* This article documents the available evidence supporting both the existence of Chronic Lyme Disease, as well as the persistence of the infection despite antibiotic therapy. The abstracts are available on U.S. Government's Public Medical Database [pubmed.gov]

The Case For Chronic Infection: Evidential persistence of Borrelia species post antibiotic exposure in vivo and in vitro.

Michael D. Parent

Introduction Summary:

There is an abundance of evidence demonstrating that Borrelia Burgdorferi, the causative agent of Lyme Disease, and related pathogenic species, can persist within specific body tissues and cells of various mammals despite adequate antibiotic therapy: ponies [93.5, 111.5], non-human primates [50, 86-87], dogs [65.5, 70, 80, 81, 82, 84], mice [44, 62, 88, 100, 107, 108, 110, 114], and humans [all others]. There is also abundant evidence that Borrelia Burgdorferi has evolved in a manner similar to other bacteria that evade the immune system via pleomorphic modification, in other words, the bacteria can change its shape beyond the conventional spirochetal form [45, 55, 61, 64, 90, 105, 109, 113, 116, 117]. L-forms, and cystic Borrelia have been identified in a number of studies [45, 68, 77, 87, 105, 109, 112, 113, 117]. When these "forms" are exposed to the typical antibiotics, such as Penicillin family antibiotics or Doxycycline, they are unaffected. When the antibiotic is removed from the environment, the bacterium will alter its form once more, morphing back into a spiral form, allowing ongoing mobility [45, 68, 87, 90, 105, 109].

I have taken the time to "bold" the conclusions and various other aspects that clearly indicate a deviation from the point of view given by a number of physicians and researchers who deny the possibility of ongoing chronic infection within the human host. The current guidelines issued by the Infectious Disease Society Of America (IDSA) are consistently used to dismiss further discussion regarding the subject of persistence. The guidelines are titled: “The Clinical Assessment, Treatment, and Prevention of Lyme Disease, Human Granulocytic Anaplasmosis, and Babesiosis” Clinical Infectious Diseases 2006; 43:1089–134.

Patients who receive a diagnosis of Lyme Disease, either based on clinical observation and/or objective indicators often improve with antibiotic therapy [1, 4, 18, 19, 26, 33, 66]. However, if they have been undiagnosed and untreated for Lyme Disease for a long period of time, it often takes longer courses of antibiotics beyond those currently recommended to see progress in symptom reduction [15, 66, 73, 93, 105, 118]. The U.S. National Institute Of Health funded a number of randomized double-blind placebo-controlled trials (RCT) regarding the long term treatment of Lyme Disease. However, these RCT's were 3 months in duration or less. Patients with documented medical records indicating Chronic Lyme Disease or a Lyme-Like

Illness who have been untreated often do not see meaningful improvement until after 4-6 months of treatment[118], and even still, the improvements are modest initially in many patients and may require an ongoing open ended treatment regimen with antibiotics [66, 93].

It is well understood and agreed upon universally that the more time Borrelia Burdorferi has had to disseminate into various ligaments, bones, collagen, muscles, and other tissues, then the higher the probability of ongoing complications or symptoms post-antibiotic therapy. Presently, studies indicate that antibiotics can not access many of the areas that Borrelia Burgdorferi disseminates to unless the bacterium itself leaves the safe haven of a Fibroblast skin cell [11, 22, 23, 24, 25, 29, 35, 52, 64, 70, 72, 80, 81, 84, 94], or synovial tissue cells and fluid [1, 7, 9, 31, 34, 37, 42, 60, 61, 69, 70, 71, 102].

Introductory Conclusion:

Therefore, we have studies demonstrating abundant persistence. We have National Institute Of Health funded studies that do not treat patients long enough to confirm whether the treatment really is effective or not. The short term studies we do have contradict other studies as well as those based on clinical reports from health care providers treating these patients with antibiotics beyond the currently accepted time frame[118]. It is unwise for the IDSA to claim that long-term antibiotic therapy doesn't work when you've only performed a study for 3 months, when the vast majority of the patients in the study have had the infection for many years and require at least 3-6 months of oral antibiotic before clinical improvements are seen. IV antibiotics may demonstrate minor to moderate symptomatic improvement after 1- 3 months, but if that treatment is only given for 3 months and then discontinued, then it will be equally ineffective and the symptoms will return to pre-treatment levels. Coincidentally, that's exactly what happened in Dr. Brian Fallon's study. Some symptoms improved, but then returned upon discontinuing therapy.

I have discussed merely one specific possibility for the failure of patients to thrive and improve during the currently available randomized double-blind placebo-controlled clinical trials (RCT). Dr. Daniel J. Cameron writes in the Journal Of Medical Hypothesis that a number of limitations exist within the currently structured (RCTs), that strongly support the position I've laid forth. Med Hypotheses. 2009 Jun;72(6):688-91. Epub 2009 Mar 5. Insufficient evidence to deny antibiotic treatment to chronic Lyme disease patients. First Medical Associates, Medicine, 175 Main Street, Mount Kisco, NY 10549, USA. Cameron@LymeProject.com

"**Evidence for the hypothesis:** There are eight limitations that support the hypothesis: (1) the power of the evidence is inadequate to draw definite conclusions, (2) the evidence is too heterogeneous to make strong recommendations, (3) the risk to an individual of facing a long-term debilitating illness has not been considered, (4) the risk to society of a growing chronically ill population has not been considered, (5) treatment delay has not been considered as

a confounder, (6) co-infections have not been considered as a confounder, (7) the design of RCTs did not address the range of treatment options in an actual practice, and (8) the findings cannot be generalized to actual practice. **Implications of the hypothesis:** This hypothesis suggests that physicians should consider the limitations of the evidence before denying antibiotic treatment for Chronic Lyme Disease (CLD). Physicians who deny antibiotic treatment to CLD patients might inform their patients that there are some clinicians who disagree with that position, and then offer to refer them for a second opinion to a doctor who could potentially present a different point of view. The hypothesis also suggests that health care insurers should consider the limitations of the evidence before adopting policies that routinely deny antibiotic treatment for CLD patients and should expand coverage of CLD to include clinical discretion for specific clinical situations."

There is more than enough information to justify at least a neutral position in respect to whether Borrelia Burgdorferi and related infectious species persist in human beings despite the Infectious Disease Society Of America's recommendations. Due to this uncertainty, treating physicians can not conclusively deny that persistence in human beings may be more problematic than assumed.

The scientific studies available on Lyme Disease contradict each other to a significant degree. Many study authors state in no uncertain terms that the discussion of Lyme Disease is a closed case. I disagree. The evidence disagrees. The Chief Medical Officer in the United Kingdom echoed the sentiments of the IDSA in 2009 stating: "There is no biological evidence of symptomatic chronic Lyme disease amongst those who have received the recommended treatment regimen." - CMO, Autum 2009, Issue 49, pg. 4. The IDSA states: "To date, there is no convincing biologic evidence for the existence of symptomatic chronic B. burgdorferi infection among patients after receipt of recommended treatment regimens for Lyme disease." - Clin Infect Dis 2006 Nov 1;43(9):1089-134

Skepticism is the heart of science. Cynicism is the death of reason.

The following studies are organized by year, page, and study title within the Study table index.

Study Table Index:

Year	Page	Study Title
1988	21	N Engl J Med. 1988 Dec 1;319,22:1441-6. Comment in: N Engl J Med. 1989 May 11;320,19:1279-80.**Seronegative Lyme disease. Dissociation of specific T- and B-lymphocyte responses to Borrelia burgdorferi.** Dattwyler RJ, Volkman DJ, Luft BJ, Halperin JJ, Thomas J, Golightly MG.
1989	21	Am J Clin Pathol. 1989 Jan;91,1:95 7. **Spirochetes in the spleen of a patient with chronic Lyme disease.** Cimmino MA, Azzolini A, Tobia F, Pesce CM Istituto Scientifico di Medicina Interna, Università di Genova, Italy.
1989	22	Conn Med. 1989 Jun;53,6:335-7. **Treatment of Lyme disease.** Schoen RT.
1989	22	Infection. 1989 Jul-Aug;17,4:216-7. **High-dose intravenous penicillin G does not prevent further progression in early neurological manifestation of Lyme borreliosis.** Kohler J, Schneider H, Vogt A.
1989	22	Dtsch Med Wochenschr. 1989 Oct 20;114,42:1602-6. **Neuro-borreliosis or intervertebral disk prolapse?** [Article in German] Dieterle L, Kubina FG, Staudacher T, Büdingen HJ.
1989	23	Infection. 1989 Nov-Dec;17,6:355-9.**Survival of Borrelia burgdorferi in antibiotically treated patients with Lyme borreliosis.** Preac-Mursic V, Weber K, Pfister HW, Wilske B, Gross B, Baumann A, Prokop J. Neurologische Klinik Grosshadern, München, FR Germany.
1990	23	Acta Trop. 1990 Dec;48, 2:89-94.**Clinical implications of delayed growth of the Lyme borreliosis spirochete, Borrelia burgdorferi.** MacDonald AB, Berger BW, Schwan TG. Department of Pathology, Southampton Hospital, New York 11968.
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1991	24	1991: Journal of Infectious Diseases, Feb;163,2:311-8 **Randomized comparison of ceftriaxone and cefotaxime in Lyme neuroborreliosis.** Pfister HW, Preac-Mursic V, Wilske B, Schielke E, SÃrgel F, EinhÃ¤upl KM.

Year	Page	Study Title
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1991	26	Arthritis Rheum. 1991 Aug;34,8:1056-60. **Treatment of refractory chronic Lyme arthritis with arthroscopic synovectomy.** Schoen RT, Aversa JM, Rahn DW, Steere AC.
1992	26	Clin Exp Rheumatol. 1992 Jul-Aug;10,4:387-90. **Molecular detection of persistent Borrelia burgdorferi in a man with dermatomyositis.** Fraser DD, Kong LI, Miller FW.
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1993	27	J Am Acad Dermatol. 1993 Feb;28,2 Pt 2:312-4. **Recurrent erythema migrans despite extended antibiotic treatment with minocycline in a patient with persisting Borrelia burgdorferi infection.** Liegner KB, Shapiro JR, Ramsay D, Halperin AJ, Hogrefe W, Kong L.
1993	27	J Infect Dis. 1993 May;167,5:1074-81.**Invasion of human skin fibroblasts by the Lyme disease spirochete, Borrelia burgdorferi.** Klempner MS, Noring R, Rogers RA.
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Year	Page	Study Title
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1993	30	J Clin Neuroophthalmol. 1993 Sep;13,3:155-61; discussion 162. 59: **First isolation of Borrelia burgdorferi from an iris biopsy.** Preac-Mursic V, Pfister HW, Spiegel H, Burk R, Wilske B, Reinhardt S, Böhmer R.
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1994	33	J Clin Microbiol. 1994 Mar;32,3:715-20.**Isolation of Borrelia burgdorferi from biopsy specimens taken from healthy-looking skin of patients with Lyme borreliosis.** Kuiper H, van Dam AP, Spanjaard L, de Jongh BM, Widjojokusumo A, Ramselaar TC, Cairo I, Vos K, Dankert J. Department of Medical Microbiology, Academic Medical Centre, University Hospital, University of Amsterdam, The Netherlands.
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Year	Page	Study Title
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1995	41	Eur Neurol. 1995;35,2:113-7. Comment in: Eur Neurol. 1996;36,6:394-5. **Seronegative chronic relapsing neuroborreliosis.**Lawrence C, Lipton RB, Lowy FD, Coyle PK.
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Year	Page	Study Title
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Year	Page	Study Title
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Year	Page	Study Title
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Year	Page	Study Title
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2007	69	Pol Merkur Lekarski. 2007 Apr;22,130:275-9. Related Articles, **Concentrations of pro-inflammatory cytokines IFN-gamma, IL-6, IL-12 and IL-15 in serum and cerebrospinal fluid in patients with neuroborreliosis undergoing antibiotic treatment.** Article in Polish. Pancewicz SA, Kondrusik M, Zajkowska J, Grygorczuk S. Akademia Medyczna w Bialymstoku, Klinika ChorÃ³b Zakaznych i Neuroinfekcji.20spancewicz@interia.pl
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2008	75	Antimicrobial Agents and Chemotherapy, May 2008, p. 1728-1736, Vol. 52, No. 50066-4804 **Persistence of Borrelia burgdorferi following Antibiotic Treatment in Mice** Emir Hodzic, Sunlian Feng, Kevin Holden, Kimberly J. Freet, and Stephen W. Barthold*
2008	76	Pol Arch Med Wewn. 2008 May;118 5:314-7. : Neuroborreliosis with extrapyramidal symptoms: a case report. Biesiada G, Czapiel J, Sobczyk-Krupiarz I, Garlicki A, Mach T. Department of Infectious Diseases, Division of Gastroenterology, Hepatology, and Infectious Diseases, Jagiellonian University School of Medicine, Kraków, Poland. gbiesiada@op.pl
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2008	77	Microb Pathog. 2008 Sep 20. **Borrelia burgdorferi expression of the bba64, bba65, bba66, and bba73 genes in tissues during persistent infection in mice.** Gilmore RD Jr, Howison RR, Schmit VL, Carroll JA.
2008	78	Med Hypotheses. 2008;70,5:967-74. Epub 2007 Nov 5. **The association between tick-borne infections, Lyme borreliosis and autism spectrum disorders.** Bransfield RC, Wulfman JS, Harvey WT, Usman AI.

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2008	79	Journal of Veterinary Diagnostic Investigation Vol. 20 Issue 3, 321-324 Copyright © 2008 by the American Association of Veterinary Laboratory Diagnosticians: Validation of an in-clinic enzyme-linked immunosorbent assay kit for diagnosis of Borrelia burgdorferi infection in horses. Amy L. Johnson1, Thomas J. Divers and Yung-Fu Chang
2009	80	J Antimicrob Chemother. 2009 Jun;63 6:1163-72. Epub 2009 Apr 17. Assessment of methylthioadenosine/S-adenosylhomocysteine nucleosidases of Borrelia burgdorferi as targets for novel antimicrobials using a novel high-throughput method. Cornell KA, Primus S, Martinez JA, Parveen N.
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2010	81	**Persistence of borrelial DNA in the joints of Borrelia burgdorferi-infected mice after ceftriaxone treatment** HETA YRJÄNÄInen 1 , JUKKA HYTÖNen 1 , PAULIINA HARTIALA 1 , JARMO OKSI 2 and MATTI K. VILJANEN Departments of 1Medical Microbiology and Immunology and 2 Medicine, University of Turku, Turku, Finland
2010	82-83	Antimicrob Agents Chemother. 2010 February; 54(2): 643–651. Published online 2009 December 7. doi: 10.1128/AAC.00788-09. **Ineffectiveness of Tigecycline against Persistent Borrelia burgdorferi :** Stephen W. Barthold,1* Emir Hodzic,1 Denise M. Imai,1 Sunlian Feng,1 Xiaohua Yang,2 and Benjamin J. Luft
2010	83	Journal of Basic Microbiology, Medical Microbiology. Volume 50, Issue Supplement 1, pages S5–S17, December 2010. **Metamorphosis of Borrelia burgdorferi organisms – RNA, lipid and protein composition in context with the spirochetes' shape**:
2011	84-85	**Evaluation of in-vitro antibiotic susceptibility of different morphological forms of Borrelia burgdorferi:** Published Date May 2011 , Volume 2011:4 Pages 97 - 113 DOI 10.2147/IDR.S19201 Eva Sapi1, Navroop Kaur1, Samuel Anyanwu1, David F Luecke1, Akshita Datar1, Seema Patel1, Michael Rossi1, Raphael B Stricker2

2011	85-86	Benefit of intravenous antibiotic therapy in patients referred for treatment of neurologic Lyme disease: Authors: Stricker RB, DeLong AK, Green CL, Savely VR, Chamallas SN, Johnson L: International Journal of General Medicine Published Date September 2011 Volume 2011:4 Pages 639 - 646
2012	86-87	Persistence of Borrelia burgdorferi in Rhesus Macaques following Antibiotic Treatment of Disseminated Infection. PLoS ONE 7(1): e29914. doi:10.1371/journal.pone.0029914

Evidential support for the case of Chronic Infection:

1: Ann Intern Med. 1986 Jun;104,6:798-800. **Borrelia burgdorferi in joint fluid in chronic Lyme arthritis.** Snydman DR, Schenkein DP, Berardi VP, Lastavica CC, Pariser KM.

