskelainen J, Viljanen MK
Inflammatory brain changes in Lyme borreliosis. A report on three patients and review of literature.
Brain **1996 Dec**; 119 (Pt 6): 2143-54
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=9010017&dopt=Abstract

Case 1:
The patient was a 51-year old woman with a history of progressive lymphoedema of the left lower limb since 1954. She had suffered from erysipelas in the left lower leg and erythema nodosum in both legs, and had also had recurrent fever episodes several times a year. Lung fibrosis, heart insufficiency and chest pain atypical of coronary heart disease developed at the age of 30-35 years. She had received long-term corticosteroids and several coureses of antimicrobial drugs.
In 1985, the patient had a 3 week period of fever and facial redness suggestive of lupoid erythema. Despite corticosteriod treatment, a spiking fever persisted. At hospital, no infection focus was found. Antimalarial drugs, combined with methylprednisolone were given for two years, but episodes of mild fever reappeared. Antinuclear antibodies and antibodies against extractable antigens were repeatedly negative. Anti-DNA-antibodies were found slightly positive.
After september 1988, she was hospitalized several times for prolonged vomiting, fatigue, fever, dizziness and progressive walking difficulties with ataxia and short gait. In addition, impairment of memory, taste, and hearing occurred. In february 1988, the erythrocyte sedimentation rate (ESR) was 125 mm/h serum C-reactive protein 83mg/ml (normal <10 mg/ml), and leucocytes 9.2 x 10^9 with 96% granulocytes. Sinus X-ray showed sinuitis, and the brain CT showed an empty sella. Despite treatment with methylprednisolone and i.v. erythromycin, the ESR and C-reactive protein remained elevated. At CSF examinations (February 1989 and January 1991), leucocyte counts and protein concentrations were normal, as was the IgG/albumin ratio, but one or two subfractions were observed with protein electrophoresis. In January 1991, MRI of the brain showed enlarged ventricles, cortical atrophy, and marked degenerative changes in the periventricular areas (Fig. 1A). Total serum immunoglobulins were normal, but immune electrophoresis showed an M-komponent (IgG lambda). Circulating immune complexes also occurred. Rheumatoid factor, antinuclear antibodies, anticardiolipin, TPHA (treponema pallidum haemagglutinations test), anti-phospholipid antibodies, and **antibodies against B. burgdorferi were negative in then se-**

rum. Leucocytes were 3.5 x 10^9 /l with an excess of band forms. In August 1991, **CSF examination showed no inflammatory cells, a slightly elevated protein concentration of 762 mg/l, and no antibodies against B. burgdorferi. Culture of CSF in BSK-II medium showed very slow growth of spirochetes during 3 months. Using monoclonal antibodies, immunoflourescence and PCR, the spirochete was identified as B. burgdorferi s.l.**
In December 1991, antimicrobial treatment with ceftriaxone (2g i.v. daily) was instituuted. The patient improved slightly, and therapy was continued after 3 weeks with oral amoxicillin (500 mg every 8 h) and oral probenecid (500 mg every 8 h). After 1 week on amoxicillin the patient developed urticaria. Oral doxycycline (100 mg every 12 h) was substituted and continued until July 1992. During this treatment, the walking difficulties and fever episodes recurred. All cultures for fungi and common bacteria were negative. In January 1992, brain MRI showed slight progression of the periventricular lesions from the image obtained 1 year earlier. In March and July 1992, subdural haemorrhages of unknown origin were evacuated.
On August 7, 1992, plasma and bone marrow specimens were positive for B. burgdorferi PCR. Treatment with ceftriaxone (2g i.v. daily) was reinstituted, the patient reacting with high fever. Empirical antifungal therapy with amphotericin B was also started. These treatments were continued until the patient died on September 12, 1992.
At autopsy, the pathological changes were slighter than expected. The spleen was slightly enlarged. Chronic liver stasis and mild pulmonary oedema were detected. No signs of fungal infection were seen. Neuropathological examination showed a chronic left-sided subdural haematoma. Its structure was compatible with the haemorrhages ocurring 6 and 12 months before death. An increased number of plasma cells were present within the organizing connective tissue of the haematoma. In subcortical and periventricular white matter, diffuse demyelination with mild perivascular inflammation was seen (Fig. 1B and C). **In one of the six analysed brain tissue specimens, B. burgdorferi DNA was detected by the PCR.**

Case 2:
This 40-year old man had previously been healthy, apart from reactivation of a genital herpes infection some weeks before. He recalled no tick bites or erythema migrans. On December 26, 1992, he had a generalized seizure and was admitted to the hospital. Another seizure ocurred on the day of admission. Brain CT was normal. On admission, CSF examination showed an unremarkable increase of protein level (688 mg/l) with no inflammatory cells. The PCR assays for herpes simlex virus (HSV) and antibodies against viruses were negative in the CSF**. Serum IgM antibodies against B. burgdorferi were found at a low level** and IgG antibodies against Chlamydia pneumoniae were moderately elevated. Serum C-reactive protein was 50 mg/l, lactic dehydrogenase 927 U7l (normal value <20 U/l), but the changes were transient. Other labororatory tests were normal including serum hepatitis B surface antigen, antibodies against HIV and herpes virus. The EEG showed an irrative focus in the left hemisphere.
On December 30, MRI of the brain showed three small frontal lesions at the bottom of the left frontal lobe near the meninges. The imaging of these lesions was enhanced using contrast medium (Fig. 3A). In the right pleural cavity, a chest X-ray examination showed fluid, which disappeared in 2 weeks. The CT showed a central cystic lesion n the left kidney, but no abnormal findings were obtained in the mediastinum, lungs, or pleural cavities. On December 31, a CSF examination showed 4 x 10^6 /l lymphocytes, but protein (373 mg/l), and angiotensin convertase enzyme and lysozyme concentrations were normal. The CSF antibodies against herpesviruses, B. burgdorferi, and Treponema pallidum were negative as was antigen for HSV and PCR for B. burgdorferi. The PCR for HSV was positive with this specimen. Culture for viruses and mycobacteria remained negative.
On January 8, 1993, a frontally located lesion was resected for suspected malignancy. Histopathological studies showed lymphocytes in the walls of leptomeningeeal and small penetrating arteries as well as in the perivascular space of the latter (Fig. 2B). The adjacent cortex was slightly oedematous with very mild astrocytic gliosis**. A PCR analysis of three separate brain specimens detected DNA of B. burgdorferi. The IgM (but not IgG) antibodies against B. burgdorferi were positive only in the first pretreatment sample serum samples, but negative thereafter.** The circulating immune complexes and complement activation products were positive. The IgG antibodies against C. pneumoniae were elevated at a constant level, but IgM antibodies remained negative, indicating that the IgG antibodies were of earlier origin. A neuropsychological investigation showed memory impairment affecting verbal function anf slightly impaired fluency of verbal expression. Anticonvulsive therapy with carbamazepine was started. Table two shows changes in the antimicrobial treatment schedule and the development of the brain lesions appearing on MRI. During the antibiotic treatment, MRI of the brain showed new lesions, one enhancing lesion (2 cm in diameter), suggestive of focal vasculitis, located medially from the postoperative area, and later, enhancing lesions at the bottom of the right frontal lobe and a frontal lobe sulcus (Fig. 3A and B). However, the initial lesion at the bottom of the lest frontal lobe behind the orbita was now markedly smaller than at previous examination. Later images showed that the first lesion was constantly reducing in size, and five months after onset of antibiotic therapy all the new foci of the putative vasculitic process had also disappeared. The antibiotic therapy was discontinued on July 5, 1993.
The patient was asymptomatic at the end of therapy. Whole body bone scanning was carried out in June 1993 because of a history of pain in the thoracic spine some months earlier. Slightly increased uptake of isotope in the thoracic spine was seen, but the finding was considered unspecific. The EEG after sleep deprivation was normal in July 1993.
Five months after the end of antibiotic therapy, brain MRI showed a new focus located adjacent to the third ventricle (Fig. 3 A and B). oral antibiotic treatment was started (the patient was asymptomatic: see table 2). The next MRI showed that the treatment had probably had beneficial effect on the former lesions, but again, a new focus in frontal sulcus and a relatively large pathological area in periventricular white matter were detected (Fig. 3B). **On May 17, 1994, DNA of B. burg-**

dorferi was detected by PCR in the patients plasma specimen (Table 2). Intravenous antibiotic therapy was reinstituted and continued for 100 days. Thereafter, on MRI studies of the brain, all lesions and perivascular enhancement have disapperared, and no new lesions have developed to date (Table 2). The antiepileptic therapy has been discontinued, and no new seizures have occurred.