Although indirect evidence suggests that chronic Lyme arthritis is caused by persistent infection with Borrelia burgdorferi, direct visualization has been lacking. **We report the demonstration of B. burgdorferi from synovial fluid aspirated from the right knee of a 31-year-old man with Lyme arthritis for more than 1 year. After 6 days, culture medium inoculated with synovial fluid showed one motile and several nonmotile spirochetes.** Direct immunofluorescence staining showed reactivity with anti-B. burgdorferi serum. Spirochetes were not seen in subcultured material. The patient's arthritis improved with high-dose intravenous penicillin. **Identification of B. burgdorferi from the joint fluid of a patient with long-standing arthritis supports the concept that the arthritis is due to persistent infection.**

2: J Am Acad Dermatol. 1986 Sep;15,3:459-63.**Treating erythema chronicum migrans of Lyme disease.** Berger BW.

The efficacy of antibiotic treatment of 117 patients with erythema chronicum migrans of Lyme disease was evaluated in terms of the necessity for retreatment and the prevention of the late manifestations of Lyme disease.**Fifty-six patients with a minor form of the illness did not require retreatment** and did not develop late manifestations following antibiotic treatment. Three pregnant patients were included in this group.**Fourteen of sixty-one patients with a major form of the illness required retreatment, and five developed posttreatment late manifestations of Lyme disease consisting of Bell's palsy and persistent joint pain.** Although the preferred antibiotic for treating erythema chronicum migrans of Lyme disease has not been conclusively established, tetracycline and penicillin proved effective. The use of probenecid plus penicillin may be of benefit to patients with the major form of the illness.

3: 1: Arthritis Rheum. 1987 Apr;30,4:448-50.**Failure of tetracycline therapy in early Lyme disease.** Dattwyler RJ, Halperin JJ.

We describe the clinical courses of 5 patients with Lyme disease who developed significant late complications, despite receiving tetracycline early in the course of their illness. All 5 patients had been treated for erythema chronicum migra ns with a course of tetracycline that met or exceeded current recommendations. The late manifestations of Lyme disease included arthritis, cranial nerve palsy, peripheral neuropathy, chronic fatigue, and changes in mental function. Our findings suggest that the use of tetracycline at a dosage of 250 mg, 4 times a day for 10 days, as a treatment for early Lyme disease should be reconsidered. To determine optimal therapy for early Lyme disease, a study that compares an increased dosage of tetracycline with alternative treatments is indicated.

4: Arthritis Rheum. 1987 Jun;30,6:705-8. **Lyme meningoencephalitis: report of a severe, penicillin-re sistant case.** Diringer MN, Halperin JJ, Dattwyler RJ.

Although Lyme disease frequently attacks the central nervous system, this involvement is rarely severe, and **high-dose intravenous penicillin usually is adequate treatment.** The patient we describe developed severe Lyme meningoencephalitis **despite receiving a full course of penicillin, and his condition continued to deteriorate after reinstitution of this treatment.** Intravenous chloramphenicol was used successfully and resulted in a substantial improvement.

5: Pediatr Infect Dis J. 1988 Apr;7,4:286-9. **Borrelia burgdorferi in a newborn despite oral penicillin for Lyme borreliosis during pregnancy.** Weber K, Bratzke HJ, Neubert U, Wilske B, Duray PH.

Department of Medicolegal Medicine, Dermatology and Microbiology, University of Munich, Federal Republic of Germany. **"We now demonstrate B. burgdorferi in the brain and liver of a newborn whose mother had been treated with oral penicillin for LB [Lyme borreliosis]** during the first trimester of pregnancy. ..The death of the newborn was probably due to a respiratory failure as a consequence of perinatal brain damage."

6: Ann N Y Acad Sci. 1988;539:346-51. **Treatment of erythema chronicum migrans of Lyme disease.** Berger BW. Department of Dermatology, New York University School of Medicine, New York 10016.

Between June 1981 and July 1987 the efficacy of antibiotic treatment of **215 patients with erythema chronicum migrans of Lyme disease was evaluated in terms of the necessity for retreatment and the prevention of the late manifestations of Lyme disease.** The principal antibiotics utilized to treat 161 patients through 1986 were varying doses of tetracycline, or penicillin alone or in combination with probenecid. Two of 8 0 patients with a minor form of the illness and 17 of 81 patients with a major form of the illness required retreatment. There were four patients who did not respond to retreatment with their original medication. A 15- to 30-day course of amoxicillin, 500 mg q.i.d., and probenecid, 500 mg q.i.d., or doxycycline, 100 mg t.i.d., and on three occasions ceftriaxone, 2-4 g/day i.v., were used to treat 54 patients in 1987. **Although it is too early to judge the efficacy of treatment in these patients, increases in the incidence of Herxheimer reactions and drug eruptions were observed**. Strict compliance with treatment protocols and the possibility of reactions to medications should be thoroughly discussed with patients.

7: 1: Arthritis Rheum. 1988 Apr;31,4:487-95. **Spirochetal antigens and lymphoid cell surface markers in Lyme synovitis. Comparison with rheumatoid synovium and tonsillar lymphoid tissue.** Steere AC, Duray PH, Butcher EC.

Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut.

Using monoclonal antibodies to spirochetal antigenes and lymphoid cell surface markers, **we examined the synovial lesions of 12 patients with Lyme disease**, and compared them with rheumatoid synovium and tonsillar lymphoid tissue. The synovial lesions of Lyme disease patients and rheumatoid arthritis patients were similar and often consisted of the elements found in normal organized lymphoid tissue. In both diseases, T cells, predominantly of the helper/inducer s ubset, were distributed diffusely in subsynovial lining areas, often with nodular aggregates of tightly intermixed T and B cells. IgD-bearing B cells were scattered within the aggregates, and a few follicular dendritic cells and activated germinal center B cells were

sometimes present. Outside the aggregates, many plasma cells, high endothelial venules, scattered macrophages, and a few dendritic macrophages were found. HLA-DR and DQ expression was intense throughout the lesions. In 6 of the 12 patients with Lyme arthritis, but in none of those with rheumatoid arthritis, **a few spirochetes and globular antigen deposits were seen in and around blood vessels in areas of lymphocytic infiltration. Thus, in Lyme arthritis, a small number of spirochetes are probably the antigenic stimulus for chronic synovial inflammation.**

8: AMA. 1988 May 13;259,18:2737-9 **Fatal adult respiratory distress syndrome in a patient with Lyme disease.** Kirsch M, Ruben FL, Steere AC, Duray PH, Norden CW, Winkelstein A.

Department of Medicine, Montefiore Hospital, University of Pittsburgh School of Medicine, PA 15213.

A dry cough, fever, generalized maculopapular rash, and myositis developed in a 67-year-old woman; she also had markedly abnormal liver function test results. **Serologic tests proved that she had an infection of recent onset with Borrelia burgdorferi, the agent that causes Lyme disease. During a two-month course of illness, her condition remained refractory to treatment with antibiotics, salicylates, and steroids.** Ultimately, fatal adult respiratory distress syndrome developed; this was believed to be secondary to Lyme disease.

9: J Infect Dis. 1988 Oct;158,4:905-6. **Cultivation of Borrelia burgdorferi from joint fluid three months after treatment of facial palsy due to Lyme borreliosis.** Schmidli J, Hunziker T, Moesli P, Schaad UB.

Attacks typically are intermittent and last from 3 days to 12 months. The knees are affected most often, but migratory arthritis is common and other large and small joints may be involved. Only very few Borrelia strains have been cultured from joint specimens worldwide However, a high percentage of **patients with Lyme arthritis, 85%, have evidence of B burgdorferi DNA, detected by PCR, in the synovial fluid The local persistence of B burgdorferi in the joint over a long period of time** might be related to the exacerbations of symptoms after chondrocyte cell transplantation. B burgdorferi is difficult to detect in synovial fluid, and cultures are positive only rarely

10: 1: N Engl J Med. 1988 Dec 1;319,22:1441-6. Comment in: N Engl J Med. 1989 May 11;320,19:1279-80.**Seronegative Lyme disease. Dissociation of specific T- and B-lymphocyte responses to Borrelia burgdorferi.** Dattwyler RJ, Volkman DJ, Luft BJ, Halperin JJ, Thomas J, Golightly MG.

Department of Medicine, State University of New York, School of Medicine, Stony Brook

11794-8161.

The diagnosis of Lyme disease often depends on the measurement of serum antibodies to Borrelia burgdorferi, the spirochete that causes this disorder.**Although prompt treatment with antibiotics may abrogate the antibody response to the infection, symptoms persist in some patients. We studied 17 patients who had presented with acute Lyme disease and received prompt treatment with oral antibiotics, but in whom chronic Lyme disease subsequently developed. Although these patients had clinically active disease, none had diagnostic levels of antibodies to B. burgdorferi** on either a standard enzyme-linked immunosorbent assay or immunofluorescence assay. On Western blot analysis, the level of immunoglobulin reactivity against B. burgdorferi in serum from these patients was no greater than that in serum from normal controls. The patients had a vigorous T-cell proliferative response to whole B. burgdorferi, with a mean, +/- SEM, stimulation index of 17.8 +/- 3.3, similar to that, 15.8 +/- 3.2, in **18 patients with chronic Lyme disease who had detectable antibodies.** The T-cell response of both groups was greater than that of a control group of healthy subjects, 3.1 +/- 0.5; P less than 0.001.**We conclude that the presence of chronic Lyme disease cannot be excluded by the absence of antibodies against B. burgdorferi and that a specific T-cell blastogenic response to B. burgdorferi is evidence of infection in seronegative patients with clinical indications of chronic Lyme disease.**

11: 1: Am J Clin Pathol. 1989 Jan;91,1:95 7. **Spirochetes in the spleen of a patient with chronic Lyme disease.** Cimmino MA, Azzolini A, Tobia F, Pesce CM Istituto Scientifico di Medicina Interna, Università di Genova, Italy.

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.**

12: 1: Conn Med. 1989 Jun;53,6:335-7. **Treatment of Lyme disease.** Schoen RT.

Lyme disease, a tick-transmitted spirochetal infection, can be divided into three stages that can overlap or occur alone. The goals of antibiotic therapy in stage one are to shorten the duration of early disease and to prevent the development of later stages20of the illness. This can usually be accomplished with oral antibiotic therapy. **Later stages of the illness are frequently more difficult to treat, requiring prolonged oral or intravenous antibiotic therapy.**

13: Infection. 1989 Jul-Aug;17,4:216-7. **High-dose intravenous penicillin G does not prevent further progression in early neurological manifestation of Lyme borreliosis.** Kohler J, Schneider H, Vogt A.

Neurologische Universitätsklinik und Poliklinik, Freiburg.

We report **two cases of Lyme borreliosis,** LB, with erythema migrans, EM, and simultaneous meningopolyneuritis with radicular pain and lymphocytic pleocytosis in the cerebrospinal fluid, CSF. EM and pain disappeared completely under high-dose penicillin G therapy within few a days. Pathological findings in CSF improved. **Nevertheless, during and after therapy, neurological signs of LB developed: cranial nerve palsies as well as paresis of extremity muscles with radicular distribution.**

14: 1: Dtsch Med Wochenschr. 1989 Oct 20;114,42:1602-6. **Neuro-borreliosis or intervertebral disk prolapse?** [Article in German] Dieterle L, Kubina FG, Staudacher T, Büdingen HJ.

Abteilung für Neurologie und klinische Neurophysiologie, St.-Elisabethen-Krankenhaus Ravensburg.

Between September 1986 and November 1988, **17 patients were hospitalized and treated for neuro-borreliosis.** Ten of them had been admitted with suspected lumbar or cervical root or compression syndrome. Only four patients recalled a tick bite, only three an erythema migrans. Uni- or bilateral facial paresis was a prominent feature in six patients. Three of 14 patients had no IgG antibodies against Borrelia, either in serum or cerebrospinal fluid at the initial examination, two had positive titres in serum only. **Despite antibiotic treatment, usually 10 mega U penicillin three times daily, six patients had a recurrence by April, 1989, treated with penicillin again or with twice daily 100 mg doxycycline or 2 g ceftriaxon. In four of them a residual painful polyneuropathy remains.**

15: 1: Infection. 1989 Nov-Dec;17,6:355-9.**Survival of Borrelia burgdorferi in antibiotically treated patients with Lyme borreliosis.** Preac-Mursic V, Weber K, Pfister HW, Wilske B, Gross B, Baumann A, Prokop J. Neurologische Klinik Grosshadern, München, FR Germany.

The persistence of Borrelia burgdorferi in patients treated with antibiotics is described. The diagnosis of Lyme disease is based on clinical symptoms, epidemiology and specific IgG and IgM antibody titers to B. burgdorferi in serum. Antibiotic therapy may abrogate the antibody response to the infection as shown in our patients. B. burgdorferi may persist as shown by positive culture in MKP-medium; patients may have subclinical or clinical disease without diagnostic antibody

titers to B. burgdorferi.**We conclude that early stage of the disease as well as chronic Lyme disease with persistence of B. burgdorferi after antibiotic therapy cannot be excluded when the serum is negative** for antibodies against B. burgdorferi.