Case 3:
In the summer of 1993, this previously healthy 11-year-old girl had visited an area in Southern Finland where Lyme borreliosis is endemic. In September 1993, occasional episodes of hyperactivity followed by headache were observed by her family. On October 1, she developed paresis of the right lowe limb. On October 7, she was admitted to a local hospital and 1 week later to the Oulu University Central Hospital. Standing on the right leg alone was difficult, and walking was slightly impaired. On October 13, CT of the brain showed a periventricular low density enhancing lesion. 10x6 mm^2 in diameter, and located in the left parietal lobe white matter. The lesion was suggestive of a neoplasm. On the next day, using MRI, the dimensions of the enhancing lesion were found to be 40x20x8 mm^3 and the surrounding oedematous area was 20-30 mm thick (fig. 4A). The EMG was normal. Abdominal ultrasonography showed mild splenomegaly. On October 22, a craniotomy was carried out. In the area of the enhancing lesion, shown by MRI, elastic and stretchy tissue with abnormal white colour was detected. On histological examination, focal necrotic areas were found, surrounded by foamy macrophages, reactive astrocytes and oedema. (Fig 4B). An increased number of small vessels with thickened walls and prominent endothelial cells were also seen. Lymphocytes occurred in the walls of some vessels.
Haemoglobin, ESR and serum C-reactive protein values were normal. Serum total immunoglobulins were normal, except for a slightly increased value of IgM at 1.94 g/l (normal value 0.35-1.63 g/l). Serum rheumatoid factor, antinuclear antibodies extractable nuclear antigens, anti-DNA and anti-phospholipid antibodies were negative, and so were antibodies against several viruses. On November 4, lumbar puncture was carried out. The CSF specimen gave a negative virus culture as HSV PCR, **but B. burgdorferi PCR was positive with two separate CSF specimens (detected at two separate runs).**
December 21, 1993, antibiotic therapy with ceftriaxone (2 g i.v. daily) was started for 4 weeks followed by therapy with oral amoxicillin (500 mg every 8 h) combined with oral probenecid (500 mg every 8 h). On February 1, the antibiotic therapy was stopped because of bloody diarrhoea. Culture and toxn detection for Clostridium difficile were negative. The diarrhoe was cured with oral metronidazole.
On Februray 1, 1994, MRI of the brain showed reduction of abnormal tissue around the operative area. At this time, no enhancement was seen in the walls of the cavity. At a follow-up of 1 year, recovery was observed witho only a slight abnormality in walking. No new symptoms developed.

Priem S, Burmester GR, Kamradt T, Wolbart K, Rittig MG, Krause A
Detection of Borrelia burgdorferi by polymerase chain reaction in synovial membrane, but not in synovial fluid from patients with persisting Lyme arthritis after antibiotic therapy.
Ann Rheum Dis **1998 Feb**;57(2):118-21
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=9613343&dopt=Abstract

Case 1: 50y M, **acute arthritis left knee. High Lyme IgG.** Doxycycline 200mg/d and diclofenac, and **intraarticular dexamethasone. Arthritis persisted.**
B. burgdorferi DNA in SF, Ceftriaxone 2g/d, 2 weeks. **Arthritis improved significantly but recurred after about six weeks.** Treated with ceftriaxone again. Although SF PCR became negative, gonarthritis persisted. A popliteal cyst developed and a bursitis of the left elbow occurred. An **arthroscopic synovectomy** of the left knee and a bursectomy were performed. Ceftriaxone 2g for 28d, doxycyclin2 200 mg for 30 d.

Case 2: 51y F, **2 month history of bilateral gonarthritis, remembered tickbite and EM-like lesion at the right thigh years ago. IgG positive ELISA & Western Blot** and **B. burgdorferi PCR in SF positive.** Doxycycline 200mg/d for 35d without effect. Ceftriaxone 2g/d for 3 wekks w only moderate success. Two mo after treatement gonarthritis persisted. An **arthrocentesis and synovial biopsy** was performed.

Case 3: 28y F, **subtotal arthroscopic synovectomy of the right knee had been performed elsewhere because of a gonarthritis with pronounced synovial proliferation… persisted for 9 months.** … **positive Lyme serology both IgM & IgG**. Ceftriaxone 2g 14 d. treatment discontinued at day 11 because of an allergic rash. Gonarthritis persisted. **PCR positive in SF** doxycycline 200mg/d for 30d. In addition corticosteroid intraarticularly. However only a temporay improvement of the arthritis was seen … **Arthroscopy … SF and SM samples.**

Case 4: 43y F, **bilateral gonarthritis 7 mo**, **ELISA & Western Blot high IgG**, **PCR for B. burgdorferi in SF and urine positive.** Ceftriaxone 2g 14d without improvement. A few weeks later the patient was seen in another hospital with an acute polyarthritis involving both wrists and several metacarpophalangeal joints and a deterioration of the right gonarthritis. Doxycycline 200mg/d 30d and prednisolone 10-20 mg/d. Again there was no sufficient response … **A closed needle arthrocentesis with synovial biopsy of the right knee was performed.**

Patients were evaluated 8 to 10 weeks after antibiotic therapy. All still seropositive and had active arthritis. Urine samples were collected and within one week SF and SM specimens were obtained ... **In none of the urine or SF samples could B. burgdorferi DNA be detected, in contrast SM samples was positive.**
Patients 1,2,4: cefotaxime 2g x3 3 weeks, followed by six weeks oral doxycycline or minocycline 200mg/d. Pt. 3: imipenem 1.0g x3 for 2 weeks, doxycycline 200mg six weeks. In all four patients arthritis completely subsided within 4-6 mo and did not recur at a median observation period of 18 mo.
No mentioning of any extraarticular symptoms.

Meier P, Blatz R, Gau M, Spencker FB, Wiedemann P
[Pars plana vitrectomy in Borrelia burgdorferi endophthalmitis][German]
Klin Monatsbl Augenheilkd **1998 Dec**;213(6):351-4
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=10048013&dopt=Abstract

BACKGROUND: Ocular manifestations of Lyme borreliose present with unusual forms of conjunctivitis, keratitis, optic nerve disease, uveitis, vitritis and rarely endophthalmitis.

CASE REPORT: A 57-year-old man working as logger in Sax-ony-Anhalt suffering from an **endophthalmitis** on his left eye was referred to us. The vision of his left eye was intact light perception and hand motions. The slit-lamp examination revealed severe inflammation of the anterior chamber with hypopyon, posterior synechiae, and opacity of the posterior lens capsule. Funduscopy showed no red reflex, no retinal details. **In the local hospital serum analysis was performed and showed in Western-Blot IgM- and IgG-antibodies against Borrelia burgdorferi. Despite of intravenous application of ceftriaxon for 14 days panuveitis persisted, and endophthalmitis developed when antibiotic therapy was finished.**
RESULTS: During pars plana vitrectomy a sharply delineated cystic lesion containing yellowish fluid was revealed, and creamy yellow fluid was aspirated. **Microscopically in hematoxylineosin stained slides of the aspirate structures consistent with Borrelia burgdorferi were found.** Postoperatively vision increased to 1/15. Despite of a second intravenous ceftriaxon treatment for 14 days we observed a retinal vasculitis in the follow up of 6 months.
CONCLUSIONS: **Despite intravenous ceftriaxon-therapy borrelia burgdorferi must have survived in the vitreous body. Further investigations are required with respect to the use of other antibiotics or immunosuppressives.**

Oksi J, Marjamaki M, Nikoskelainen J, Viljanen MK
Borrelia burgdorferi detected by culture and PCR in clinical relapse of disseminated Lyme borreliosis.
Ann Med 1999 Jun;31(3):225-32
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=99369287&dopt=Abstract
http://www.annmed.org/duo/annmed.check_login?fname=1999_31_3_225-32.html

A total of **165 patients** with disseminated Lyme borreliosis (diagnosed in 1990-94, all seropositive except one culture-positive patient) were followed after antibiotic treatment .....

32/165 = **19,4% had clinical relapse after more than 3 months antibiotic treatment for borreliosis.**
In **13/32 (40,6%) could the relapse be verified by either positive PCR (12) and/or positive culture (3) for B. burgdorferi.**
2/104 (1,9%) of the asymptomatic had positive PCR. These were not treated and didn't have sign of relapse since, according to personal communication (120900) with Oksi.
At time of proven relapse 6/13 (46%) were seronegative! (12/13 were seropositive at initial diagnosis, i.e. 5 pts. developed seronegativity despite proven persistency !!)
5/13 (38%) had circulating immunecomplexes, of these 3 were seronegative.

1 patient (10) was seronegative throughout the whole course of illness despite both positive culture and PCR in CSF and positive biopsy and plasma PCR at relapse ! This patient had been treated with ceftriaxone IV 2g for 3 weeks, followed by 24 weeks of doxycycline 100 g bid and amoxicillin 1 week - a total of 28 weeks (6-7 months).
1 patient (8) had been treated for as long as 47 weeks (11 months) including 7 weeks of intravenous ceftriaxone - primary diagnosis was confirmed by positive biopsy and the relapse 44 weeks after treatment confirmed by a positive plasma PCR.
1 patient (2) had relapse 130 weeks after 1. treatment, that had lasted 16 weeks. Pt. was seropositive initially (both IgM and IgG), but seronegative at relapse, relapse confirmed by positive PCR, no history of reinfection in the meantime.

Hulinska D, Votypka J, Valesova M
Persistence of Borrelia garinii and Borrelia afzelii in patients with Lyme arthritis.
Int J Med Microbiol Virol Parasitol Infect Dis **1999 Jul;**289(3):301-18
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=10467661&dopt=Abstract

We repeatedly detected DNA of Borrelia garinii or B. afzelii and Borrelia-like structures in the blood, joint fluid or in the synovium of 10 patients with Lyme arthritis by means of the polymerase chain reaction and immunoelectron microscopy at 2-4-month intervals in the course of two years.