[Persistence:] However, **some patients later developed symptoms of the disease despite antibiotic treatment,** 9-11. Because of these observations **it has become questionable if a definite eradication of B. burgdorferi with antibiotics is possible,** p.357. ..The central nervous system invasion by spirochetes and a persistence of Treponema pallidum after penicillin G therapy is common in neurosyphilis, 22,23, p.358.[Treatment:] In view of the hitherto failure of treatment, low CSF concentration of penicillin G, **survival of B. burgdorferi in patients treated with antibiotics, the moderate penicillin G susceptibility o f the organism and unpredictable progression of the disease, it seems appropriate to treat patients with substantially larger doses of antibiotics and/or longer than is provided in present treatment regimens.** p.358.[Seronegativity:] As shown, negative antibody-titers do not provide evidence for successful therapy; antibody-titers may become negative despite persistence.

16: Acta Trop. 1990 Dec;48, 2:89-94.**Clinical implications of delayed growth of the Lyme borreliosis spirochete, Borrelia burgdorferi.** MacDonald AB, Berger BW, Schwan TG. Department of Pathology, Southampton Hospital, New York 11968.

Lyme borreliosis, a spirochetal infection caused by Borrelia burgdorferi, may become clinically active after a period of latency in the host.**Active cases of Lyme disease may show clinical relapse following antibiotic therapy.** The latency and relapse phenomena suggest that the Lyme disease spirochete is capable of survival in the host for prolonged periods of time. We studied 63 patients with erythema migrans, the pathognomonic cutaneous lesion of Lyme borreliosis, and examined in vitro cultures of biopsies from the active edge of the erythematous patch. Sixteen biopsies yielded spirochetes after prolonged incubations of up to 10.5 months, suggesting that **Borrelia burgdorferi may be very slow to divide in certain situations. Some patients with Lyme borreliosis may require more than the currently recommended two to three week course of antibiotic therapy to eradicate strains of the spirochete which grow slowly.**

17: Infect Immun. 1991 Feb;59,2:671-8. **Intracellular localization of Borrelia burgdorferi within human endothelial cells.** Ma Y, Sturrock A, Weis JJ.

Department of Pathology, University of Utah School of Medicine, Salt Lake City 84132.

The later stages of infection by the Lyme disease pathogen, Borrelia burgdorferi, are characterized by the persistence of the organism in individuals possessing a strong anti-Borrelia immune response. This suggests that the organism is sequestered in a tissue

protected from the immune system of the host or there is a reservoir of the organism residing within the cells of the host. In this report, the ability of B. burgdorferi to gain entrance into human umbilical vein endothelial cells was explored as a model for invasion. Incubation of B. burgdorferi with human umbilical vein endothelial cells at ratios ranging from 200:1 to 5,000:1 resulted in the intracellular localization of 10 to 25% of B. burgdorferi in 24 h. The intracellular location of the spirochetes was demonstrated by the incorporation of radiolabeled B. burgdorferi into a trypsin-resistant compartment and was confirmed by double-immunofluorescence staining which differentiated intracellular from extracellular organisms. Actin-containing microfilaments were required for the intracellular localization, indica ting that the host cell participates in the internalization process. Activation of endothelial cells by agents known to increase the expression of several adhesion molecules had no effect on the interaction of B. burgdorferi with the endothelial monolayer. This indicates that the endothelial receptor for B. burgdorferi is constitutively expressed and that internalization is not dependent upon adhesion molecules whose expression is induced by inflammatory mediators. The demonstration of B. burgdorferi within endothelial cells suggest that intracellular localization may be a potential mechanism by which the organism escapes from the immune response of the host and may contribute to persistence of the organism during the later stages of Lyme disease.

18: 1991: Journal of Infectious Diseases, Feb;163,2:311-8 **Randomized comparison of ceftriaxone and cefotaxime in Lyme neuroborreliosis.** Pfister HW, Preac-Mursic V, Wilske B, Schielke E, SÃrgel F, EinhÃ¤upl KM.

Neurological Department, Klinikum Grosshadern, University of Munich, Federal Republic of Germany.

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 2 7 patients examined were clinically asymptomatic. 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.**

19: Medicine, Baltimore. 1991 Mar;70,2:83-90. **Lyme disease: clinical features, classification,**

and epidemiology in the upper midwest. Agger W, Case KL, Bryant GL, Callister SM.

Section of Infectious Disease, La Crosse Lutheran Hospital, Wisconsin.

Lyme disease can be classified using the terminology of syphilis. In this series of **95 cases from the upper midwest**, early cases, defined as an illness of less than 2 months, were more likely to have lived in or recently visited a highly endemic area. Unlike late cases, early cases presented entirely in the nonwinter months, p less than .001. Early disease was further subdivided into primary and secondary disease. Ninety percent of primary and 43% of secondary cases had erythema migrans, while no late cases had active erythema migrans, p less than .001. Clinical manifestations of nonspecific inflammation, except for arthralgia, were more common in early than late disease, p less than .01. In secondary cases, monoarticular arthritis was slightly more common than polyarticular arthritis, with the reverse occurring in late disease, p less than .05. Indirect fluorescent antibody testing revealed a ratio of IgM to IgG antibodies to be helpful in distinguishing early from late disease. **Antibacterial therapy in early, primary cases caused Jarisch-Herxheimer reaction 7% of the time. Despite longer and more frequent parenteral therapy, late Lyme disease frequently required retreatment, owing to poor clinical response, p less than .05.**

19.5: N Engl J Med. 1991 Apr 18;324(16):1137. Chronic neurologic manifestations of Lyme disease. Logigian EL, Kaplan RF, Steere AC. Department of Neurology, Tufts University School of Medicine, Boston, MA 02111.

BACKGROUND AND METHODS. Lyme disease, caused by the tick-borne spirochete Borrelia burgdorferi, is associated with a wide variety of neurologic manifestations. To define further the chronic neurologic abnormalities of Lyme disease, we studied 27 patients, age range, 25 to 72 years, with previous signs of Lyme disease, current evidence of immunity to B. burgdorferi, and chronic neurologic symptoms with no other identifiable cause. Eight of the patients had been followed prospectively for 8 to 12 years after the onset of infection. RESULTS. Of the 27 patients, 24, 89 percent, had a mild encephalopathy that began 1 month to 14 years after the onset of the disease and was characterized by memory loss, mood changes, or sleep disturbance. Of the 24 patients, 14 had memory impairment on neuropsychological tests, and 18 had increased cerebrospinal fluid protein levels, evidence of intrathecal production of antibody to B. burgdorferi, or both. Nineteen of the 27 patients,70 percent, had polyneuropathy with radicular pain or distal paresthesias; all but two of these patients also had encephalopathy. In 16 patients electrophysiologic testing showed an axonal polyneuropathy. One patient had leukoencephalitis with asymmetric spastic diplegia, periventricular white-matter lesions, and intrathecal production of antibody to B. burgdorferi. Among the 27 patients, associated symptoms included fatigue, 74 percent, headache, 48 percent, arthritis, 37 percent, and hearing loss, 15 percent. At the time of

examination, chronic neurologic abnormalities had been present from 3 months to 14 years, usually with little progression. Six months after a two-week course of intravenous ceftriaxone, 2 g daily, 17 patients, 63 percent, had improvement; 6, 22 percent, had improvement but then relapsed; and 4,15 percent, had no change in their condition. CONCLUSIONS. **Months to years after the initial infection with B. burgdorferi, patients with Lyme disease may have chronic encephalopathy, polyneuropathy, or less commonly, leukoencephalitis. These chronic neurologic abnormalities usually improve with antibiotic therapy.**

20: Arthritis Rheum. 1991 Aug;34,8:1056-60. **Treatment of refractory chronic Lyme arthritis with arthroscopic synovectomy.** Schoen RT, Aversa JM, Rahn DW, Steere AC.

Department of Medicine, Yale University School of Medicine, New Haven, Connecticut 06510.

Of 20 patients who underwent arthroscopic synovectomy for refractory chronic Lyme arthritis of the knee, 16, 80%, had resolution of joint inflammation during the first month after surgery or soon thereafter, and they have remained well during the 3-8-year followup period. Three of these 16 patients who were more disabled preoperatively, still had mild functional limitation at long-term followup. The remaining 4 patients, 20%, had persistent or recurrent synovitis. We conclude that arthroscopic synovectomy is effective in treating chronic Lyme arthritis in patients in whom the disease does not respond to antibiotic therapy.

21: 1: Clin Exp Rheumatol. 1992 Jul-Aug;10,4:387-90. **Molecular detection of persistent Borrelia burgdorferi in a man with dermatomyositis.** Fraser DD, Kong LI, Miller FW.

National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, Bethesda, Maryland.

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, p robenecid 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 sero-negative Lyme disease**

which may occur in immunocompromised individuals.

22: 1:20 J Infect Dis. 1992 Aug;166,2:440-4.**Fibroblasts protect the Lyme disease spirochete, Borrelia burgdorferi, from ceftriaxone in vitro.** Georgilis K, Peacocke M, Klempner MS. Department of Medicine, New England Medical Center, Boston, Massachusetts.

The Lyme disease spirochete, Borrelia burgdorferi, can be recovered long after initial infection, even from antibiotic-treated patients, indicating that it resists eradication by host defense mechanisms and antibiotics. Since B. burgdorferi first infects skin, the possible protective effect of skin fibroblasts from an antibiotic commonly used to treat Lyme disease, ceftriaxone, was examined. Human foreskin fibroblasts protected B. burgdorferi from the lethal action of a 2-day exposure to ceftriaxone at 1 microgram/mL, 10-20 x MBC. In the absence of fibroblasts, organisms did not survive. Spirochetes were not protected from ceftriaxone by glutaraldehyde-fixed fibroblasts or fibroblast lysate, suggesting that a living cell was required. The ability of the organism to survive in the presence of fibroblasts was not related to its infectivity.**Fibroblasts protected B. burgdorferi for at least 14 days of exposure to ceftriaxone.** Mouse keratinocytes, HEp-2 cells, and Vero cells but not Caco-2 cells showed the same protective effect. Thus, **several eukaryotic cell types provide the Lyme disease spirochete with a protective environment contributing to its long-term survival.**

23: J Am Acad Dermatol. 1993 Feb;28,2 Pt 2:312-4. **Recurrent erythema migrans despite extended antibiotic treatment with minocycline in a patient with persisting Borrelia burgdorferi infection.** Liegner KB, Shapiro JR, Ramsay D, Halperin AJ, Hogrefe W, Kong L.

Department of Medicine, Northern Westchester Hospital Center, Mount Kisco, NY.

Erythema migrans recurred in a patient 6 months after a course of treatment with minocycline for Lyme disease. Polymerase chain reaction on heparinized peripheral blood at that time demonstrated the presence of Borrelia burgdorferi-specific DNA. The patient was seronegative by Lyme enzyme-linked immunosorbent assay but showed suspicious bands on Western blot. Findings of a Warthin-Starry stain of a skin biopsy specimen of the eruption revealed a Borrelia-compatible structure. **Reinfection was not believed to have occurred. Further treatment with minocycline led to resolution of the erythema migrans.**

24: 1: J Infect Dis. 1993 May;167,5:1074-81.**Invasion of human skin fibroblasts by the Lyme disease spirochete, Borrelia burgdorferi.** Klempner MS, Noring R, Rogers RA.

Division of Geographic Medicine and Infectious Diseases, New England Medical Center, Tufts University School of Medicine, Boston, Massachusetts 02111.

The ability of Borrelia burgdorferi to attach to and invade human fibroblasts was investigated by scanning electron and confocal microscopy. By scanning electron microscopy, **B. burgdorferi were tightly adherent to fibroblast monolayers after 24-48 h but were eliminated from the cell surface by treatment with ceftriaxone, 1 microgram/mL, for 5 days.** Despite the absence of visible spirochetes on the cell surface after antibiotic treatment, viable B. burgdorferi were isolated from lysates of the fibroblast monolayers. B. burgdorferi were observed in the perinuclear region within human fibroblasts by laser scanning confocal microscopy.**Intracellular spirochetes specifically labeled with monoclonal anti-flagellin antibody were also identified by fluorescent laser scanning confocal microscopy. These observations suggest that B. burgdorferi can adhere to, penetrate, and invade human fibroblasts in organisms that remain viable.**

25: Infection. 1993 Mar-Apr;21,2:83-8. **Azithromycin versus doxycycli ne for treatment of erythema migrans: clinical and microbiological findings.** Strle F, Preac-Mursic V, Cimperman J, Ruzic E, Maraspin V, Jereb M.

Department of Infectious Diseases, University Medical Center, Ljubljana, Slovenia.

The effectiveness of azithromycin and doxycycline in the treatment of erythema migrans was compared in a prospective randomized trial. One hundred seven adult patients with typical erythema migrans, examined in the Lyme Borreliosis Outpatients' Clinic, University Department of Infectious Diseases in Ljubljana, were included in the study. Fifty-five patients received azithromycin, 500 mg twice daily for the first day, followed by 500 mg once daily for four days, and 52 patients received doxycycline, 100 mg twice daily for 14 days. The mean duration of skin lesions after the beginning of treatment was 7.5 +/- 5.9 days, median value 5, range 2-28 days, in the azithromycin group and 11.4 +/- 7.8 days, median value 9, range 2 days--8 weeks, in the doxycycline group, $p < 0.05$. Borrelia burgdorferi was isolated from erythema migrans in 28 patients before therapy: in 13 out of 52 in the doxycycline group and in 15 out of 55 in the azithromycin group. **Three months after therapy, the culture was positive in four out of 13 patients treated with doxycycline and in one of the 15 patients who received azithromycin. A biopsy was repeated in all the patients with a positive isolation from the first skin specimen. During the first 12 months' follow-up, three patients treated with doxycycline but none in the azithromycin group developed major manifestations of Lyme borreliosis, while 15 doxycycline recipients and 10 azithromycin recipients developed minor consecutive manifestations.**

26: 1: J Neurol. 1993 May;240,5:278-83. **Borrelia burgdorferi myositis: report of eight patients.** Reimers CD, de Koning J, Neubert U, Preac-Mursic V, Koster JG, Müller-Felber W, Pongratz DE, Duray PH.

Friedrich-Baur-Institute, Clinic for Internal Medicine Innenstadt, Munich, Germany.

Myositis is a rare manifestation of Lyme disease of unknown pathogenesis. **This study describes the course of disease in eight patients with Lyme disease, aged 37-70 years, all of whom were suffering from histologically proven myositis.** The clinical, electrophysiological, and myopathological findings are reported. One patient showed signs and symptoms of myositis of all limbs. **In six patients myositis was localized in the vicinity of skin lesions, arthritis or neuropathy caused by Borrelia burgdorferi.** In another patient suffering from pronounced muscle weakness of the legs and cardiac arrest, inflammation of the myocardium, the conducting system and skeletal muscles was revealed at autopsy. Muscle biopsy revealed lymphoplasmocellular infiltrates combined with few fibre degenerations in three patients. The lymphoplasmocellular infiltrates were found predominantly in the vicinity of small vessels. **Several spirochetes were stained in six of seven muscle biopsy samples by means of the immunogold-silver technique.** Culturing of20B. Burgdorferi from the muscle biopsy samples was, however, unsuccessful. **Antibiotic treatment succeeded in curing the myositis in four of six patients.** In one patients signs and symptoms improved. One patient died from cardiac arrest caused by myocarditis and Guillain-Barré syndrome. The outcome is unknown in one patient. Clinical and myopathological findings indicate that Lyme myositis can be caused either by local spreading of B. burgdorferi or an unknown antigen or toxin from adjacent tissues or haematogenously.

27: Zhonghua Yan Ke Za Zhi. Sep;29,5:271-3. **Lyme disease in China and its ocular manifestations** [Article in Chinese] Liu AN.

Department of Ophthalmology, Chinese Navy General Hospital, Beijing.

The authors report **30 chinese patients of ocular Lyme borreliosis**, which is a tick-borne spirochaetal disease involving multiple organ systems. The ocular manifestations begin as conjunctivitis, and then as uveitis, choroidoretinitis, keratitis and vitritis. Diagnosis is based on case history and clinical and laboratory findings. **Early cases may be cured by oral antibiotics while intravenous drip of large dosage is needed for advanced cases, with a relapsing rate of 16%.** Prolonged systemic corticosteroids may predispose the patient to antibiotic failure; however, topical corticosteroids in combination with antibiotics may minimize ocular inflammation and complications.

28: Arthritis Rheum. 1993 Nov;36,11:1621 6. **Persistence of Borrelia burgdorferi in ligamentous tissue from a patient with chronic Lyme borreliosis.** Häupl T, Hahn G, Rittig M, Krause A, Schoerner C, Schönherr U, Kalden JR, Burmester GR.

Department of Medicine III, University of Erlangen-Nuremberg, Germany.

OBJECTIVE. **To document the persistence of Borrelia burgdorferi in ligamentous tissue samples obtained from a woman with chronic Lyme borreliosis.** METHODS. Spirochetes were isolated from samples of ligamentous tissue, and the spirochetes were characterized antigenetically and by molecular biology techniques. The ligamentous tissue was examined by electron microscopy. Humoral and cellular immune responses were analyzed. RESULTS. Choroiditis was the first recognized manifestation of Lyme disease in this patient. **Despite antibiotic therapy, there was progression to a chronic stage, with multisystem manifestations.** The initially significant immune system activation was followed by a loss of the specific humoral immune response and a decrease in the cellular immune response to B burgdorferi over the course of the disease. "Trigger finger" developed, and a portion of the flexor retinaculum obtained at surgery was cultured. Viable spirochetes were identified. Ultramorphologically, the spirochetes were situated between collagen fibers and along fibroblasts, **some of which were deeply invaginated by these organisms. The cultured bacteria were identified as B burgdorferi** by reactions with specific immune sera and monoclonal antibodies, and by polymerase chain reaction amplification and Southern blot hybridization techniques. CONCLUSION. To our knowledge, this is the first report of the isolation of B burgdorferi from ligamentous tissue. This suggests that **tendon tissues serve as a specific site of spirochete residence in human hosts.**

29: J Clin Neuroophthalmol. 1993 Sep;13,3:155-61; discussion 162. 59: **First isolation of Borrelia burgdorferi from an iris biopsy.** Preac-Mursic V, Pfister HW, Spiegel H, Burk R, Wilske B, Reinhardt S, Böhmer R.

Max v. Pettenkofer Institut für Hygiene u. Medizinische Mikrobiologie, LM-Universität München, Germany.

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.**

30: Repeat

31: Cent Eur J Public Health. 1993 Dec;1,2:81-5. Electron microscopy and the polymerase chain

reaction of spirochetes from the blood of patients with Lyme disease. Hulínská D, Krausová M, Janovská D, Rohácová H, Hancil J, Mailer H.

Department of Electron Microscopy, National Institute of Public Health, Prague, Czech Republic.

Results of studies using direct antigen detection suggest that seronegative Lyme borreliosis is not rare and support the hypothesis that Borrelia antigens can persist in humans. We report three successful cultures from blood out of 30 attempts from 96 Lyme disease patients. The proof of borreliaemia in early or late phases of Lyme disease by immuno-capture electron microscopy has practical importance for subsequent cultivation. The polymerase chain reaction with oligonucleotide sequences directed against 16S rRNA identified two of our blood isolates as Borrelia burgdorferi genospecies III., VS 461 group, and one as Borrelia garinii sp. nov. All of the three isolates were reactive with monoclonal antibody H9724 against flagellin and with antibody against main extracellular protein at 83 kDa. Borrelia garinii had a single predominant protein OspA at 33.5 kDa and reacted with monoclonal antibody H5332 in contrast to two isolates of the VS 461 group with two major proteins OspA and OspB at 32.5 and 35 kDa. **We conclude that isolation of spirochetes from the blood might prove successful in clinically selected cases of Lyme borreliosis.** Immuno-capture electron microscopy has proved to be a sensitive assay for monitoring and studying Lyme borreliosis.Clin Orthop Relat Res. 1993 Dec;,297:238-41. **Chronic septic arthritis caused by Borrelia burgdorferi.** Battafarano DF, Combs JA, Enzenauer RJ, Fitzpatrick JE.

Department of Medicine, Fitzsimons Army Medical Center, Aurora, Colorado 80045-5001.

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.

32: 1: Neurology. 1993 Dec;43,12:2705-7. **Stroke due to Lyme disease.** Reik L Jr. Department of Neurology, University of Connecticut Health Center, Farmington 06030-1845.

A 56-year-old Connecticut woman suffered multiple strokes 18 months after antibiotic treatment for early Lyme disease with facial palsy. Pleocytosis, intrathecal synthesis of anti-Borrelia burgdorferi antibody, and **the response to antibiotic treatment substantiated the diagnosis of neuroborreliosis. This is the first report of stroke caused by Lyme disease acquired in North America.**

33: Lancet, Vol 345: 1436-37 Lopez-Andreu JA; Salcede-Vivo J; . Our patient received during 2 years seven short-term antibiotic treatments, achieving transitory improvements. Nonetheless, his condition greatly deteriorated. In October, 1993, **he started a different antibiotic regimen, ceftriaxone, 2 g per day intravenously for 12 months, oral roxithromycin 150 mg per day for 2 months, and oral ciprofloxacin, 500 mg per 12 hours for 2 months. After ceftriaxone he has continued with oral minocycline, 100 mg per 12 hours for 7 months. His quality of life has greatly improved and the treatment is more tolerable than the borreliosis. We add, however, in accord with the advice of others that antibiotics should be continued in the long term, until we achieve cure or delay the progression of the disease.**

34: N Engl J Med. Jan 27; 330,4:282-3.**Detection of Borrelia burgdorferi DNA by polymerase chain reaction in synovial fluid from patients with Lyme arthritis.** Nocton JJ, Dressler F, Rutledge BJ, Rys PN, Persing DH, Steere AC.

Division of Rheumatology/Immunology, New England Medical Center, Boston, MA 02111.

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.**

35: 1: J Clin Microbiol. 1994 Mar;32,3:715-20.**Isolation of Borrelia burgdorferi from biopsy specimens taken from healthy-looking skin of patients with Lyme borreliosis.** Kuiper H, van

Dam AP, Spanjaard L, de Jongh BM, Widjojokusumo A, Ramselaar TC, Cairo I, Vos K, Dankert J. Department of Medical Microbiology, Academic Medical Centre, University Hospital, University of Amsterdam, The Netherlands.

Erythematous skin lesions due to infection with Borrelia burgdorferi will often disappear without antibiotic treatment. The aim of the study was to assess whether after disappearance of the erythematous skin lesion **B. burgdorferi is still present in the healthy-looking skin of untreated patients.** In six patients, a skin biopsy specimen was taken at the site of a previous erythematous skin lesion 1 to 6 months after disappearance of the lesion. Four of them presented with early disseminated Lyme borreliosis. In one additional patient with early disseminated Lyme borreliosis, the site of a previous tick bite was biopsied. None of these patient s had been treated with antibiotics before presentation.**The cultures of the skin biopsy specimens of the seven patients showed growth of Borrelia species**. By rRNA gene restriction analysis and genospecies-specific PCR, six isolates were classified as Borrelia garinii and one as Borrelia group VS461.**These results show that B. burgdorferi can still be cultured** from the skin after disappearance of the erythematous skin lesion or at the site of a previous tick bite.

36: J Rheumatol. 1994 Mar;21,3:454-61. **Lyme disease: an infectious and postinfectious syndrome.**Asch ES, Bujak DI, Weiss M, Peterson MG, Weinstein A.

Department of Medicine, New York Medical College, Valhalla 10595.

OBJECTIVE. **To determine chronic morbidity and the variables that influence recovery in patients who had been treated for Lyme disease.** METHODS. Retrospective evaluation of 215 patients from Westchester County, NY, who fulfilled Centers for Disease Control case definition for Lyme disease, were anti-Borrelia antibody positive and were diagnosed and treated at least one year before our examination. RESULTS. Erythema migrans had occurred in 70% of patients, neurological involvement in 29%, objective cardiac problems in 6%, arthralgia in 78% and arthritis in 41%. Patients were seen at a mean of 3.2 years after initial treatment. A history of relapse with major organ involvement had occurred in 28% and a history of reinfection in 18%. Anti-Borrelia antibodies, initially present in all patients, were **still positive in 32%. At followup, 82, 38%, patients were asymptomatic and clinically active Lyme disease was found in 19, 9%. Persistent symptoms of arthralgia, arthritis, cardiac or neurologic involvement with or without fatigue were present in 114, 53%, patients. Persistent symptoms correlated with a history of major organ involvement or relapse but not the continued presence of anti-Borrelial antibodies.** Thirty-five of the 114, 31%, patients with persistent symptoms had predomina ntly arthralgia and fatigue. Antibiotic treatment within 4 weeks of disease onset was more likely to result in complete recovery. Children did not significantly differ from adults in disease manifestations or in the frequency of relapse, reinfection or complete recovery.

CONCLUSION. Despite recognition and treatment, **Lyme disease is associated with significant infectious and postinfectious sequelae.**

37: Ann Intern Med. 1994 Mar 15;120,6:487-9. **The persistence of spirochetal nucleic acids in active Lyme arthritis.** Bradley JF, Johnson RC, Goodman JL.

Section of Infectious Diseases, University of Minnesota School of Medicine, Minneapolis 55455.

Six of seven patients with Lyme arthritis were positive by PCR. In contrast, all 18 synovial fluid samples from patients with other disorders, including rheumatoid arthritis, spondyloarthropathy, gout, pseudogout, hemarthrosis, degenerative joint disease, lupus, papillary synovitis, and trauma, were negative by PCR, $P < 0.001$, Lyme arthritis compared with controls, Fisher exact test. All 38 laboratory controls were negative by PCR. **The assay reproducibly detected 20 or fewer B. burgdorferi cells directly or when added to extracted synovial fluid that was previously negative by PCR.** Polymerase chain reaction was done four times with identical results, including analyses with both outer surface protein A primer sets.

38: Ann Intern Med. 1994 Oct 15;121,8:560-7.**The long-term clinical outcomes of Lyme disease. A population-based retrospective cohort study.** Shadick NA, Phillips CB, Logigian EL, Steere AC, Kaplan RF, Berardi VP, Duray PH, Larson MG, Wright EA, Ginsburg KS, Katz JN, Liang MH.

Department of Rheumatology-Immunology, Brigham & Women's Hospital, Boston, MA 02115.

OBJECTIVE: To ascertain the prevalence of and risk factors for long-term sequelae from acute Lyme disease. DESIGN: Population-based, retrospective cohort study. SETTING: A coastal region endemic for Lyme disease. PARTICIPANTS: **Patients with a history of Lyme disease who were previously treated with antibiotics were compared with randomly selected controls.** MEASUREMENTS: A standardized physical examination, health status measure, Short Form 36, psychometric test battery, and serologic analysis. RESULTS: Compared with the control group, n = 43, the Lyme group, n = 38; mean duration from disease onset to study evaluation, 6.2 years, had more arthralgias, 61% compared with 16%; $P < 0.0001$; distal paresthesias, 16% compared with 2%; $P = 0.03$; concentration difficulties, 16% compared with 2%; $P = 0.03$; and fatigue, 26% compared with 9%; $P = 0.04$, and they had poorer global health status scores, $P = 0.04$. The Lyme group also had more abnormal joints, $P = 0.02$, and more verbal memory deficits, $P = 0.01$, than did the control group. Overall, 13 patients, 34%; 95% CI, 19% to 49%, had long-term sequelae from Lyme20disease, arthritis or recurrent arthralgias [n = 6], neurocognitive impairment [n = 4], and neuropathy or myelopathy [n = 3]. Compared with controls, patients who had long-term sequelae had higher IgG antibody titers to the spirochete, $P = 0.03$, and received treatment later,

34.5 months compared with 2.7 months; P < 0.0001. CONCLUSIONS: **Persons with a history of Lyme disease have more musculoskeletal impairment and a higher prevalence of verbal memory impairment when compared with those without a history of Lyme disease. Our findings suggest that disseminated Lyme disease may be associated with long-term morbidity.**

39: Infect. 1994 Nov;29,3:255-61.**Treatment of late Lyme borreliosis.** Wahlberg P, Granlund H, Nyman D, Panelius J, Seppälä I.

Department of Medicine, Aland Central Hospital, Mariehamn, Finland.

The aim of this study was to develop a treatment for late Lyme borreliosis and to compare the clinical results with serological findings before and after treatment. It was done in the Aland Islands, population 25,000, a region endemic for Lyme borreliosis.**The patients were the first consecutive 100 patients from the Aland Islands with late Lyme borreliosis.** They were followed for at least **1 year after treatment.** The clinical results of treatment were compared with results of analyses of flagellar IgG antibodies to Borrelia burgdorferi done at the time of diagnosis before treatment and up to 12 months afterwards.**Short periods of treatment were not generally effective. The outcome was successful in four of 13 treatments with 14 days of intravenous ceftriaxone alone, in 50 of 56 assessable treatments with ceftriaxone followed by 100 days of amoxycillin plus probenecid, and in 19 of 23 completed treatments with ceftriaxone followed by 100 days of cephadroxil.** Titres of IgG antibodies to B. burgdorferi flagella declined significantly after 6 and 12 months in the patients who had successful treatments. All patients whose final titres were less than 30% of the initial titre were in the successful group. Their titres usually remained above the upper limit of normal for a long time but a decline to a value of less than 30% of that before treatment was always a sign of cure.

Symptoms and signs often improve temporarily shortly after treatment but reappear within weeks or months. ..To conclude, we have shown that long-term treatments beginning with intraveous ceftriaxone and continuing with amoxycillin plus probenecid or with cephadroxil were useful in the treatment of late Lyme borreliosis.

40: **Late complaints after erythema migrans** Herta Klade, MD and Elizabeth Aberer, MD. JSTD 1994; 1:52-56.