All samples were analyzed using primers which amplified the 16S rRNA gene sequence of Borrelia burgdorferi sensu lato and nucleotide sequences for the OspA gene. No cross hybridization occurred with DNA from human cells and with DNA from other bacteria. **Capture and labelling with monoclonal antibodies of aggregated antigens, membranes and flagellae were evident in the blood of 7 patients, in 4 synovial membranes and 2 synovial fluids. Borreliae were found in blood capillaries, in collagen and in clusters surrounding inflammatory cells in the synovium of patients with recurrent infections who carried IgM and IgG antibodies to OspA and to 83 kDa core protein.**
After significant improvement for several weeks after treatment, arthritis recurred in six patients. Synoviocyte hyperplasia, inflammatory infiltration and concentric adventitial fibroplasia were seen in the synovium of the patients with persisting borreliae. Only two patients were infected with B. afzelii, the others with B. garinii.

Albert S, Schulze J, Riegel H, Brade V
Lyme arthritis in a 12-year-old patient after a latency period of 5 years.
Infection 1999;27(4-5):286-8
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=10885847&dopt=Abstract

Lyme arthritis (LA) may be confused with other rheumatic diseases, particularly in the absence of a history of erythema migrans (EM). We report the case of a 12-year-old patient who developed a large effusion of the right knee joint. The titer for antinuclear antibodies was 1:80 and the test for rheumatoid factor was negative. **Investigations for antibody response to Borrelia burgdorferi demonstrated remarkable elevation of IgG antibody and no specific IgM response.These results were confirmed by immunoblotting reactivity with the bands p83/100, p58, p43, p41, p39, OspA, p30, OspC, p21, and p17. We subsequently learned that the child had suffered a tick bite followed by an EM 5 years earlier and had been treated with trimethoprim/sulfamethoxazole at that time. The patient now was given intravenous ceftriaxone, 2 g daily for 14 days. In the absence of clinical improvement 3 weeks later a knee joint aspiration was performed which resulted in a positive polymerase chain reaction (PCR) test for B. burgdorferi DNA (OspA) in the synovial fluid.**The patient fully recovered 2 months later without further treatment. The case indicates that the latency period between EM and onset of LA may last up to 5 years. In addition to serologic test methods, analysis of synovial fluid using PCR may be decisive for making the final diagnosis of LA.

Honegr K, Hulinska D, Dostal V, Gebousky P, Hankova E, Horacek J, Vyslouzil L, Havlasova J.
[Persistence of Borrelia burgdorferi sensu lato in patients with Lyme borreliosis][Czech]
Epidemiol Mikrobiol Imunol 2001 Feb;50(1):10-16
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=11233667&dopt=Abstract

In **18 patients** with Lyme borreliosis the authors **proved the persistence of Borrelia burgdorferi sensu lato by detection of the causal agent by immune electron microscopy or of its DNA by PCR in plasma or cerebrospinal fluid after an interval of 4-68 months.** Clinical manifestations common in Lyme borreliosis were present in only half the patients, in the remainder non-specific symptoms were found. **In nine subjects with confirmed Borrelia burgdorferi sensu lato in the cerebrospinal fluid the cytological and biochemical finding was normal. Examination of antibodies by the ELISA method was negative in 7 of 18 patients during the first examination and in 12 of 18 during the second examination. In all negative examinations the specific antibodies were assessed by the Western blot or ELISA method after liberation from the immunocomplexes.** In the authors' opinion it is advisable to examine repeatedly plasma and other biological material from potentially affected organs by PCR and subjects with persisting or relapsing complaints after the acute form of Lyme borreliosis as well as to examine cerebrospinal fluid in case on non-specific symptoms and concurrent pathic EEG or MR findings.

back - European cases - American cases - Animal studies
American cases
Kirsch M, Ruben FL, Steere AC, Duray PH, Norden CW, Winkelstein A
Fatal adult respiratory distress syndrome in a patient with Lyme disease.
JAMA **1988** May 13; 259(18): 2737-9
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=88188323&dopt=Abstract

Case
A 67-year-old woman who lived on a farm near Pittsburgh first noted lethargy, weakness, lower-extremity stiffness, and myalgias on May 16, 1986, one week after a weekend trip to the Maryland shore. Soon thereafter, a dry cough, swelling of her hands, fever, anorexia, and a rash on her extremities and trunk developed. Although she had no known history of a tick bite, a presumptive diagnosis of Rocky Mountain spotted fever was made, and a two-week course of tetracycline hydrochloride, 250 mg orally four times a day, was prescribed.
Because of persistent symptoms, she was referred to Montefiore Hospital in Pittsburgh. Examination showed a maculopapular eruption on her trunk and hands. Although antibody titers for Rocky Mountain spotted fever were negative, both the IgM and IgG antibody titers in response to the Lyme disease spirochete *Borrelia burgdorferi* were markedly elevated (Table), as determined by enzyme-linked immunosorbent assay.2 She completed her tetracycline course without improvement in her condition.
She was admitted to the hospital on June 17, 1986. Her temperature was 16.7°C. Liver function test results were abnormal

(lactic dehydrogenase [LDH], 419 U/L; aspartate aminotransferase [ASAT], 639 U/L; alanine aminotransferase [ALAT], 418 U/L; and alkaline phosphatase, 223 U/L), but results of the remainder of the admission studies, including a serum VDRL test, chest roentgenogram, and electrocardiogram, were unremarkable. A skin biopsy specimen revealed nonspecific inflammatory changes. Because of the lack of response to tetracycline, she was given a ten-day course of intravenous penicillin G potassium, 5 million U every six hours. Her fevers resolved, and her rash, joint complaints, and liver enzyme abnormalities improved (LDH, 338 U/L; ASAT, 184 U/L; ALAT, 111 U/L; and alkaline phosphatase, 198 U/L). She was discharged on June 28.

At home, her rash worsened, and periorbital edema developed. Her constitutional symptoms persisted, and she was readmitted to the hospital five days later. Examination revealed an oral temperature of 38.1°C, a pulse rate of 112 beats per minute, and respirations of 20/min. In addition to the maculopapular rash and periorbital edema, there was moderate swelling and tenderness over the carpal and interphalangeal joints. There was no muscle tenderness or pedal edema, and the lungs were clear. The white blood cell count was 6.0 x 10^9/L (6.0 x 10^3/mm^3); the erythrocyte sedimentation rate (Wintrobe) was 47 mm/h; and liver and muscle enzyme concentrations were elevated (LDH, 508 U/L; ASAT, 429 U/L; ALAT, 162 U/L; alkaline phosphatase, 286 U/L; and creatine kinase, 279 U/L). A chest roentgenogram showed atelectasis at the left base (Fig 1). The level of immune complexes was shown to be elevated by the C1q binding assay result of 36% (norms), <13%) and by the Raji cell assay result of 315 mg of aggregated human gamma globulin equivalents per liter (normal, <50 mg of aggregated human gamma globulin equivalents per liter). Serum cryoglobulins were absent. Antinuclear antibody was present in low titer (1:10), and rheumatoid factor was positive (1:40). The level of the C3 component of complement was mildly decreased, at 0.85 g/L (35 g/dl) (normal range, 1.15 to 1.85 g/L [115 to 185 mg/dl]). Titers for hepatitis A, hepatitis B, cytomegalovirus, Epstein-Barr virus, *Trichinella*, *Legionella pneumophila*, and febrile agglutinins were negative. The patient was given a second course of intravenous penicillin C potassium, 5 million U every six hours, and enteric coated aspirin 650 mg orally every four hours.

Initially, her rash, edema, and arthralgias improved, but her marked lethargy and dry cough persisted. On her tenth day in the hospital, bibasilar rales were noted, and a chest roentgenogram demonstrated new findings of bibasilar atelectasis with bilateral peripheral infiltrates. Prednisone therapy, 20 mg orally three times a day, was begun. While her rash and edema improved, her liver and muscle enzyme abnormalities worsened. An abdominal computed tomographic scan showed a nonhomogeneous liver and was otherwise unremarkable.

Four days later, her oral temperature rose to 38.9°C, and her respirations were 34/mm. Increased rales were present bilaterally. An arterial blood gas test done while the patient was breathing 35% 0_2 showed the following values: Arterial oxygen pressure, 68 mm Hg; arterial carbon dioxide pressure, 27 mm Hg; and pH, 7.55. The white blood cell count was 12.4 x 10^9/L (12.4 10 10^3/mm^3), with 0.04 (4%) band forms. A chest roentgenogram showed diffuse, bilateral, patchy infiltrates (Fig 1). Blood and urine cultures showed no growth. Lyme disease antibody titers were still markedly elevated (Table). Immunoblotting demonstrated IgG antibodies against at least 14 polypeptides of *B burgdorferi*, including the 31-kilodalton outer-membrane component. Nafcillin and tobramycin therapy was started empirically.

On the following day, during bronchoalveolar lavage, the patient became tachypnoic and cyanotic and underwent intubation. A chest roentgenogram now showed bilateral central infiltrates. The patient was given intravenous trimethoprim with sulfamethoxazole, and nafcillin therapy was discontinued. She became increasingly more hypoxemic, requiring a positive end-expiratory pressure of 15 cm H_2O and a fraction of inspired oxygen in the range of 0.8 to 0.9. On her 19th day in the hospital, an open-lung biopsy specimen revealed severe, acute, diffuse alveolar damage, compatible with the adult respiratory distress syndrome (ARDS). There was no evidence of tumor, infection, or vasculitis. She became progressively more hypoxemic and hypotensive, and she died six days later.

Pathologic Findings

The cause of death was diffuse alveolar damage of the lungs (ARDS). Additional findings were cardiomegaly (370 g with borderline right ventricular hypertrophy (0.3 cm), fatty liver (1600 g), and acute tubular necrosis of the kidneys. **Lymph nodes showed a transformed lymphocytic response, and, when Dieterle silver stain was used,[3] spirochetes compatible with *B burgdorferi* infection were demonstrated (Fig 2).** Lung tissue was submitted for virus isolation and was found to be negative for influenza A and B, parainfluenza, respiratory syncytial virus, adenovirus, and coxoackievirus. Antibodies to *Leptospira canicola* and *Leptospira icterohaemorrhoragiae* antibodies were present at less than 1:8 by complement fixation testing and were not detectable by direct agglutination testing using serum from July 21, 1986, three days before the patient died. [**Anti-borrelia-antibodies were present, both IgM and IgG, in four samples taken over 6 weeks, IgM showed increasing, while IgG titer showed falling tendency on the last sample**].

Comment

After the first description of Lyme disease in 1975, the clinical spectrum was expanded to include cardiac and neurologic features.[4] This disorder is now recognized to be a multisystemic disease that triggers a complex immune response to a spirochetal infection.

The patient described herein presented with cough, fever, a diffuse rash, and myositis, and she also had abnormal liver function test results. Her hospital course was essentially characterized by progressive respiratory failure and fatal ARDS. Levels of IgM and IgG antibodies were markedly increased in response to *B burgdorferi*, the agent that causes Lyme disease. This level of titers has been shown to exhibit cross-reactivity in patients with syphilis or relapsing fever. We

excluded syphilis with a nonreactive VDRL test and relapsing fever with immunoblotting studies. Antibodies were demonstrated to 14 polypeptides of *B burgdorferi*, including the 31-kilodalton polypeptide, a pattern thought to be specific for Lyme disease.[5] In addition, lymph node sections examined after the patients death demonstrated spirochetes morphologically compatible with *B burgdorferi*. **We are confident that the serologic and histologic evidence described supports the diagnosis of Lyme disease.**

The clinical features of our patient were, however, highly atypical for Lyme disease. Her rash was generalized and prominent on her hands. This distribution has not previously been reported in Lyme disease and contrasts with erythema chronicum migrans, the unique clinical marker for this disorder.[6] Although mild hepatitis has been described,[6] markedly abnormal liver enzyme levels, myositis, and edema of the hands and face are not characteristic of Lyme disease. Most important was her progressive and fatal respiratory failure. The only respiratory symptom described in human Lyme disease is a nonproductive cough, although spirochetes have been demonstrated in the lungs of experimentally infected hamsters.[7] Since it is recognized that ARDS follows a wide variety of predisposing conditions we believe that Lyme disease triggered this fatal complication.

How can we explain our patients variant clinical syndrome? This case is atypical in its presentation, course, response to therapy, and outcome. **This patient received a course tetracycline followed by two courses of high-dose intravenous penicillin, without improvement.** Both of these antibiotic regimens are considered to be established antispirochetal therapy for Lyme disease. This suggests that the spirochete was particularly virulent, that the host defenses were impaired, or that the disease state no longer solely depended on live spirochetes for its expression. We do not believe that our patient was immunocompromised. The mechanism by which *B burgdorferi* causes Lyme disease is still under study. It is unclear whether certain manifestations of Lyme disease require a live spirochete for continued disease activity or whether they result from immune-mediated mechanisms.[1] **Although spirochetes were identified in lymph nodes, the transformed lymphocytic response, the presence of circulating immune complexes and the progression of disease despite antibiotic treatment indicate an immune-mediated disease.**

Lyme disease has become a prevalent and serious infection. In addition to chronic arthritis,[8] this disease has been shown to be the cause of fatal myocarditis,[9] panophthalmitis leading to blindness,[10] fetal death,[11] and central nervous system syndromes suggestive of demyelination.[12]To these complications we add respiratory failure and ARDS, which are refractory to known therapy. Although Lyme disease is endemic in the Northeast, the Midwest, and the Far West of the United States, it has been reported throughout the country.[13]

With an incubation period that ranged from three to 12 days[6] and no documented tick bite, we are uncertain whether our patient contracted the disease in Maryland or in western Pennsylvania.

We urge physicians throughout the United States to consider the multisystemic features of Lyme disease and to recognize its lethal potential.

Schoen RT, Aversa JM, Rahn DW, Steere AC
Treatment of refractory chronic Lyme arthritis with arthroscopic synovectomy.
Arthritis Rheum **1991 Aug**; 34(8): 1056-60
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=1859481&dopt=Abstract

All seropositive …

18/20 patients received oral or i.v. antibiotic treatment most both, but treatment drug, dose and duration not specified ...
[thus 2/20 (10%) did not receive any antibiotic treatment (?), which makes the 'refractory' a bit inappropiate].
Synovia from 12/20 [60%] patients have been analyzed in detail and were similar to 12 rheumatoid synovia used for comparison … moderate-to-marked synovial cell hyperplasia, vascular proliferation, and mononuclear cell infiltration, sometimes with pseudolymphoid follicles ..
In 6/12 patients w/ Lyme arthritis, but in none of those w/ rheumatoid arthritis, **a few spirochetes and globular antigen deposits were seen in and around blood vessels in areas of lymphocytic infiltration** [i.e. **6/20 (30%) had proven persistent infection in the joint.** The paper doesn't tell if 2 of these patients were those that didn't receive antibiotics, but at least 4(-6) obviously had persistent infection despite previous antibiotic treatment.]
80% had resolution of joint inflammation .. and they have not had recurrences during a followup period of 3-8 y …
15% of these patients all of whom were more disabled preoperatively, had no further synovitis, but still had mild functional limitations at long-term followup.
Only [!!!] 20% of the patients had persisitent or recurrent synovitis .. [is synovectomy a certain cure for a persistent systemic infection?]
Authors say they did not find symptoms of extraarticular Lyme borreliosis ... but 1 had facial palsy ….
And the postoperative assessment only includes joint examination not a neurological examination … a (rheumatological) focus problem ??
Attempts to do culture or PCR were not done.

Liegner KB, Rosenkilde CE, Campbell GL, Guam TJ, Dennis DT
Culture-confirmed Treatment Failure of Cefotaxime and Minocycline in a Case of Lyme Meningoencephalomyelitis in the US.
1992 V Int Conf Lb abs #63

In 1987,. 37-year-old woman living in Westchester County, NY, developed spastic paraparesis, bilateral Babinski reflexes, and cranial nerve and bulbar dysfunction characterized by dysphagia, dysphonia. diplopia, absent gag reflex, and dysfunction of bowel and bladder control. CSF contained 19 WBC/mm (86% lymphs). **A test for antibodies to Borrelia burgdorferi (Bb) in serum was negative.** No etiology was established despite an extensive workup. Symptoms and signs reportedly worsened gradually from 1988 to present. There was a past history of splenectomy for idiopathic thrombocytopenic purpura diagnosed in 1975. In 1989, the right frontal region and right basal ganglia were abnormal on brain MRI. In January 1990 CSF contained 6 WBC/mm3 (93% lymphs), but no oligoclonal bands or myelin basic protein. **Paired CSF and serum tests for antibodies to Bb and PCR for Bb-specific oligonucleotides in CSF were negative.** An empiric 21-day course of cefotaxime (3g/l2 hr i.v.) was given in January, 1990 with no clear clinical benefit. Following treatment, CSF contained 9 WBC/mm3 (93% lymphs). Four months of minocycline (200 mg/day p.o.) begun in November, 1990 also yielded no clear clinical benefit. **In December, 1990 a T-cell stimulation test with Bb antigens was strongly positive**. In December, 1991 CSF contained 6 WBC/mm3 (89% lymphs) and elevated IgG. **Paired serum and CSF samples were strongly positive for antibodies to Bb, with a CSF-to-serum index of 1.04. Culture of this CSF specimen in BSK-II yielded a strain of Bb.** Culture--confirmed treatment failures have been previously reported for three Lyme neuroborreliosis cases in Europe. The present case apparently is the first of this type to be reported from the United States.

Drulle J, Eiras E
Persistence of Borrelia burgdorferi Antigens in Patients receiving Long-Term Antibiotic Therapy.
1992 V Int Conf Lb abs#70E

25 Patients, previously diagnosed with Lyme Disease and treated with long-term antibiotic therapy provided samples of urine, spinal fluid, synovial fluid, breast milk or tears. All but 3 of the patients were symptomatic at the time of the testing. Samples were tested by employing a polyclonal antibody tagged with colloidal gold which is specific to an 83 kilodalton vescicular protein, also known as a “bleb” and thought to be specific to Borrelia burgdorferi. **In 13 patients antigen was detected [13/25 = 52%].** Persistence of antigen may be related to persisting infection or may represent lengthy clearance of dead spirochetal debris.

Fraser DD, Kong LI, Miller FW
Molecular detection of persistent Borrelia burgdorferi in a man with dermatomyositis.
Clin Exp Rheumatol **1992 Jul-Aug**; 10(4): 387-90
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=1395222&dopt=Abstract

A 40-year-old white man with a several year history of various immunologic disorders, including anti-Jo-1 autoantibody positive dermatomyositis, developed clinical Lyme disease after being biten by a tick. **The patient was treated with oral tetracycline and his initial symptoms resolved;** however, he suffered an exacerbation of his muscle disease which was difficult to control despite cytotoxic therapy. **Antibiotic therapy was reinstituted after Borrelia burgdorferi was detected in the patient's peripheral blood leukocytes by the polymerase chain reaction (PCR). All serologic, T-cell stimulation, and western blot analyses, however, were negative.** The patient's disease responded to oral ampicillin, probenecid therapy and concurrent cytotoxic therapy. Subsequent leukocyte PCR testing has been negative for the causative agent of Lyme disease. This case may provide an example of the in vivo immuno-modulatory effects of spirochetes in human autoimmune disease. In addition, this case emphasizes the potential clinical utility of PCR technology in evaluating the persistent seronegative Lyme disease which may occur in immunocompromised individuals.