A lot of treatment studies have been carried out, but no antibiotic has been proved to avoid late manifestations of Lyme disease. Our interest focused on late manifestations following20uncomplicated erythema migrans, UEM, and complicated erythema migrans, CEM, after a median observation period of 30 months. To compare the therapeutic, serological, and clinical outcome, 161 patients were re-examined prospectively. **Late complaints could be**

observed in 31/161, 19%, of patients, more often in CEM than in UEM, 36% versus 12%. Patients with late sequelae were more often seropositive than the total collective, 77% versus 67%, at least once during the observation period, as against **12 of 13 patients who needed several therapy cycles, 92%. Seven seropositive patients did not respond to oral antibiotic treatment even after several cycles.** Amoxicillin/clavulanic acid treated patients had late complaints in 8% in contrast to penicillin V, 15%, and doxycycline, 17%, treated persons.

Seropositivity before treatment has a negative influence on the course of erythema migrans, EM, disease. **Immunogenetic disposition might be responsible for repeated infections and for treatment failures** in a certain patient group.

41: **Borrelia burgdorferi - Seek and ye shall find. Expanding the envelope** Kenneth Liegner, MD. JSTD 1994; 1:79-81.

With recognition of chronic persistent infection, we will begin to look at disease pathogenesis quite differently. **A persisting pathogen may induce noxious injure over not just days, weeks, or months, but years and decases and even the naturel life of the host.** Slowly simmering infection can induce a wide variety of host responses, both directly and immune mediated. The treatment approach may need to be very different in this circumstance than for a readily extirpated bacterium such as staphylococcus or streptococcus. **Chronic infection may require chronic treatment.** Definitive cure, while theoretically possible, may not be achieved using currently available methods in chronically infected patients. This dilemma should prompt a determined effort to develop definitive means of curing the infection. **We will begin to perceive just how daunting an adversary the borreliae really are how commonly affected the populace of** endemic regions. **Borrelia are resilient.**

42: **Psychiatric aspects of Lyme disease in children and adolescents: A community epidemiologic study in Westchester, New York** Brian A. Fallon, MD, MPH; Hector Bird, MD; Christina Hoven, DrPH; Daniel Cameron, MD, MPH; Michael R. Liebowitz, MD; and David Shaffer, MD. JSTD 1994; 1:98-100.

To date, no community study has examined the psychiatric aspects and or sequelae of Lyme disease, LD, among children. As part of a community epidemilogic study of psychiatric disorders among children ages 9 through 17 in a Lyme endemic county, parents were asked whether their child had ever been diagnosed as having LD, and 10.1%, 36/357, responded yes to the LD question. Of the 36, 29 also agreed to take part in a follow-up interview. Sixteen of the 29 children had physician-diagnosed LD as well as either an erythema migrans rash or a positive serology. Fifteen of these 16 rec eived treatment within 1 month of symptom onset; none of these 15 children were symptomatic longer than 4 months. Only one child had physical symptoms at the

time of the interview; **she was not treated until 4 months after symptom onset. The child experienced 5 years of intermittent arthritis, cognitive deficits, emotional problems, severe fatigue, and a deterioration in school performance. Courses of oral antibiotics were at first associated with a good response, followed by a resurgence of symptoms months later**

The lifetime prevalence of LD by history among children ages 9 through 17 in an endemic area may be at least 44.8/1000. In general, when LD is diagnosed early, it responds well to treatment. **Delayed diagnosis and treatment may lead to a chronic course.**

43: **Persistence of Borrelia burgdorferi despite antibiotic treatment** Michael A. Patmas, MD. JSTD 1994; 1:101.

It has been suggested that Lyme Disease may trigger fibromyalgia and that antibiotic therapy beyond 30 days is almost always unnecessary. Recently, two cases demonstrated persistence of borrelia burgdorferi despite lengthy antibiotic treatment were noted.

Case number: 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 500mg 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 normal gestation, delivered a health 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 failed treatment with nonsterodal anti-inflammatories and remittive agents, the author saw the patient for the first time in 1990. Aspiration of fluid from the right knee was **positive by specific antibody ratio for Lyme Disease** as 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 i ntravenously 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 center 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 her hips. A right total hip replacement was performed and a 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 continous oral antibiotic treatment with Azithromycin 250mg 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 aboth 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 because a patient has received 30 days of treatment. Careful histopathologic examination by modified dieterle silver stain my suggest otherwise.

44: J Infect Dis. 1994 Nov;170,5:1312-6 Comment in: J Infect Dis. 1995 May;171,5:1379-80. **Fate of Borrelia burgdorferi DNA in tissues of infected mice after antibiotic treatment.** Malawista SE, Barthold SW, Persing DH. Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut.

Persistence of Borrelia burgdorferi DNA in tissues following antibiotic treatment was evaluated in C3H mice inoculated intradermally with 10,3, B. burgdorferi N40 or sterile medium. Half of the infected mice and all of the uninfected mice were treated with ceftriaxone 15 days after inoculation for 5 days. Ear and urinary bladder samples were collected on days 20, 30, and 60 after inoculation for culture and for extraction of DNA and amplification of specific spirochetal DNA by polymerase chain reaction, PCR. PCR primers were specific for a 280-bp portion of a highly conserved region of the gene encoding outer surface protein, Osp, A of B. burgdorferi and for a 328-bp part of the OspB gene. There was excellent concordance between culture and PCR for ears, 35/36 mice, and bladders, 33/36. Both tissues became uniformly negative at the earliest interval tested after antibiotic treatment. Thus, the ability to amplify B. burgdorferi DNA quickly disappeared from tissues that had become culture-negative after antibiotic treatment, suggesting that serial study of PCR-positive tissues and fluids may be useful for evaluating the efficacy of antibiotic therapy in human Lyme disease. 2 out of 5 mice tested 60 days after treatment were found to be positive on culture; 1 of these mice was also positive by PCR. **The authors speculate that this could be due to:, a, reinfection, which they consider .highly unlikely.,, b, contamination, or, c, the .resurgence of spirochetes in animals not completely sterilized by antibiotics. This last possibility will bear further scrutiny because late recurrences of Lyme**

disease without obvious reinfection may occur in humans.[Diagnosis:] Positive PCR results were found to suggest active infection. .Unless some patients with Lyme disease have a defect in their ability to degrade spirochetal DNA, these results suggest that persisting PCR positivity indicates persisting infection.

45: Antimicrob Agents Chemother. 1995 May;39,5:1127-33. **Effects of penicillin, ceftriaxone, and doxycycline on morphology of Borrelia burgdorferi.** Kersten A, Poitschek C, Rauch S, Aberer E.

Department of Dermatology, University of Vienna, Austria.

Antibiotic therapy with penicillin, doxycycline, and ceftriaxone has proven to be effective for the treatment of Lyme borreliosis. **In some patients, however, it was noticed that borreliae can survive in the tissues in spite of seemingly adequate therapy**. For a better understanding of this phenomenon, we investigated the different modes of degeneration of Borrelia burgdorferi suspensions during a 96-h exposure to various antibiotics. By dark-field microscopy and ultrastructural investigations, increasing blebbing and the gradual formation of granular and cystic structures could be followed during the exposure time. Although antibiotic concentrations at the MIC at which 90% of organisms are inhibited after 72 h were 80% or even greater, **motile organisms were still present after incubation with penicillin and doxycycline** but not after incubation with ceftriaxone. By transmission electron microscopy, **intact spirochetal parts, mostly situated in cysts, were seen up to 96 h after exposure with all three antibiotics tested.** According to experiences from studies with other spirochetes **it is suggested that encysted borreliae, granules, and the remaining blebs might be responsible for the ongoing antigenic stimulus leading to complaints of chronic Lyme borreliosis.**

46: **Persistent PCR positivity in a patient being treated for Lyme disease**. Kornelia Keszler, MD and Richard C. Tilton, PhD. JSTD 1995; 2:57-58.

A 30-year-old white female presented **with worsening clinical symptoms suggestive of Lyme disease** while on antibiotic therapy. Results of enzyme-linked immunosorbent assay, ELISA, and of western blot tests for IgG and IgM antibody were equivocal. However, **Borrelia burgdorferi DNA detected by the polymerase chain reaction, PCR, was detected in whole blood on two separate occasions, 1 month apart, while the patient was on oral doxycycline**, 100 mg b.i.d. This report questions the significance of persistent Borrelia burgdorferi DNA in a patient who is not responding to antibiotic therapy.

47: **Neuroborreliosis in Texas** Audrey Stein Goldings, MD. JSTD 1995; 2:59-61.

Chronic persistent symptoms after treatment for Lyme disease, LD, are common. Early effective treatment is the only known way to avoid this possibility. despite early recognition of the infection, **patients still may not do well due to failure to eradicate the spirochete.** This is a case study of one such patient.

48: Vartiovaara I. 1995 Living with Lyme. Lancet, 345:842-4 **A Finnish physician 's account of his experiences that beginning with a tick bite in Vancouver in 1987.**

Dr. Vartiovaara resigned from his position with the Finnish Medical Journal in 1992, due to disabilities caused by Lyme disease. [Persistence:] After that [a positive result on a T-cell proliferation test at Stony Brook Hospital] **I had two months' heavy treatment with oral doxycycline 300mg a day. I was a little better after it, but only for about two months. Then it started all over again, and got worse. ..We sent blood and spinal fluid to Dr. Oksi and they turned out to be positive [by PCR]--in other words, the spirochaete was still alive in my body after six years, despite the antibiotics.** Dr. Vartiovaara was then treated aggressively with a combination of antibiotics, including four weeks of ceftriaxone, for six months. Some time after the cessation of treatment however, he found that .My symptoms are on the move again. [Diagnosis:] **What should be done when a patient has the typical Lyme disease history but negative serology?** This is still a hot question especially in the USA. **My strong opinion is that oral antibiotics should be given in such cases. Ordinary laboratory tests cannot be relied upon and the PCR is too expensive** for routine use. When the whole picture leans towards Lyme borreliosis it is both ethically and medically right to treat.

49: J Neuropsychiatry Clin Neurosci. 1995 Summer;7,3:345-7. **Rapidly progressive frontal-type dementia associated with Lyme disease.** Waniek C, Prohovnik I, Kaufman MA, Dwork AJ.

New York State Psychiatric Institute, NY 10032, USA.

The authors report **a case of fatal neuropsychiatric Lyme disease,** LD, that was expressed clinically by progressive frontal lobe dementia and pathologically by severe subcortical degeneration. **Antibiotic treatment resulted in transient improvement, but the patient relapsed after the antibiotics were discontinued.** LD must be considered even in cases with purely psychiatric presentation, and **prolonged antibiotic therapy may be necessary.**

50: Ann Neurol. 1995 Oct;38,4:667-9. Comment in: Ann Neurol. 1995 Oct;38,4:560-2.**Neuroborreliosis in the nonhuman primate: Borrelia burgdorferi persists in the central nervous system.** Pachner AR, Delaney E, O'Neill T.

Department of Neurology, Georgetown University School of Medicine, Washington, DC 20007,

USA.

Neurological involvement in Lyme disease is common, and is frequently difficult to diagnose and treat. Little is known about the fate of the causative spirochete Borrelia burgdorferi in the ce ntral nervous system, CNS. To determine the frequency of parenchymal infection and to determine localization of the organism, polymerase chain reaction/hybridization assays were performed in a newly described model of Lyme neuroborreliosis in nonhuman primates infected with B. burgdorferi. Polymerase chain reaction/hybridization of CNS tissues from 5 infected nonhuman primates was performed.**Substantial amounts of B. burgdorferi DNA were detected in the CNS in all infected animals, with a predilection toward subtentorial structures. These data suggest that Lyme neuroborreliosis represents persistent infection with B. burgdorferi.**

51: 1: Eur Neurol. 1995;35,2:113-7. Comment in: Eur Neurol. 1996;36,6:394-5. **Seronegative chronic relapsing neuroborreliosis.**Lawrence C, Lipton RB, Lowy FD, Coyle PK.

Department of Medicine, Albert Einstein College of Medicine, New York, N.Y., USA.

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.

52: Infection. 1996 Jan-Feb;24,1:64-8. **Azithromycin and doxycycline for treatment of Borrelia culture-positive erythema migrans.** Strle F, Maraspin V, Lotric-Furlan S, Ruzi·-Sablji· E, Cimperman J.

Dept. of Infectious Diseases, University Medical Centre Ljubljana, Japlijeva, Slovenia.

Adult patients with typical solitary erythema migrans, participating in prospective therapeutic studies on early Lyme borreliosis at the Lyme borreliosis Outpatient's Clinic, University Department of Infectious Diseases in Ljubljana, in 1991 to 1993, **and followed up for 1 year, were included in the study. Only patients who were treated with azithromycin or doxycycline and in whom Borrelia burgdorferi was isolated from the border of the skin lesion prior to institution of antibiotic treatment were selected for presentation in this report.** Fifty-eight patients received azithromycin, 500 mg twice daily for the first day, followed by 500 mg once daily for 4 days, and 42 patients received doxycycline, 100 mg twice daily for 14 days. The median duration of skin lesions after the beginning of treatment was 6.5, 2-30, days in the azithromycin

group and 8, 2-35, days in the doxycycline group, non-significant difference. **During the follow-up of 12 months one patient in each group developed major later manifestations of Lyme borreliosis and in 19 patients minor manifestations appeared: in nine, 20, 15.5%, treated with azithromycin and in ten, 23.8%, receiving doxycycline. In one patient in the azithromycin group and in one patient in the doxycycline group B. burgdorferi was isolated from normal appearing skin at the site of previous erythema migrans 2 months after the institution of antibiotic therapy. Five, 8.6%, patients receiving azithromycin and nine, 21.4%, patients receiving doxycycline reported mild to moderate gastrointestinal discomfort. In addition, five patients treated with doxycycline developed photosensitivity.**

53: 1: Infection. 1996 Jan-Feb;24,1:9-16. Erratum in: Infection 1996 Mar-Apr;24,2:169.**Kill kinetics of Borrelia burgdorferi and bacterial findings in relation to the treatment of Lyme borreliosis.** Preac Mursic V, Marget W, Busch U, Pleterski Rigler D, Hagl S. Max v. Pettenkofer Institut, Ludwig-Maximilians-Universität München, Germany.

For a better understanding of the persistence of Borrelia burgdorferi sensu lato, s.l., after antibiotic therapy the kinetics of killing B. burgdorferi s.l. under amoxicillin, doxycycline, cefotaxime, ceftriaxone, azithromycin and penicillin G were determined. The killing effect was investigated in MKP medium and human serum during a 72 h exposure to antibiotics. Twenty clinical isolates were used, including ten strains of Borrelia afzelii and ten strains of Borrelia garinii. The results show that the kinetics of killing borreliae differ from antibiotic to antibiotic. The killing rate of a given antibiotic is less dependent on the concentration of the antibiotic than on the reaction time. Furthermore , the data show that the strains of B. afzelii and B. garinii have a different reaction to antibiotics used in the treatment of Lyme borreliosis and that different reactions to given antibiotics also exist within one species.**The B. garinii strains appear to be more sensitive to antibiotics used in therapy. Furthermore, the persistence 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 titers to B. burgdorferi.**

54: Infection. 1996 Jan-Feb;24,1:73-5. **Treatment failure in erythema migrans--a review.** Weber K. Dermatologische Privatpraxis, München, Germany.