Reimers CD, de Koning J, Neubert U, Preac Mursic V, Koster JG, Muller Felber W, Pongratz DE, Duray PH
Borrelia burgdorferi myositis: report of eight patients.
J Neurol **1993 May**; 240(5): 278-83
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=93316101&dopt=Abstract

8 patients w histologic proven myositis of which on was seronegative - one fatal case
5/8 had normal CK and none (2) or slight abnormality in other laboratory findings (3 had ESR in range 26-38) see table 2
2 had normal EMG
Patients 4 & 6 were culture positive in skin but not in muscle biopsies
Treated 10-14 days, patient 1 had persistent fatique, patient 8 died from cardiac arrest caused by B. burgdorferi myocarditis, myocardial inflammation at autopsy, where spirochetes were demonstrated.

Battafarano DF, Combs JA, Enzenauer RJ, Fitzpatrick JE
Chronic septic arthritis caused by Borrelia burgdorferi.
Clin Orthop **1993 Dec**(297): 238-41
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=8242938&dopt=Abstract

Chronic arthritis occurs in 10% of Lyme disease patients. **A patient had chronic septic Lyme arthritis of the knee for seven years despite multiple antibiotic trials and multiple arthroscopic and open synovectomies. Spirochetes were documented in synovium and synovial fluid (SF). Polymerase chain reaction (PCR) analysis of the SF was consistent with Borrelia infection.** Persistent infection should be excluded with silver stains and cultures in any patient with chronic monoarticular arthritis and a history of Lyme disease.

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 [see comments]
N Engl J Med **1994 Jan** 27; 330(4): 229-34
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=94097367&dopt=Abstract

BACKGROUND. Borrelia burgdorferi is difficult to detect in synovial fluid, which limits our understanding of the pathogenesis of Lyme arthritis, particularly when arthritis persists despite antibiotic therapy.
METHODS. Using the polymerase chain reaction (PCR), we attempted to detect B. burgdorferi DNA in joint-fluid samples obtained over a 17-year period. The samples were tested in two separate laboratories with four sets of primers and probes, three of which target plasmid DNA that encodes outer-surface protein A (OspA).
RESULTS. B. burgdorferi DNA was detected in 75 of 88 patients with Lyme arthritis (85 percent) and in none of 64 control patients. Each of the three OspA primer-probe sets was sensitive, and the results were moderately concordant in the two laboratories (kappa = 0.54 to 0.73). **Of 73 patients with Lyme arthritis that was untreated or treated with only short courses of oral antibiotics, 70 (96 percent) had positive PCR results. In contrast, of 19 patients who received either parenteral antibiotics or long courses of oral antibiotics (> or = 1 month), only 7 (37 percent) had positive tests (P < 0.001).** None of these seven patients had received more than two months of oral antibiotic treatment or more than three weeks of intravenous antibiotic treatment. Of 10 patients with chronic arthritis (continuous joint inflammation for one year or more) despite multiple courses of antibiotics, 7 had consistently negative tests in samples obtained three months to two years after treatment.
CONCLUSIONS. PCR testing can detect B. burgdorferi DNA in synovial fluid. This test may be able to show whether Lyme arthritis that persists after antibiotic treatment is due to persistence of the spirochete.

Patmas MA
Persistence of Borrelia burgdorferi despite antibiotic treatment.
J Spiro Tick Diseases **1994**; 1:101
http://www.medscape.com/SLACK/JSTD/1994/v01.n04/std0104.06.patm/pnt-std0104.06.patm.html

To the Editor:
It has been suggested that Lyme disease may trigger fibromyalgia and that antibiotic therapy beyond 30 days is almost always unnecessary [1]. Recently, two cases demonstrating persistence of Borrelia burgdorferi despite lengthy antibiotic treatment were noted.

Case Number 1:
In October 1991, a 35-year-old Caucasian female, registered nurse, was referred for evaluation. She had reported a lesion compatible with erythema chronicum migrans about one year earlier. After a short course of oral antibiotics, she noted fatigue, myalgia, and arthralgias and was given 2 weeks of intravenous ceftriaxone 1 g daily with resolution of her symptoms. Over the next several months, however, her symptoms gradually returned. **An ELISA titer was elevated**, and she was started on ceftriaxone 2 g intravenously daily. After 10 days, the patient developed a vigorous Jarisch-Herxheimer reaction and was referred to the author. The patient was switched to cefotaxime 3 g intravenously every 12 hours with improvement in symptoms. After 6 weeks, the intravenous cefotaxime was changed to oral clarithromycin 500 mg daily for 6 more weeks with complete resolution of all signs and symptoms. One week later, the patient discovered that she was 1 month pregnant and, after a normal gestation, delivered a healthy male infant. **The placenta was examined at Brigham and Women's Hospital in Boston, Massachusetts, where several spirochetes were noted in perivascular and intervillous spaces on modified Dieterle silver stain.**

Case Number 2:
A 47-year-old Caucasian female was well until an untreated tick bite in 1985. She subsequently developed a progressive arthritis diagnosed as rheumatoid. After failing treatment with nonsteroidal anti-inflammatories and remittive agents, the author saw the patient for the first time in l990. **Aspiration of fluid from the right knee was positive by specific antibody ratio for Lyme disease** at the University of Medicine and Dentistry of New Jersey-Robert Wood Johnson University Hospital Lyme Disease Research Center. The patient was started on ceftriaxone 2 g intravenously daily for 4 weeks. She had a significant objective response to treatment but quickly relapsed after it was discontinued. A second 4-week course of ceftriaxone was given with only moderate improvement. The patient then sought treatment at several university centers, where she received experimental treatment for rheumatoid arthritis including monoclonal antibody therapy. There was no improvement in her condition. **By July 1992, the patient developed bilateral aseptic necrosis of the hips. A right total hip replacement was performed and histopathologic examination revealed several spirochetes on modified Dieterle silver stain of synovial tissue performed at the Brigham and Women's Hospital.** The patient was then started on continuous oral antibiotic treatment with azithromycin 250 mg daily. **Approximately 6 months later, the patient underwent left total knee replacement and once again spirochete-like structures were observed in synovial tissue on modified Dieterle silver stain.**

These two cases suggest that despite lengthy courses of both intravenous and oral antibiotics, Borrelia burgdorferi may persist. The presumption that residual symptoms are due to fibromyalgia may not always be true and is not assured simply be-

cause a patient has received 30 days of treatment. Careful histopathologic examination by modified Dieterle silver stain may suggest otherwise.

Keszler K, Tilton RC
Persistent PCR Positivity in a Patient Being Treated for Lyme Disease.
J Spiro Tick Diseases **1995**; 2:57-58
http://www.medscape.com/SLACK/JSTD/1995/v02.n03/std0203.05.kesz/pnt-std0203.05.kesz.html

Before contracting a present illness, 30-year-old white female occupational therapist was healthy and active. She bicycled regularly in a Lyme endemic area. **In early July 1994** she had 3 days of flu-like symptoms with a temperature of 101[ring]F for three consecutive nights. At the end of August 1994, she developed fatigue, and could not ride her bicycle as long as she was used to. She did not recall either a tick bite or a rash. By the beginning of September 1994, she had trouble concentrating, experienced short-term memory problems, and was increasingly fatigued. She had bilateral knee pain without redness or swelling. She noted a lot of "crunching" in the joints. She went to see her primary physician. Tests for infectious mononucleosis and rheumatoid arthritis were all negative. Because she lived in an area endemic for Lyme disease and spent much time outdoors, the physician performed a **Lyme disease antibody test in October 1994. The test was positive,** and she was started on oral doxycycline, 100 mg b.i.d. for 30 days. Her symptoms persisted and antibiotic treatment was extended for a total of 3 months. At the end of this period, she felt better but reported that she was not "normal." Her physician felt that additional treatment was unnecessary. In March 1995, the patient complained of recurrent frontal headaches, vertigo, shooting pains in her right ear, neck stiffness, pain near the paravertebral area of the upper thoracic spine, arthralgia, paresthesia of the right hand, and weakness in her thigh muscles. She felt heaviness in her chest and exertional dyspnea climbing a flight of stairs. She had memory problems, difficulty concentrating, and irritability when referred.
Her past medical history and physical examination were unremarkable. **Lyme antibody tests were repeated at North American Clinical Laboratories. The IgG ELISA titer was 1:160, and the IgM < 1: 160. The IgG test was interpreted as equivocal, and the IgM as nonreactive. The IgG western blot showed 50,41,23 Kda bands. The IgM blot showed a 31 Kda band. Both western blots were interpreted as equivocal. A PCR was done on whole blood (N.A.C.L.) and was positive.** The PCR on whole blood utilized a 20 kb primer, which is a protein of the 350 kb Osp A sequence. Positive hybridization controls (HLA), DQ alpha negative controls, and inhibition controls were used in each PCR run. Amplified products were detected by both southern blotting and a nonradioisotopic DNA capture technique. Patient was restarted on oral doxycycline 100 mg b.i.d. The patient continued to have the same symptoms with **exacerbations once a week while on the oral doxycycline. The PCR test on whole blood was repeated 1 month later while on doxycycline. It was again positive for Bb.** After the second positive DNA-PCR test result, the patient was switched to intravenous Ceftriaxone 2 gm q.d. for 4 weeks. At the end of 21/2 weeks, she developed an allergic rash, and the I.V. therapy was discontinued. As of this writing, she is being continued on oral Biaxin (500 mg b.i.d.). The patient has improved significantly and is 95% better.

Lawrence C, Lipton RB, Lowy FD, Coyle PK
Seronegative chronic relapsing neuroborreliosis [see comments]
Eur Neurol **1995**; 35(2): 113-7
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=7796837&dopt=Abstract

We report an unusual patient with evidence of Borrelia burgdorferi infection who experienced repeated neurologic relapses despite aggressive antibiotic therapy. Each course of therapy was associated with a Jarisch-Herxheimer-like reaction. Although **the patient never had detectable free antibodies to B. burgdorferi in serum or spinal fluid, the CSF was positive on multiple occasions for complexed anti-B. burgdorferi antibodies, B. burgdorferi nucleic acids and free antigen.**

Cimperman J, Strle F, Maraspin V, Lotric S, Ruzic Sabljic E, Picken RN
Repeated Isolation of Borrelia burgdorferi from the Cerebrospinal Fluid of Two Patients Treated for Lyme Neuroborreliosis.
1996 VII Int Conf Lb abs #D657 University of California, Berkeley, p 181.

The clinical diagnosis of neuroborreliosis (NB) remains challenging and the efficacy of antibiotic therapy in its treatment is uncertain. We present the case histories of two patients with NB who had unusual clinical presentations and failed to respond to conventional antibiotic therapy.

Patient #1 was a 20 year old woman who presented with lymphocytic meningitis. **No antibodies against Borrelia burgdorferi (BB) were detectable in her serum and cerebrospinal fluid (CSF).** Subsequently, **1.5 months later, BB was cultured from the CSF** and the patient was treated with ceftriaxone (2g/day for 14 days). The patient was asymptomatic for 3.5 months; thereafter, she developed a right lumboischialgia. Analgesic therapy was unsuccessful. **The CSF showed increased proteins (0.98 g/L), but again no antibodies against BB were detectable in serum and CSF. However, BB was once again cultured from the CSF.** The patient was treated again with ceftriaxone and subsequently recovered. Currently, the patient is lost for follow-up.

Patient #2 was a 51 year old female who developed erythema migrans (EM) following a tick bite. At that time she received no antibiotic treatment. Two months later she developed multiple neurologic symptoms including headache, vertigo, nausea,

left nervus trigeminus paresthesia, memory/ concentration disorders and depression, as well as myalgia, arthralgia, and ocular symptoms. **Low titres of IgM antibodies against BB were detected in the serum;** serologic tests for neurotropic viruses and syphilis were negative. **Routine CSF tests were normal; no anti-BB antibodies were detectable.** VEP were abnormal, while CT, MRI of the brain and EEG were all normal. **BB was cultured from the CSF 9 months after the initial presentation with EM.** The patient was treated with ceftriaxone (2g/day) for 14 days. **Two months after treatment, BB was again isolated from the CSF.** Four months after the initial therapy, the symptoms persisted and, in addition, monoarthritis developed. Antibiotic therapy was repeated and included ceftriaxone (2g/day) for 21 days. The patient improved approximately one month after completion of the latter therapy.

The above results demonstrate that: (1) negative serology does not exclude diagnosis of NB, (2) BB can persist in the CSF following antibiotic treatment, (3) standard ceftriaxone therapy (2g/day for 14 days) may not be sufficient for the treatment of NB

Manak MM, González-Villaseñor LI,Crush-Stanton S, Tilton RC
Use of PCR assays to monitor the clearance of Borrelia burgdorferi DNA from blood following antibiotic therapy
J Spiro Tick Diseases **1997**; 4:11-20
http://www.medscape.com/SLACK/JSTD/1997/v04.n01/jst0401.03.mana/pnt-jst0401.03.mana.html

Seven of 21 (33%) early Lyme disease patients and 10 of 20 (50%) late-stage patients not on antibiotic therapy at the time of enrollment were PCR positive.
All seven PCR-positive early Lyme disease patients and 8 of the 10 PCR-positive late-stage patients became PCR negative within 2 weeks of antibiotic treatment.
Of an additional eight late-stage patients who had been on therapy for more than 2 months at the time of enrollment, three were PCR positive. All five late-stage patients who remained PCR positive while under therapy (two from the newly treated group and three from the previously treated group) became PCR negative when alternative therapy was administered. **[table 2. Two of the patients with 'Late Lyme Disease Before Therapy' were seronegative before treatment, one PCR-negative seroconverted, while one PCR-positive remained seronegative.]**

This report demonstrates several phenomena:
1. **Despite aggressive IV and oral antimicrobial treatment, B. burgdorferi may persist in sequestered areas of the body such as bone marrow**
2. The yield on PCR testing for Lyme disease may be enhanced by testing bone marrow specimens or, by extrapolation, testing leucocytes, ie, buffy coat, where the organism may reside intracellularly protected from immune defenses. The bone marrow provides a specimen resource rich in nucleated cells, which may harbor the spirochete in dormant or active form. We clearly do not advocate this invasive testing for all patients but rather those who are refractory to standard therapy and have concomitant immunologic or hematologic abnormalities.
3. There does not appear to be a clear correlation between reactive Lyme serology and PCR positivity, although one patient had a persistently positive IgG and IgM Western Blot and the other a positive ELISA test.

In conclusion, these cases reports suggest that bone marrow may be an important site for detection of B. burgdorferi DNA.

Fein L, Tilton R
Bone Marrow as a Source for Borrelia burgdorferi DNA.
J Spiro Tick Diseases **1997**; 4:58-60
http://www.medscape.com/SLACK/JSTD/1997/v04.n03/jst0403.03.fein/pnt-jst0403.03.fein.html

Case 1: 51y F, lupus-like disease NSAID. Frequent infections, joint pains, headaches and fatigue. Plaquenil plus intermittent antibiotics. Steroids. ANA 1:40. Biopsy of mediastinal glands: noncaseating granulomas, atypical for sarcoidosis. Progressive improvement ... Jan 1993 influenzalike illness ass w severe arthritis and neck stiffness. Biaxin (clarithromycin) and suprax (cefixime) for **presumptive seronegative Lyme disease**. Initial flare … progressively worse … march 1993 i.v. claforan (cefotaxime). ANA repeatedly negative., experienced multiple JHR requiring adjustment of dose. After 2 months on i.v. antimicrobials her condition improved. She had no additional antimicrobials until March 1994 when she developed gradually recurring symptoms. ANA's negative or weak. **ELISA reactive, but not confirmed by Western Blot.** She was maintained on zithromax (azithromycin) and plaquenil. Immunological studies normal except for IgG and lambda monoclonal gammopathy. **In september bone marrow biopsy was performed. PCR for B. burgdorferi was reactive.** IM bicillin 1.2 mu and oral zithromax 250mg x2. The patient was maintained on the above regimen until clinical remission with progressive improvement of all her symptoms.

Case 2: 59y F seen April 1994 after being treated by another physician for Lyme disease. Multiple influenza-like illnesses in 1992 through 1993 and was treated with short courses of oral antibiotics. She went to Spain in 1993. This trip was followed by the onset of severe pain and weakness. ….**IgM Western Blot was reactive**. …. IV Claforan (cefotaxime) for 8 weeks. She initially felt better but relapsed. Intravenous ampicillin was administered for 1 mo followed by claforan 9g/d for 2 weeks which was reduced to 6g/d. By May 1994, there was lack of response … IV Vancomycin. The patient responded well but re-

lapsed when vancomycin was discontimued…. Restarted Vancomycin June 1994 and responded well. Therapy was continued through July followed by pulse vancomycin and bicillin LA. By august 1994 she was very symptomatic with fevers and arthritis. IV vancomycin was stopped and plaquenil startedin addition to IM bicillin. The patient was noted to have hypogammaglobulinaemia and anemia and was treated with IV gammaglobulin and transfusions. The patient did well until October 1994 when both symptoms and anemia recurred. **The consulting hematologist performed a bone marrow biopsy that was unremarkable except for a positive PCR (B. burgdorferi).** By November 1994 she had recurrent fevers and arthritis. Her anemia is stable on Epogen (epoetin alpha) and her strength is improving on Plaquenil. She has had two gammaglobulin transfusions for recurrent hypogammaglobulinaemia. **Throughout this time, both her IgG and IgM Western Blot have remained consistently positive.** The patient is now reporting she is feeling better.

Liegner KB, Duray P, Agricola M, Rosenkilde C, Yannuzzi LA, Ziska M, Tilton RC, Hulinska D, Hubbard J, Fallon BA
Lyme Disease and the clinical Spectrum of Antibiotics responsive Chronic Meningoencephalomyelitides
J Spiro Tick Diseases **1997**; 4:61-73
http://www.medscape.com/SLACK/JSTD/1997/v04.n03/jst0403.04.lieg/pnt-jst0403.04.lieg.html

Intensive study of four patients with chronic meningoencephalomyelitis believed due to Lyme disease revealed seronegativity and/or variable seroreactivity and chronic persistent infection as common threads. Evaluation of these complex cases required determined study over time using all known methods (i.e., culture isolation, histologic, immunohistochemical, electron micrographic, direct antigen detection as well as standard serologic methods) on tissues as well as serial study of blood, cerebrospinal fluid (CSF) and urine.