Patients with erythema migrans can fail to respond to antibiotic therapy. Persistent or recurrent erythema migrans, major sequelae such as meningitis and arthritis, **survival of Borrelia burgdorferi and significant and persistent increase of antibody titres against B. burgdorferi after antibiotic therapy are strong indications of a treatment failure.** Most, if not all, antibiotics used so far have been associated with a treatment failure in patients with erythema migrans. Roxithromycin and erythromycin are definitely or probab ly ineffective. However, doxycycline,

amoxicillin, cefuroxime, ceftriaxone, azithromycin and high-dose penicillin V perform comparably well.

55: Infection. 1996 May-Jun;24,3:218-26. Erratum in: Infection 1996 Jul-Aug;24,4:335. **Formation and cultivation of Borrelia burgdorferi spheroplast-L-form variants.** Mursic VP, Wanner G, Reinhardt S, Wilske B, Busch U, Marget W.

Max von Pettenkofer-Institut, Ludwig-Maximilians-Universität München, Germany.

As clinical persistence of Borrelia burgdorferi in patients with active Lyme borreliosis occurs despite obviously adequate antibiotic therapy, in vitro investigations of morphological variants and atypical forms of B. burgdorferi were undertaken. In an attempt to learn more about the variation of B. burgdorferi and the role of atypical forms in Lyme borreliosis, borreliae isolated from antibiotically treated and untreated patients with the clinical diagnosis of definite and probable Lyme borreliosis and from patient specimens contaminated with bacteria were investigated. Furthermore, the degeneration of the isolates during exposure to penicillin G in vitro was analysed. Morphological analysis by darkfield microscopy and scanning electron microscopy revealed diverse alterations. Persisters isolated from a great number of patients, 60-80%, after treatment with antibiotics had an atypical form. **The morphological alterations in culture with penicillin G developed gradually and increased with duration of incubation. Pleomorphism, the presence of elongated forms and spherical structures, the inability of cells to replicate, the long period of adaptation to growth in MKP-medium and the mycoplasma-like colonies after growth in solid medium, PMR agar, suggest that B. burgdorferi produce spheroplast-L-form variants.** With regard to the polyphasic course of Lyme borreliosis, these forms without cell walls can be a possible reason why **Borrelia survive in the organism for a long time, probably with all beta-lactam antibiotics, [corrected] and the cell-wall-dependent antibody titers disappear and emerge after reversion.**

56: JAMA. 1996 Jun 5; 275,21, :1657-60. **Concurrent Lyme disease and babesiosis. Evidence for increased severity and duration of illness.** K rause PJ, Telford SR 3rd, Spielman A, Sikand V, Ryan R, Christianson D, Burke G, Brassard P, Pollack R, Peck J, Persing DH.

Department of Pediatrics, University of Connecticut School of Medicine, Farmington 06030, USA.

OBJECTIVE--To determine whether patients coinfected with Lyme disease and babesiosis in sites where both diseases are zoonotic experience a greater number of symptoms for a longer period of time than those with either infection alone. DESIGN--Community-based, yearly serosurvey and clinic-based cohort study. SETTING--Island community in Rhode Island and 2 Connecticut

medical clinics from 1990 to 1994. STUDY PARTICIPANTS--Long-term residents of the island community and patients seeking treatment at the clinics. MAIN OUTCOME MEASURES--Seroreactivity to the agents of Lyme disease and babesiosis and number and duration of symptoms. RESULTS--Of 1156 serosurvey subjects, 97, 8.4%, were seroreactive against Lyme disease spirochete antigen, of whom 14, 14%, also were seroreactive against babesial antigen. Of 240 patients diagnosed with Lyme disease, 26, 11%, were coinfected with babesiosis. Coinfected patients experienced fatigue, P = .002, headache, P < .001, sweats, P < .001, chills, P = .03, anorexia, P = .04, emotional lability, P = .02, nausea, P = .004, conjunctivitis, P = .04, and splenomegaly, P = .01, more frequently than those with Lyme disease alone. Thirteen, 50%, of 26 coinfected patients were symptomatic for 3 months or longer compared with 7,4%, of the 184 patients with Lyme disease alon e from whom follow-up data were available, P < .001.**Patients coinfected with Lyme disease experienced more symptoms and a more persistent episode of illness than did those, n = 10, experiencing babesial infection alone. Circulating spirochetal DNA was detected more than 3 times as often in coinfected patients as in those with Lyme disease alone, P = .06.**

CONCLUSIONS-- **Approximately 10% of patients with Lyme disease in southern New England are coinfected with babesiosis** in sites where both diseases are zoonotic. The number of symptoms and duration of illness in patients with concurrent Lyme disease and babesiosis are greater than in patients with either infection alone. In areas where both Lyme disease and babesiosis have been reported, the possibility of concomitant babesial infection should be considered when moderate to severe Lyme disease has been diagnosed.

57: 1: Antimicrob Agents Chemother. 1996 Jun;40,6:1552-4. **Eucaryotic cells protect Borrelia burgdorferi from the action of penicillin and ceftriaxone but not from the action of doxycycline and erythromycin.** Brouqui P, Badiaga S, Raoult D. Unité des Rickettsies, Faculté de Médecine, Centre National de la Recherche Scientifique, Marseille, France.

Despite appropriate antibiotic treatment, Lyme disease patients may have relapses or may develop chronic manifestations. The intracellular location of Borrelia burgdorferi suggests that antibiotics that penetrate cells will have greater efficiency. Doxycycline or erythromycin was more effective than penicillin or ceftriaxone in killing B. burgdorferi when the organism was grown in the presence of eucaryotic cells

58: Repeat

59: 1: Infection. 1996 Sep-Oct;24,5:347-53.**Borrelia burgdorferi DNA in the urine of treated patients with chronic Lyme disease symptoms. A PCR study of 97 cases.**
Bayer ME, Zhang L, Bayer MH. Fox Chase Cancer Center, Philadelphia, PA 19111, USA.

The presence of Borrelia burgdorferi DNA was established by PCR from urine samples of 97 patients clinically diagnosed as presenting with symptoms of chronic Lyme disease. All patients had shown erythema chronica migrans following a deer tick bite. Most of the patients had been antibiotic-treated for extended periods of time. We used three sets of primer pairs with DNA sequences for the gene coding of outer surface protein A, OspA, and of a genomic sequence of B. burgdorferi to study samples of physician-referred patients from the mideastern USA. Controls from 62 healthy volunteers of the same geographic areas were routinely carried through the procedures in parallel with patients' samples.**Of th e 97 patients, 72, 74.2%, were found with positive PCR and the rest with negative PCR. The 62 healthy volunteers were PCR negative. It is proposed that a sizeable group of patients diagnosed on clinical grounds as having chronic Lyme disease may still excrete Borrelia DNA, and may do so in spite of intensive antibiotic treatment.**

The urine of **74.2% of patients previously treated with antibiotics for Lyme disease was found to be positive for B. burgdorferi** DNA using PCR testing. All patients, n=97, had prior documented EM rash and had received a minimum of **3 weeks to 2 months oral or intravenous antibiotics. In 4 patients, PCR results were temporarily negative after treatment, but became positive again 4-6 weeks later. All patients suffered continuing, often gradually worsening Lyme disease-like symptoms. ..it seems to be characteristic for most of the patients in our study that, after antibiotic-free periods of a few months, they had again become increasingly ill with neurological and arthritic symptoms, so that treatment had been resumed.**

60: Hum Pathol. 1996 Oct;27,10:1025-34.**Ultrastructural demonstration of spirochetal antigens in synovial fluid and synovial membrane in chronic Lyme disease: possible factors contributing to persistence of organisms.** Nanagara R, Duray PH, Schumacher HR Jr. Allergy-Immunology-Rheumatology Division, Department of Medicine, Faculty of Medicine, KhonKaen University, Thailand.

To perform the first systematic electronmicroscopic, EM, and immunoelectron microscopy, IEM, study of the pathological changes and the evidence of spirochete presence in synovial membranes and synovial fluid, SF, cells of patients with chronic Lyme arthritis. EM examination was performed on four synovial membrane and eight SF cell samples from eight patients with chronic Lyme disease. Spirochetal antigens in the samples were sought by IEM using monoclonal antibody to Borrelia burgdorferi outer surface protein A, OspA, as the immunoprobe. Prominent ultrastructural findings were surface fibrin-like material, thickened synovial lining cell layer and signs of vascular injury. Borrelia-like stru ctures were identified in all four synovial membranes and in two of eight SF cell samples. The presence of spirochetal antigens was confirmed by IEM in all four samples studied, one synovial membrane and three SF cell samples. OspA labelling was

in perivascular areas, deep synovial stroma among collagen bundles, and in vacuoles of fibroblasts in synovial membranes; and in cytophagosomes of mononuclear cells in SF cell samples.**Electron microscopy adds further evidence for persistence of spirochetal antigens in the joint in chronic Lyme disease. Locations of spirochetes or spirochetal antigens both intracellulary and extracellulary in deep synovial connective tissue as reported here suggest sites at which spirochaetes may elude host immune response and antibiotic treatment.**

61: Rheumatol Int. 1996;16,3:125-32.**Intracellular persistence of Borrelia burgdorferi in human synovial cells.** Girschick HJ, Huppertz HI, Rüssmann H, Krenn V, Karch H.

Children's Hospital, University of Würzburg, Germany.

To investigate if Borrelia burgdorferi can persist in resident joint cells, an infection model using cell cultures of human synovial cells was established and compared to the interaction of Borrelia burgdorferi and human macrophages. Borrelia burgdorferi were found attached to the cell surface or folded into the cell membrane of synovial cells analysed by transmission electron and confocal laser scanning microscopy. In contrast to macrophages, morphologically intact Borrelia burgdorferi were found in the cytosol of synovial cells without engulfment by cell membrane folds or phagosomes. Borrelia burgdorferi were isolated from parallel cultures.**Treatment with ceftriaxone eradicated extracellular Borrelia burgdorferi, but spirochetes were reisolated after lysis of the synovial cells. Borrelia burgdorferi persisted inside synovial cells for at least 8 weeks. These data suggested that Borrelia burgdorferi might be able to persist within resident joint cells in vivo.**

62: Antimicrob Agents Chemother. 1996 Nov;40 11 :2632-6.**In vivo activities of ceftriaxone and vancomycin against Borrelia spp in the mouse brain and other sites.** Kazragis RJ, Dever LL, Jorgensen JH, Barbour AG.

Department of Medicine Infectious Diseases, University of Texas Health Science Center at San Antonio 78284, USA.

Borrelia burgdorferi, the agent of Lyme disease, and B. turicatae, a neurotropic agent of relapsing fever, are susceptible to vancomycin in vitro, with an MIC of 0.5 microgram/ml. To determine the activity of vancomycin in vivo, particularly in the brain, we infected adult immunocompetent BALB/c and immunodeficient CB-17 scid mice with B. burgdorferi or B. turicatae. The mice were then treated with vancomycin, ceftriaxone as a positive control, or normal saline as a negative control. The effectiveness of treatment was assessed by cultures of blood and brain and other tissues. Ceftriaxone at a dose of 25 mg/kg of body weight administered every 12 h for 7 to 10 days eliminated cultivable B. burgdorferi or B. turicatae from all BALB/c or

scid mice in the study.**Vancomycin at 30 mg/kg administered every 12 h was effective in eliminating infection from immunodeficient mice if treatment was started within 3 days of the onset of infection. If treatment with vancomycin was delayed for 7 days or more, vancomycin failed to eradicate infection with B. burgdorferi or B. turicatae from immunodeficient mice. The failure of vancomycin in eradicating established infections in immunodeficient mice was associated with the persistence of viable spirochetes in the brain during antibiotic treatment.**

63: Brain. 1996 Dec;119, Pt 6:2143-54. **Inflammatory brain changes in Lyme borreliosis. A report on three patients and review of literature.** Oksi J, Kalimo H, Marttila RJ, Marjamäki M, Sonninen P, Nikoskelainen J, Viljanen MK.

Department of Internal Medicine, Turku University Central Hospital, Finland.

Despite a rapid increase in the number of patients with Lyme neuroborreliosis, LNB, its neuropathological aspects are poorly understood. The objective of this study was evaluation of neuropathological, microbiological, and magnetic resonance imaging, MRI, findings in three patients with the **Borrelia burgdorferi infection and neurological disease from whom brain tissue specimens were available.** Perivascular or vasculitic lymphocytic inflammation was detected20in all specimens. Large areas of demyelination in periventricular white matter were detected histologically and by MRI in one patient. **The disease had a fatal outcome in this patient. Brain MRI suggested malignancies in two patients before histopathological studies were carried out. One of these two patients was a child with sudden hemiparesis. Another was a 40-year-old man presenting with epileptic seizures and MRI-detected multifocal lesions, which disappeared after repeated courses of antibiotics.** We conclude that cerebral lymphocytic vasculitis and multifocal encephalitis may be associated with B. burgdorferi infection. The presence of B. burgdorferi DNA in tissue samples from areas with inflammatory changes indicates that direct invasion of B. burgdorferi may be the pathogenetic mechanism for focal encephalitis in LNB.

"In one of the six analysed brain tissue specimens [from a patient who had received more than six months of antibiotic treatment prior to death, including two 3-week courses of IV ceftriaxone], B. burgdorferi DNA was detected by PCR."

64: Am J Dermatopathol. 1996 Dec;18,6:571-9. **Heterogeneity of Borrelia burgdorferi in the skin.** Aberer E, Kersten A, Klade H, Poitschek C, Jurecka W.

Department of Dermatology, University of Vienna, Austria.

The reliability of various in vitro techniques to identify Borrelia burgdorferi infection is still unsatisfactory. Using a high-power resolution videomicroscope and staining with the borrelia genus-specific monoclonal flagellar antibody H9724, we identified borrelial structures in skin biopsies of erythema chronicum migrans, from which borrelia later was cultured, of acrodermatitis chronica atrophicans, and of morphea. In addition to typical borreliae, we noted stained structures of varying shapes identical to borreliae found in a "borrelia-injected skin" model; identical to agar-embedded borreliae; **and identical to cultured borreliae following exposure to hyperimmune sera and/or antibiotics. We conclude that the H9724-reactive structures represent various forms of B. burgdorferi rather than staining artifacts. These "atypical" forms of B. burgdorferi may represent in vivo morphologic variants of this bacterium.**

Neuralgias arising 6 months after ECM in spite of antibiotic therapy were evident in a seronegative patient who showed perineural rod-like borrelia structures.

65: 328: Semin Neurol. 1997 Mar;17,1:25-30.**Peripheral nervous system Lyme borreliosis.** Logigian EL.

Harvard Medical School, Clinical Neurophysiology Laboratory, Brigham and Women's Hospital, Boston, Massachusetts 02115, USA.