Prolonged intravenous antibiotic therapy conferred clinical benefit in each case and withholding of treatment resulted in clinical deterioration.
These 4 cases demonstrate:
1. **Long term seronegativity for borrelia despite longterm symptomatology consistent with borreliosis**
2. Repeatedly antibiotic responsive while relapses occurred when treatment was paused or decreased
3. Difficulty in discriminating chronic active Lyme borreliosis from MS (case 4) and lupus (case 1).
4. Severe worsening whenever steroid treatment is used in chronic active Lyme borreliosis.
5. **Severe debilitating illness from borreliosis and death following vascular hemorrhage**

Case #1
A 39-year-old woman with a **two-year history of progressing spastic quadraparesis, cranial nerve palsies, and persistent unexplained CSF pleocytosis was evaluated beginning in 1989.** She had been diagnosed with idiopathic thrombocytopenic purpura (ITP) in 1975 and underwent splenectomy in 1976. She had lived in northern Westchester county, New York and northern California but gave no history of tick attachments or of erythema migrans. No diagnosis was established after a year of observation and testing, and **serologic studies for Lyme disease in serum and CSF were repeatedly negative.** CSF examination in 1990 showed lymphocytic pleocytosis, elevated IgG, and absence of oligoclonal bands or myelin basic protein. Anticardiolipin and antinuclear antibodies were present and Raji cell assay and C1Q immune complexes were elevated. HIV and HTLV-1 antibodies were negative.
An empiric trial of intravenous antibiotic treatment with cefotaxime (CFOTX) for 21 days in April 1990 resulted in no clinical improvement and no change in CSF pleocytosis. Thereafter she was treated with 4 months of minocycline with no clinical benefit. The patient remained wheelchair-bound.
B. burgdorferi grew from CSF in December 1991 at which time the patient first became seropositive despite at least 4 years of clinical illness. She was treated with CFOTX (4 g IV Q 8 hrs once weekly) with complete resolution of pleocytosis after 13 weeks and constitutional symptoms improved. Despite continuation of once weekly IV therapy for 10 months, there was gradual neurologic deterioration. Intravenous antibiotics were discontinued December 1992.
Methylprednisolone sodium succinate was given intravenously, 1 g daily for 5 days, followed by prednisone over a six-week period for the possibility of systemic lupus erythematosus. Pleural effusions developed within one week of starting steroids along with severe encephalopathy and debilitation. She could not remember conversations held minutes earlier and was unable to hold a cup, roll over in bed, or transfer from bed to wheelchair. Computed axial tomography of the chest revealed pleuropericardial effusions (Fig 1). A pleuropericardial window was created for diagnostic and therapeutic purposes. **Fibrinous pericarditis was present with infiltration of plasma cells and macrophages and spirochete-compatible structures were seen with modified Steiner silver and phycoerythrin stains, as well as a touch preparation (Figs 2-5).** Intravenous CFOTX 6 g daily was administered for the next 3fi months with dramatic improvement of her encephalopathy. The pleuropericardial effusions improved (Fig 6). The patient was able to walk 500 feet with a rolling walker and was able to go home. A further 3 months of daily CFOTX was administered but the patient's health insurer refused authorization for any subsequent intravenous antibiotic therapy. The patient became increasingly encephalopathic over the next 6 months. Daily intravenous CFOTX was reinstituted in June 1994 and mental status improved as confirmed by serial neuropsychological testing before and after 4 months of treatment. **Several specimens of plasma and urine between February and July of 1995 were found to be PCR positive for B. burdorferi-specific DNA.** From July 1995 through April 1996 the patient was treated with intramuscular benzathine penicillin. On this treatment she felt poorly, encephalopathy worsened, and she lost the ability to ambulate. **Plasma PCR for B. burdorferi-specific DNA was again positive February 1996.** CSF analysis March 1996

showed 14 lymphocytes/mm3, elevated protein (57 mg %) and slight elevation of IgG. Oligoclonal bands were present in both CSF and serum. Myelin basic protein was absent. **CSF Lyme PCR and OspA antigen were negative as were Lyme-specific immune complexes in serum and CSF.** Authorization for additional intravenous antibiotic therapy was refused by the insurer. Encephalopathy and debilitation worsened (Table I).

Case #2
In the fall of 1985 a 61-year-old outdoorsman residing in the Catskill region of New York State **developed a large round rash on one thigh. A physician was consulted but no treatment was given. The following winter unrelenting headache, low grade fever, paresthesias and truncal instability developed**. Lumbar puncture demonstrated lymphocytic pleocytosis. **Lyme ELISA was negative.** Dysphasia and a progressive stroke syndrome developed. A diagnosis of "vasculitis" was given and the patient was treated with steroids and cyclophosphamide for a number of months with **progressive deterioration to a level of functioning slightly above a persistent vegetative state. Lyme ELISA was positive in 1988.** Treatment with intramuscular ceftriaxone (CFTRX) for 14 days resulted in slight improvement. In 1992, computed axial tomography of the brain showed massive hydrocephalus (Fig 7). Electroencephalogram revealed status epilepticus and phenobarbital was prescribed. **Lyme serology was negative in one laboratory, yet positive in another. Western blot was nondiagnostic, showing only a 41 kiloDalton band.** CSF examination revealed the presence of oligoclonal bands without myelin basic protein and very elevated CSF IgG. Serum showed elevated C1Q immune complexes. OspA antigen capture assay in CSF was strongly positive. The patient was given daily intravenous CFTRX for one month, then weekly CFOTX (4 g IV Q 8 hr x 3 doses) for one year, with modest improvement in his neurologic status. **The patient succumbed to his disease July 1993. Autopsy revealed severe hydrocephalus (Figs 8,9) and florid meningoencephalomyelitis and ependymitis (Figs 10-13). The CSF was positive for OspA antigen and Lyme-specific immune complexes.** Spirochetes were not visualized on histopathologic and immunohistochemical study by light microscopy but **borrelia-compatible structures were visualized in formalin-fixed tissues studied by electron microscopy** (Figs 14-16) and **brain tissue and dura mater were PCR positive for detection of B. burgdorferi-specific oligonucleotides** (Figs 17A,B)[7] (Table II).

About case 3 & 4 from discussion (for full story see above link):

Case 3 had a clear clinical history indicating Lyme disease. Despite intensive study laboratory corroboration for the diagnosis could not be obtained for some 13 years. A prior six-week course of intravenous CFTRX did not prevent the development of meningoencephalomyelitis. An eight-month course of intravenous CFOTX was required to resolve disturbed CSF parameters. Lyme-specific immune complexes were demonstrable in the final three of five cerebrospinal fluid examinations and key Lyme disease-compatible bands finally developed on Western blot in serum thereafter. **She has been seronegative by ELISA throughout, calling into serious question the validity of using this assay alone as a screening test.**

Case 4 demonstrates how closely neuroborreliosis can mimic multiple sclerosis. Given now that seronegativity occurs in Lyme disease, distinguishing the two disorders may be a daunting task. **The patient showed resolution of markers thought to be pathognomonic for multiple sclerosis in CSF along with clinical improvement following intensive intravenous antibiotic treatment. Relapse of abnormal CSF findings and of neurologic signs occurred with suspension of intensive treatment. Resolution again followed a second course of intravenous therapy. This case suggests that neuroborreliosis may be misdiagnosed as multiple sclerosis.**[18,19]

Phillips SE, Mattman LH, Hulinska D, Moayad H
A proposal for the reliable culture of Borrelia burgdorferi from patients with chronic Lyme disease, even from those previously aggressively treated.
Infection **1998 Nov-Dec**;26(6):364-7
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=9861561&dopt=Abstract

Since culture of Borrelia burgdorferi from patients with chronic Lyme disease has been an extraordinarily rare event, clarification of the nature of the illness and proving its etiology as infectious have been difficult. A method for reliably and reproducibly culturing B. burgdorferi from the blood of patients with chronic Lyme disease was therefore sought by making a **controlled blood culture trial studying 47 patients with chronic Lyme disease. All had relapsed after long-term oral and intravenous antibiotics.** 23 patients with other chronic illness formed the control group. **Positive cultures were confirmed by fluorescent antibody immuno-electron microscopy using monoclonal antibody directed against Osp A, and Osp A PCR.**
43/47 patients (91%) cultured positive. 23/23 controls (100%) cultured negative. Although persistent infection has been, to date, strongly suggested in chronic Lyme disease by positive PCR and antigen capture, there are major problems with these tests. This new method for culturing B. burgdorferi from patients with chronic Lyme disease certainly defines the nature of the illness and establishes that it is of chronic infectious etiology. This discovery should help to reestablish the gold standard in laboratory diagnosis of Lyme disease.