There are acute and chronic Lyme neuropathies. The seasonal acute syndromes of cranial neuritis or radiculoneuritis are generally quite distinctive, but may cause diagnostic difficulty when one syndrome occurs without the other, when erythema migrans is absent or missed, and when meningeal signs are minimal or absent. The chronic Lyme radiculoneuropathies are less severe, and less distinctive. Their recognition depends on eliciting a history of earlier classical manifestations of Lyme disease and by labor atory testing. In both acute and chronic Lyme radiculoneuropathy, electrophysiologic testing often proves the presence of a sensorimotor, axon loss polyradiculoneuropathy. Both acute and chronic Lyme radiculoneuropathy have similar pathologic features and can be classified as a nonvasculitic mononeuritis multiplex. The pathogenesis is uncertain; both direct infection as well as parainfectious mechanisms may play a role.**The treatment with which we have the most experience is intravenous ceftriaxone 2 g/day for 2 to 4 weeks. Improvement occurs rapidly over days to weeks in early Lyme neuroborreliosis, but slowly over many months in chronic neuroborreliosis.**

65.5: J Clin Microbiol. 1997 January; 35(1): 111–116. Persistence of Borrelia burgdorferi in experimentally infected dogs after antibiotic treatment. R K Straubinger, B A Summers, Y F Chang, and M J Appel Institute for Animal Health, College of Veterinary Medicine, Cornell University, Ithaca, New York 14853, USA. rks4@cornell.edu

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.**

66: Clin Infect Dis. 1997 Jul;25 Suppl 1:S52-6. **Tetracycline therapy for chronic Lyme disease.** Donta ST.

Boston University Medical Center and Boston Veterans Affairs Medical Center, Massachusetts 02118, USA.

Two hundred seventy-seven patients with chronic Lyme disease were treated with tetracycline for 1 to 11 months, mean, 4 months; the outcomes for these patients were generally good. Overall, 20% of the patients were cured; 70% of the patients' conditions improved, and treatment failed for 10% of the patients. Improvement frequently did not take place for several weeks; after 2 months of treatment, 33% of the patients' conditions were significantly improved, degree of improvement, 75%-100%, and after 3 months of treatment, 61% of the patients' conditions were significantly improved. Treatment outcomes for seronegative patients, 20% of all patients, were similar to those for seropositive patients. Western immunoblotting showed reactions to one or more Borrelia burgdorferi-specific proteins for 65% of the patients for whom enzyme-linked immunosorbent assays were negative. Whereas age, sex, and prior erythema migrans were not correlated with better or worse treatment outcomes, **a history of longer duration of symptoms or antibiotic treatment was associated with longer treatment times to achieve improvement and cure. These results support the use of longer courses of treatment in the management of patients with chronic Lyme disease. Controlled trials need to be conducted to validate these observations.**

67: 1: Clin Infect Dis. 1997 Jul;25 Suppl 1:S64-70.**Why is chronic Lyme borreliosis chronic?** Aberer E, Koszik F, Silberer M.

Department of Dermatology, University of Graz Medical School, Austria.

Chronic Lyme borreliosis, CLB, can present not only in different organs but also in different patterns. Although many theories exist about the mechanisms leading to CLB, it is known that viable Borrelia burgdorferi can persist for decades and cause late skin manifestations of acrodermatitis chronica atrophicans, ACA. Thus, the immunopathogenetic findings in ACA can serve as a model for studying the chronic course of Lyme borreliosis. Recent findings indicate that the most important cell for antigen presentation, the epidermal Langerhans cell, LC, is invaded by B. burgdorferi in early Lyme borreliosis. Therefore, LCs were stained immunohistochemically with different markers to investigate their functional activity. Numbers of CD1a+ LCs were reduced in erythema migrans but normal or slightly elevated in ACA. In both diseases there was also a marked downregulation of major histocompatibility complex class II molecules on LCs, as measured by staining of human leukocyte antigen DR.**This phenomenon might be a mechanism that protects against the presentation of autoantigens and may be the cause of the impaired capacity of LCs to eliminate B. burgdorferi antigens, thus explaining why CLB is chronic.**

68: 1: Infection. 1997 Jul-Aug;25,4:240-6.**Transformation of cystic forms of Borrelia burgdorferi to normal, mobile spirochetes.** Brorson O, Brorson SH.

Dept. of Microbiology, Ullevål University Hospital, Oslo, Norway.

The purpose of this study was to evaluate the behaviour of Borrelia burgdorferi under controlled conditions. The occurrence o f cystic forms of Borrelia burgdorferi in vitro was noted, and these cysts were able to be transformed to normal, mobile spirochetes. B. burgdorferi was cultivated in a commercial culture medium without serum. The spirochetes multiplied only slowly in this medium, and transformation to encysted forms was observed after 1 week. When these cysts were transferred to the same culture medium with rabbit serum, the encysted forms developed into regular, mobile spirochetes after 6 weeks, and their regeneration time was normal. Examination of these cysts in the transmission electron microscope revealed transverse fission inside the cysts. It is probable that similar phenomena may occur in vivo under conditions unfavourable for spirochetes. **These observations may help to explain why diagnosis and treatment of B. burgdorferi infections in humans can be difficult.**

69: American College of Rheumatology, Vol 40,9, Branigan P; Rao J; 1997 **PCR evidence for Borrelia burgdorferi DNA in synovium in absence of positive serology.** Suppl, Rao J; Gerard H; Sept, p.S270 Hudson A; Williams W; Arayssi T; Pando J; Bayer M; Rothfuss S; .PCR evidence for Borrelia has been identified in synovial biopsies of patients with clinical pictures that had not

initially suggested Lyme disease. Clayburne G; Sieck M; Schumacher HR.

All [6 PCR-positive] patients were negative for antibodies to Borrelia and some were **PCR positive in synovium despite previous treatment with antibiotics.**

70: Journal of Spirochetal & Tick-borne Diseases, Vol. 4, No. 1/2 **Two lessons from the canine model of Lyme Disease: migration of Borrelia burgdorferi in tissues and persistence after antibiotic treatment.** Straubinger RK; 1997 Straubinger AF; Jacobson RH; Chang Y; Summer BA;

[Persistence:] **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.

[Diagnostic issues:] In the dog model, we detected B. burgdorferi reliably in skin but infrequently in blood by culture and polymerase chain reaction, PCR. **We found the organism in the synovium of joints but not in synovial fluids, and in meninges but not in cerebrospinal fluid.**

71: 1: Ann Rheum Dis. 1998 Feb;57,2:118-21.**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.** Priem S, Burmester GR, Kamradt T, Wolbart K, Rittig MG, Krause A.

Charité University Hospital, Department of Medicine III, Rheumatology and Clinical Immunology, Berlin, Germany.

OBJECTIVES: **To identify possible sites of bacterial persistence in patients with treatment resistant Lyme arthritis. It was determined whether Borrelia burgdorferi DNA may be detectable by polymerase chain reaction, PCR, in synovial membrane, SM, when PCR results from synovial fluid, SF, had become negative after antibiotic therapy.** METHODS: Paired SF and SM specimens and urine samples from four patients with ongoing or recurring Lyme arthritis despite previous antibiotic therapy were investigated. A PCR for the detection of B burgdorferi DNA was carried out using pri mer sets specific for the ospA gene and a p66 gene of B burgdorferi. RESULTS: In all four cases, PCR with either primer set was negative in SF and urine, but was positive with at least one primer pair in the SM specimens. In all patients arthritis completely resolved after additional antibiotic treatment. CONCLUSIONS: **These data suggest**

that in patients with treatment resistant Lyme arthritis negative PCR results in SF after antibiotic therapy do not rule out the intraarticular persistence of B burgdorferi DNA. Therefore, in these patients both SF and SM should be analysed for borrelial DNA by PCR as positive results in SM are strongly suggestive of ongoing infection.

72: Med J Aust. 1998 May 18;168,10:500-2. Comment in: Med J Aust. 1998 May 18;168,10:479-80. **Culture-positive Lyme borreliosis.** Hudson BJ, Stewart M, Lennox VA, Fukunaga M, Yabuki M, Macorison H, Kitchener-Smith J.

Microbiology Department, Royal North Shore Hospital, Sydney, NSW.
bhudson@med.usyd.edu.au

We report a case of Lyme borreliosis. Culture of skin biopsy was positive for Borrelia garinii, despite repeated prior treatment with antibiotics. The patient had travelled in Europe 17 months before the onset of symptoms, but the clinical details indicate that the organism could have been acquired in Australia. The results of conventional serological and histopathological tests were negative, despite an illness duration of at least two years.

73: Acta Clin Belg. 1998 Jun;53,3:178-83.**Lyme borreliosis--a review of the late stages and treatment of four cases.** Petrovic M, Vogelaers D, Van Renterghem L, Carton D, De Reuck J, Afs chrift M. Department of Internal Medicine, University Hospital Ghent, Belgium.

Difficulties in diagnosis of late stages of Lyme disease include low sensitivity of serological testing and late inclusion of Lyme disease in the differential diagnosis.**Longer treatment modalities may have to be considered in order to improve clinical outcome of late disease stages.** These difficulties clinical cases of Lyme borreliosis. The different clinical cases illustrate several aspects of late borreliosis: false negative serology due to narrow antigen composition of the used ELISA format, **the need for prolonged antibiotic treatment in chronic or recurrent forms and typical presentations of late Lyme disease**, such as lymphocytic meningo-encephalitis and polyradiculoneuritis.

A five-week treatment with doxycycline at a dose of 200 mg daily was prescribed. Fatigue, arthralgia en myalgia seemed to respond positively to Carton D; et al. the initiated therapy.**However, they reappeared two weeks after cessation of doxycycline. ..it was decided to treat with ceftriaxone IM 2 g daily for three weeks. This resulted in a complete resolution of the general symptoms. However, three weeks later arthralgia of the knees and myalgia in both legs recurred.** Symptoms and signs may improve only temporarily shortly after treatment, but re-emerge within weeks or months.

74: 1: Eur J Clin Microbiol Infect Dis. 1998 Oct;17,10:715-9.**Comparison of oral cefixime and intravenous ceftriaxone followed by oral amoxicillin in disseminated Lyme borreliosis.** Oksi J, Nikoskelainen J, Viljanen MK. Department of Medicine, Turku University Central Hospital, Finland.

Two treatment regimens for disseminated Lyme borreliosis, mainly neurologic and musculoskeletal manifestations, were compared in a randomized trial. A group of 30 patients received oral cefixime 200 mg combined with probenecid 500 mg three times daily for 100 days. Another group of 30 patients received intravenous ceftriaxone 2 g daily for 14 days followed by oral amoxicillin 500 mg combined with probenecid 500 mg three times daily for 100 days. There was no statistically significant difference in the outcome of infection between the two groups. However, the total number of patients with relapses or no response at all and the number of positive polymerase chain reaction findings after therapy were greater in the cefixime group. The general outcomes of infection in patients with disseminated Lyme borreliosis after **3-4 months of therapy indicate that prolonged courses of antibiotics may be beneficial in this setting, since 90% of the patients showed excellent or good treatment responses.**

75: Neurology. 1998 Nov;51,5:1489-91. Comment in: Neurology. 1999 Sep 11;53,4:895-6. **Clinical and serologic follow-up in patients with neuroborreliosis.** Treib J, Fernandez A, Haass A, Grauer MT, Holzer G, Woessner R.

Department of Neurology, University of the Saarland, Homburg, Germany.

The authors performed a clinical and serologic follow-up study after 4.2 +/- 1.2 years in 44 patients with clinical signs of neuroborreliosis and specific intrathecal antibody production. All patients had been treated with ceftriaxone 2 g/day for 10 days. Although neurologic deficits decreased significantly, **more than half the patients had unspecific complaints resembling a chronic fatigue syndrome and showed persisting positive immunoglobulin M serum titers for Borrelia in the Western blot analysis.**

76: 1: Infection. 1998 Nov-Dec; 26,6:364-7.**A proposal for the reliable culture of Borrelia burgdorferi from patients with chronic Lyme disease, even from those previously aggressively treated.** Phillips SE, Mattman LH, Hulínská D, Moayad H. Greenwich Hospital, CT 06830, USA.

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 persist ent 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.

77: Klin Monatsbl Augenheilkd. 1998 Dec;213,6:351-4. **Pars plana vitrectomy in Borrelia burgdorferi endophthalmitis** [Article in German] Meier P, Blatz R, Gau M, Spencker FB, Wiedemann P.

Klinik und Poliklinik für Augenheilkunde der Universität Leipzig.

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.

78: Ann Med. 1999 Jun; 3,3:225-32. **Borrelia burgdorferi detected by culture and PCR in clinical relapse of disseminated Lyme borreliosis.** Oksi J, Marjamäki M, Nikoskelainen J, Viljanen MK.

Department of Medicine, Turku University Central Hospital, Finland. jarmo.oksi@utu.fi

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, and **32 of them were regarded as having a clinically defined treatment failure. Of the 165 patients, 136 were tested by polymerase chain reaction, PCR, during the follow-up. PCR was positive from the plasma of 14 patients 0-30 months after discontinuation of the treatment, and 12 of these patients had a clinical relapse.** In addition, Borrelia burgdorferi was cultured from the blood of three patients during the follow-up. All three patients belonged to the group with relapse, and two of them were also PCR positive. This report focuses on the 13 patients with clinical relapse and culture or PCR positivity. Eight of the patients had culture or PCR-proven initial diagnosis, the diagnosis of the remaining five patients was based on positive serology only.**All 13 patients were primarily treated for more than 3 months with intravenous and/or oral antibiotics,11 of them received intravenous ceftriaxone, nine for 2 weeks, one for 3 weeks and one for 7 weeks, followed by oral antibiotics. The treatment caused only temporary relief in the symptoms of the patients.**All but one of them had negative PCR results immediately after the first treatment. The patients were retreated usually with intravenous ceftriaxone for 4-6 weeks. None of them was PCR positive after the retreatment.**The response to retreatment was considered good in nine patients. We conclude that the treatment of Lyme borreliosis with appropriate antibiotics for even more than 3 months may not always eradicate the spirochete.**By using PCR, it is possible to avoid unnecessary retreatment of patients with 'post-Lyme syndrome' and those with 'serological scars' remaining detectable for months or years after infection.

79: Zentralbl Bakteriol. 1999 Jul;289,3:301-18. **Persistence of Borrelia garinii and Borrelia afzelii in patients with Lyme arthritis.** Hulínská D, Votýpka J, Valeso vá M.

National Institute of Public Health, Prague, Czech Republic.

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.**

80: J Infect Dis. 2000 Mar;181,3:1069-81. **Status of Borrelia burgdorferi infection after antibiotic treatment and the effects of corticosteroids: An experimental study.** Straubinger RK, Straubinger AF, Summers BA, Jacobson RH.