Concerns regarding this paper:
Unanswered question 'Why does the culture medium contain Detroit tap water?'. Does tap water contains some minerals needed by Bb?

back - European cases - American cases - Animal studies

Animal studies

Pachner AR, Schaefer H, Amemiya K, Cadavid D, Zhang WF, Reddy K, O'Neill T
Pathogenesis of neuroborreliosis--lessons from a monkey model.
Wien Klin Wochenschr **1998 Dec** 23;110(24):870-3
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=10048168&dopt=Abstract

The diagnosis of human LNB can be difficult, because its major clinical manifestations--meningitis, facial palsy, radiculitis, and neuritis--are non-specific and the characteristic skin lesion is usually absent at the time of neurological involvement. Thus, CSF assays are often used in diagnosis. Culture of CSF is rarely performed because it has a low yield and requires special culture medium. PCR of the CSF identified spirochetal DNA in clinical specimens with greater sensitivity, but it suffers from a number of disadvantages. Measurement of specific antibody in the CSF also has its limitations. The role of available assays for LNB has not been studied carefully in a comparative investigation. The recent development of the nonhumane primate (NHP) model of LNB allows us to address this need in a faithful model of human LNB. We compared PCR and culture in their ability to detect spirochetal presence in the CSF and brain tissue of infected NHPs, and related these measures of infection to the development of anti-B. burgdorferi antibody. We also tested a bioassay, the mouse infectivity test (MIT), in this model. **Using these four assays (PCR, culture, MIT, and CSF Ab) at least one assay for spirochetal presence in CSFs from NHPs was positive in 87% of CSFs tested during early infection in the CNS. Detection of spirochetal presence by PCR, MIT, and culture in the CSF was inversely related to the concomitant presence of anti-B. burgdorferi antibody intrathecally.** The performance of any particular test was associated with the strength of the host immune response. **In early CNS infection, when anti-B. burgdorferi antibody had not yet appeared, or in immunocompromised hosts, the MIT compared favorably to culture and PCR in infected NHPs; antibody in the CSF was the most useful assay in immunocompetent NHPs.** This is the first study demonstrating that a bioassay using inoculation of mice, the mouse infectivity test (MIT), has potential as a useful adjunct in the diagnosis of LNB. The MIT for LNB was modeled after the rabbit infectivity test or RIT, which is considered the "gold standard" for the diagnosis of the related CNS infection, neurosyphilis, and felt to be very sensitive and specific. The presence of specific anti-B. burgdorferi antibody in the CSF is the most widely used assay for Lyme neuroborreliosis. **In the immunocompetent NHPs in our study it was a very successful assay for detection of CNS invasion. However, it is frequently false-negative, especially early in the course of the infection, or if there is transient immunosuppression. Transient suppression of the anti-B. burgdorferi immune response in the human could occur in instances of co-infection, i.e. simultaneous transmission via the tick of another pathogen other than B. burgdorferi.** Thus, mild immunosuppression as accomplished in our NHPs with corticosteroids was designed to mimic conditions in the human host which allow B. burgdorferi in the natural state to gain a firm foothold in the central nervous system in the 10-15% of B. burgdorferi-infected patients who develop clinically symptomatic nervous system disease. **This study is the first to compare utility of available diagnostic techniques in LNB in which necropsy proved presence of infection in the CNS. None of the assays was ideal for all conditions, and the utility of the assay was associated with the host immune status. The differences in the responses of immunocompromised and immunocompetent NHPs in this study were striking.** In immunocompetent NHPs the window of opportunity for CNS invasion prior to the development of CSF antibody was brief, and the chance of detection of spirochete low by any of the three techniques used (i.e. culture, PCR, or MIT); in this group, measurement of CSF antibody was generally diagnostic. **In immunocompromised NHPs, intrathecal antibody production was delayed, and this helpful diagnostic assay was false-negative; diagnosis required more labor-intensive assays such as PCR, culture, and MIT during weeks 3.5 to 9.5 after infection.** It is likely that had the experiment been allowed to proceed longer in the immunosuppressed NHPs, antibody would have eventually been produced intrathecally.
The clinical relevance of the data on comparison of diagnostic assays is clear. The appearance of anti-B. burgdorferi antibody in CSF may be delayed especially when there is interference with the anti-B. burgdorferi immune response. In these circumstances, or for a short time early in CNS invasion in immunocompetent individuals, the measurement of anti-B. burgdorferi antibody in CSF may be negative; under these circumstances the likelyhood of detecting spirochete by PCR, culture, or MIT is at its highest. Conversely, detecting spirochetal presence by clture, PCR, or MIT will be least likely to be successful when anti-B. burgdorferi antibody is present.
(the last part of abstract was truncated in Medline but added here)

Straubinger RK, Summers BA, Chang YF, Appel MJ
Persistence of Borrelia burgdorferi in experimentally infected dogs after antibiotic treatment.
J Clin Microbiol **1997 Jan**; 35(1): 111-6
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=8968890&dopt=Abstract

In specific-pathogen-free dogs experimentally infected with Borrelia burgdorferi by tick exposure, **treatment with high doses of amoxicillin or doxycycline for 30 days diminished but failed to eliminate persistent infection.** Although joint

disease was prevented or cured in five of five amoxicillin- and five of six doxycycline-treated dogs, **skin punch biopsies and multiple tissues from necropsy samples remained PCR positive and B. burgdorferi was isolated from one amoxicillin- and two doxycycline-treated dogs following antibiotic treatment.** In contrast, B. burgdorferi was isolated from six of six untreated infected control dogs and joint lesions were found in four of these six dogs. **Serum antibody levels to B. burgdorferi in all dogs declined after antibiotic treatment. Negative antibody levels were reached in four of six doxycycline- and four of six amoxicillin-treated dogs. However, in dogs that were kept in isolation for 6 months after antibiotic treatment was discontinued, antibody levels began to rise again, presumably in response to proliferation of the surviving pool of spirochetes.** Antibody levels in untreated infected control dogs remained high.

Straubinger RK, Straubinger AF, Jacobson RH, Chang YF, Summers BA, Erb HM, Appel MJG
Two lessons from the canine model of Lyme disease: Migration of Borrelia burgdorferi in tissues and persistence after antibiotic treatment
J Spiro Tick Diseases 1997; 4: 24-31.
http://www.medscape.com/SLACK/JSTD/1997/v04.n01/jst0401.05.stra/pnt-jst0401.05.stra.html

..... Tissues closest to the infection site harbored spirochetes more frequently than did more distant tissues. Of all tested tissues taken from the front quadrant (synovium, lymph node, fascia, and muscle) that contained the site of inoculation, 75% were culture positive. In the opposite front quarter, 60% of the tissues were positive for B. burgdorferi, tissues from the hind quarters showed 26% and 16% culture positivity when they originated from the side of exposure or from the opposite side, respectively. The development of severe arthritis with clinically evi-dent lameness was associated with the side of infection. Of 70 tick-infected and lame dogs, 80% developed the first episode of acute arthritis in joints closest to the tick bites after a median incubation of 68 days. Ten percent of the dogs showed clinically evident lameness in the limb of the opposite front quadrant after 121 days, 8.6% in the ipsilateral hind quadrant after 103 days, and 1.4% in the opposite hind quadrant after 123 days posttick exposure. We have shown that **in untreated dogs, B. burgdorferi can persist in connective tissue for at least a year and perhaps for life. In two studies, antibiotic treatment with amoxicillin or doxycycline for 30 days failed to eliminate persistent infection in 11 dogs. Immediately after treatment, borreliae could not be demonstrated, antibody levels declined, and joint lesions were prevented or cured. Live spirochetes, however, persisted in the tissue of at least three dogs as B. burgdorferi DNA was detected in all 11 treated dogs for up to 6 months after treatment, at which time antibody levels again began to rise.** Additional therapeutic studies using intravenously administered ceftriaxone or oral azithromycin are underway in an attempt to identify a successful treatment regime.

Straubinger RK, Straubinger AF, Summers BA, Jacobson RH, Erb HN
Clinical manifestations, pathogenesis, and effect of antibiotic treatment on Lyme borreliosis in dogs.
Wien Klin Wochenschr **1998 Dec** 23;110(24):874-81
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&list_uids=10048169&dopt=Abstract

BACKGROUND: Borrelia burgdorferi, the causative agent of Lyme disease, infects humans and animals. In humans, the disease primarily affects the skin, large joints, and the nervous system days to months after infection. Data generated with appropriate animal model help to understand the fundamental mechanisms of the disease.
OBJECTIVE:
1) More clearly define the clinical manifestation and pathogenetic mechanisms of Lyme disease in dogs;
2) evaluate the effect of antibiotics in dogs infected with B. burgdorferi;
3) describe the effects of corticosteroids on dogs persistently infected with B. burgdorferi.
DESIGN: Specific-pathogen-free beagles were infected with B. burgdorferi using ticks collected in an endemic Lyme disease area. Clinical signs were recorded daily. Antibody titers were measured by ELISA at two-week intervals. B. burgdorferi organisms were detected in tissues by culture and PCR. Synovial fluids were evaluated microscopically and with a chemotaxis cell migration assay. Histological sections were examined for pathological lesions. Specific cytokine up-regulation in tissues was detected by RT-PCR.
INTERVENTIONS:
In three separate experiments, B. burgdorferi-infected dogs received antibiotic treatment (amoxicillin; azithromycin; ceftriaxone; doxycycline) for 30 consecutive days. Two subclinical persistently infected dogs received oral prednisone for 14 consecutive days starting at day 420 post-infection.
RESULTS: Dogs developed acute arthritis in the joints closest to the tick bites after a median incubation period of 68 days. Synovial membranes of lame and non-lame dogs produced the chemokine IL-8 in response to B. burgdorferi. **Antibiotic treatment prevented or resolved episodes of acute arthritis, but failed to eliminate the bacterium from infected dogs. Corticosteroid treatment reactivated Lyme disease in persistently infected dogs, which had not received antibiotics previously.**
CONCLUSIONS: **B. burgdorferi disseminates through tissue by migration following tick inoculation, produces episodes of acute arthritis, and establishes persistent infection. The spirochete survives antibiotic treatment and disease can be reactivated in immunosuppressed animals.**

Mit Fragen wenden Sie sich bitte an Kroun@Ulmar.dk oder an borreliose.shv.berlin@t-online.de