James A. Baker Institute for Animal Health, College of Veterinary Medicine, Cornell University, Ithaca NY, 14853, USA. rks4@cornell.edu

Sixteen specific-pathogen-free beagles were infected with Borrelia burgdorferi. Three groups of 4 dogs were treated with antibiotics for 30 consecutive days starting 120 days after tick exposure; 4 dogs were untreated controls. At day 420 after tick exposure and again before euthanasia, 2 dogs of each group were treated with prednisone for 14 days. All dogs contracted infection and 11 developed acute arthritis 50-120 days after exposure. After day 120, one of 12 antibiotic-treated dogs and 2 of 4 untreated dogs became lame. Antibiotic therapy reduced the freq uency of Borrelia-positivity in subsequent skin biopsy samples. After prednisone treatment, both control dogs developed severe polyarthritis. At euthanasia, single tissues of the antibiotic-treated dogs and multiple tissues of all control dogs were Borrelia-positive by polymerase chain reaction. Viable spirochetes were not recovered from antibiotic-treated dogs. Two antibiotic-treated dogs showed histologic evidence of minimal lesions, whereas all control dogs had mild polyarthritis with periarteritis.

16 dogs were infected with Borrelia burgdorferi. 120 days after tick exposure, 12 dogs were treated with antibiotics for 30 days; 4 control dogs were not treated. .At euthanasia, single tissues of the antibiotic-treated dogs and multiple tissues of all control dogs were Borrelia-positive by polymerase chain reaction.[Persistence:] .Do the data indicate an ongoing persistent infection in these animals or only the presence of DNA remnants of dead Borrelia..? From this study and our previous investigations, 20, it appears likely that **B. burgdorferi maintains a persistent infection with live organisms albeit at a very low level,** p.1079, [Diagnosis:] As demonstrated by the injection of heat-killed B. burgdorferi organisms into the skin of an uninfected animal, DNA of dead organisms was detectable in our hands only for 3 weeks. These results are in concordance with a study in which persistent experimental infection with Treponema pallidum, the spirochetal agent of syphilis, was identified by PCR, 21. Wicher et al.[1998] discovered that DNA of dead Treponema organisms was removed from or degraded within rabbit tissue within 15-30 days after syringe inoculation, p.1079, Our studies show that at least in the dog, blood is an unreliable tissue to demonstrate B. burgdorferi infection., p.1080

81: J Clin Microbiol. 2000 Jun; 38,6, :2191-9. **PCR-Based quantification of Borrelia burgdorferi organisms in canine tissues over a 500-Day postinfection period.** Straubinger RK. James A. Baker Institute for Animal Health, College of Veterinary Medicine, Cornell University, Ithaca, New York 14853, USA. rks4@cornell.edu

Borrelia burgdorferi infection in beagle dogs was studied quantitatively with skin punch biopsy samples and blood samples collected at 4- and 2-week intervals, respectively, over a 500-day period. Thereafter, **25 tissue samples of each dog were collected for further analysis.** Starting at day 120 after tick challenge, **12 dogs were treated with antibiotics, azithromycin, ceftriaxone, or doxycycline, for 30 consecutive days. Four dogs received no antibiotic therapy.** Quantification of B. burgdorferi DNA was done with an ABI Prism 7700 Sequence Detection System with oligonucleotide primers and a fluorescence-labeled probe designed to specifically amplify a fragment of the ospA gene of B. burgdorferi strain N40. All 16 dogs became infected with B. burgdorferi after tick challenge. In skin biopsy samples, **spirochete numbers peaked at day 60 postinfection**, <1.5 x 10, 6, organisms per 100 microgram of extracted DNA, at the same time when **clinical signs of arthritis developed in 11 of 16 dogs, and decreased to almost undetectable levels during the following 6 months. The number of B. burgdorferi organisms detected in skin biopsy samples was inversely correlated with the antibody levels measured by enzyme-linked immunosorbent assay. Antibiotic treatment reduced the amount of detectable spirochete DNA in skin tissue by a factor of 1,000 or more. At the end of the experiment, B. burgdorferi DNA was detectable at low levels, 10, 2, to 10, 4, organisms per 100 microgram of extracted DNA, in multiple tissue samples regardless of treatment.** However, more tissue samples of untreated dogs than of antibiotic-treated dogs were positive, and tissue samples of untreated dogs also were positive by culture. Only 1.6% of 576 blood samples of all dogs were positive for B. burgdorferi by PCR.

82: Straubinger RK, Straubinger AF, Summers BA, Jacobson RH, Erb HN. James A. Baker Institute for Animal Health, Ithaca, New York, USA. rks4@cornell.edu

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 approp riate 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 pe rsistently 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.**

83: Kaiser R. Neurologische Klinik, Städtisches Klinikum Pforzheim. K aiser.Neurologische_Klinik@Stadt-Pforzheim.de

Between 1990 and 2000, a total of 101 patients with acute, n=86, or chronic, n=15, neuroborreliosis, proven by clinical data, pleocytosis in the CSF, and **elevated Borrelia burgdorferi-specific antibody indices, were treated with 2 g of ceftriaxone per day for either 2 or 3 weeks**. The patients were reexamined clinically and serologically after 3, 6, and 12 months. Six, 12, months after the antibiotic treatment, about 93%, 95%, of the patients with acute neuroborreliosis and 20%, 66%, of the patients with chronic neuroborreliosis were cured. One year after treatment, four patients with acute neuroborreliosis still suffered from facial palsy and five with chronic neuroborreliosis still had moderate spastic ataxic gait disturbance.**The prognosis of facial palsy in neuroborreliosis is quite similar to that in idiopathic facial palsy, while that in chronic neuroborreliosis largely depends on the time elapsed before diagnosis.**

84: 1: Br J Dermatol. 2001 Feb;144,2:387-92. **Isolation and polymerase chain reaction typing of Borrelia afzelii from a skin lesion in a seronegative patient with generalized ulcerating bullous lichen sclerosus et atrophicus.** Breier F, Khanakah G, Stanek G, Kunz G, Aberer E, Schmidt B, Tappeiner G.

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A 64-year-old woman presented with bullous and ulcerating lichen sclerosus et atrophicus,

LSA, on the neck, trunk, genital and perigenital area and the extremities. Histology of lesional skin showed the typical manifestations of LSA; in one of the biopsies spirochaetes were detected by silver staining. Despite treatment with four courses of ceftriaxone with or without methylprednisone for up to 20 days, progression of LSA was only stopped for a maximum of 1 year. **Spirochaetes were isolated from skin cultures obtained from enlarging LSA lesions. These spirochaetes were identified as Borrelia afzelii** by sodium dodecyl sulphate--polyacrylamide gel electrophoresis and polymerase chain reaction, PCR, analyses. However, serology for B. burgdorferi sensu lato was repeatedly negative. After one further 28-day course of ceftriaxone the lesions stopped expanding and sclerosis of the skin was diminished. At this time cultures for spirochaetes and PCR of lesional skin for B. afzelii DNA remained negative. These findings suggest a pathogenetic role for B. afzelii in the development of LSA and a beneficial effect of appropriate antibiotic treatment.

[From the article:] **The relapses she repeatedly suffered despite initially successful antibiotic treatment** could be related to the observation **that Borrelia may possibly be able to remain dormant in certain tissue compartments, thus escaping bactericidal antibiotic activity.** This would be consistent with the fact that these relapses were always able to be treated successfully with a course of the same antibiotics as before; this is **corroborated by a recent report that Bb may persist in experimentally infected dogs despite antibiotic treatment with doxycycline or amoxycillin.**

85: Epidemiol Mikrobiol Imunol. 2001 Feb;50,1:10-6.**Persistence of Borrelia burgdorferi sensu lato in patients with Lyme borreliosis** [Article in Czech] Honegr K, Hulínská D, Dostál V, Gebouský P, Hanková E, Horácek J, Vyslouzil L, Havlasová J. Infekcní klinika, Fakultní nemocnice, Hradec Králové.

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.

86: 1: Ann Neurol. 2001 Sep;50,3, :330-8.**Central and peripheral nervous system infection, immunity, and inflammation in the NHP model of Lyme borreliosis.** Pachner AR, Cadavid D, Shu G, Dail D, Pachner S, Hodzic E, Barthold SW. Department of Neurosciences, UMDNJ-New Jersey Medical School, Newark 07103, USA. pachner@umdnj.edu

The relationship between chronic infection, antispirochetal immunity, and inflammation is unknown in Lyme neuroborreliosis. In the nonhuman primate model of Lyme neuroborreliosis, we measured spirochetal density in the nervous system and othe r tissues by polymerase chain reaction and correlated these values to anti-Borrelia burgdorferi antibody in the serum and cerebrospinal fluid, and to inflammation in tissues. Despite substantial presence of Borrelia burgdorferi, the causative agent of Lyme borreliosis, in the central nervous system, only minor inflammation was present there, though skeletal and cardiac muscle, which contained similar levels of spirochete, were highly inflamed. Anti-Borrelia burgdoferi antibody was present in the cerebrospinal fluid but was not selectively concentrated. All infected animals developed anti-Borrelia burgdorferi antibody in the serum, but increased amplitude of antibody was not predictive of higher levels of infection. **These data demonstrate that Lyme neuroborreliosis is a persistent infection, that spirochetal presence is a necessary but not sufficient condition for inflammation, and that antibody measured in serum may not predict the severity of infection.**

87: Wien Klin Wochenschr. 2002 Jul 31;114,13-14:574-9. **Cystic forms of Borrelia burgdorferi sensu lato: induction, development, and the role of RpoS.** Murgia R, Piazzetta C, Cinco M.

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It has been demonstrated recently that cells of Borrelia burgdorferi sensu lato, the etiological agent of Lyme disease, **transform from mobile spirochetes into nonmotile cystic forms** in the presence of certain unfavourable conditions, and that cystic forms are able to reconvert to vegetative spirochetes in vitro and in vivo. **T he purpose of this study was to investigate the kinetics of conversion of borreliae to cysts in different stress conditions such as starvation media or the presence of different antibiotics.** Using the same experimental conditions we also investigated the possible role in cyst formation of RpoS, an alternative sigma factor that controls a regulon in response to starvation and transition to stationary phase. **We observed that beta-lactams penicillin G and ceftriaxone, the antibiotics of choice in Lyme borreliosis treatment, favoured the production of cysts** when used with serum-depleted BSK medium. In contrast, we observed a low level of cyst formation in the presence of macrolides and tetracyclines. In order to elucidate the role of the rpoS gene in cyst formation we analyzed the reaction of the rpoS mutant strain in comparison with its wild-type in different conditions.

Under the same stimuli, both the wild-type borrelia and the rpoS knock-out isogenic strain produced cystic forms with similar kinetics, thus excluding the participation of the gene in this phenomenon. **Our findings suggest that cyst formation is mainly due to a physical-chemical rearrangement of the outer membrane of Borrelia burgdorferi sensu lato leading to membrane fusion and controlled by different regulation mechanisms.**

87.5: Acta Neurol Scand. 2002 Oct;106(4):205-8. **Chronic symptoms are common in patients with neuroborreliosis -- a questionnaire follow-up study**. Vrethem M, Hellblom L, Widlund M, Ahl M, Danielsson O, Ernerudh J, Forsberg P.

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OBJECTIVES: **The existence of chronic neuroborreliosis is controversial. The aim of our study was to investigate the existence and kind of persistent symptoms in patients previously treated because of neurological symptoms as a result of neuroborreliosis.** MATERIALS AND METHODS: A total of 106 patients with neuroborreliosis, according to established criteria, and a control group of 123 patients with Borrelia induced erythema migrans diagnosed in a general practitioner office were studied. A questionnaire was sent to patients and controls concerning their health situation. Time from onset of neurological symptoms to the questionnaire send out was 32 months, mean, for the patients with neuroborreliosis and 33 months , mean, for the controls. RESULTS: **Fifty per cent of the individuals in the patient group compared with 16% of the individuals in the control group showed persistent complaints after their Borrelia infection**, $P < 0.0001$. **The most significant differences between the groups were the presence of neuropsychiatric symptoms such as headache, attention problems, memory difficulties and depression. Paresthesia, pain and persistent facial palsy was also significantly more common in patients treated because of neuroborreliosis.** CONCLUSION: **Our study shows that persisting neurological symptoms are common after a neuroborreliosis infection**. The pathological mechanisms that lay behind the development of chronic symptoms, however, are still uncertain.

88: J Infect Dis. 2002 Nov 15;186,10:1430-7. Epub 2002 Oct 23. **Detection of attenuated, noninfectious spirochetes in Borrelia burgdorferi-infected mice after antibiotic treatment.** Bockenstedt LK, Mao J, Hodzic E, Barthold SW, Fish D.

Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut 06520-8031, USA. linda.bockenstedt@yale.edu

Xenodiagnosis by ticks was used to determine whether spirochetes **persist in mice after 1 month of antibiotic therapy, Doxycyline and Ceftriaxone, for vectorborne Borrelia burgdorferi infection**. Immunofluorescence and polymerase chain reaction, PCR, were used to show that spirochetes could be found in Ixodes scapularis ticks feeding on 4 of 10 antibiotic-treated mice up to 3 months after therapy. These spirochetes could not be transmitted to naive mice, and some lacked genes on plasmids correlating with infectivity. By 6 months, antibiotic-treated mice no longer tested positive by xenodiagnosis, and cortisone immunosuppression did not alter this result. **Nine months after treatment, low levels of spirochete DNA could be detected by real-time PCR in a subset of antibiotic-treated mice.** In contrast to sham-treated mice, antibiotic-treated mice did not have culture or histopathologic evidence of persistent infection. **These results provide evidence that noninfectious spirochetes can persist for a limited duration after antibiotics but are not associated with disease in mice.**

89: Antimicrob Agents Chemother. 2002 Nov;46,11:3637-40. **Erythromycin resistance in Borrelia burgdorferi**. Terekhova D, Sartakova ML, Wormser GP, Schwartz I, Cabello FC.

Departments of Microbiology and Immunology, New York Medical College, Valhalla, New York 10595, USA.

Susceptibility testing of laboratory strains and clinical isolates of Borrelia burgdorferi indicates that resistance to erythromycin is present in them. Evaluation of the MICs, minimal bactericidal concentrations, and kinetics of bacterial killing of erythromycin suggests that this resistance is increased by preexposure to the antibiotic, is dependent on inoculum size, and may be the result of selection of subpopulations of bacterial cells with increased resistance.

90: 1: Przegl Epidemiol. 2002;56 Suppl 1:57-67.**New aspects of the pathogenesis of lyme disease** [Article in Polish] Zajkowska JM, Hermanowska-Szpakowicz T. Klinika Chorób Zaka·nych i Neuroinfekcji AM w Bia ymstoku.

Morphological changes of B. burgdorferi as well as changes in expression of surface proteins caused by environmental determinants are essential in pathogenesis of Lyme disease.**Cysts, spherical form, spheroplasts, L-form, and "blebs", gemmae, can be responsible for long lasting antigenic stimulation, signs of chronic borreliosis, and even probably connected with MS and Alzheimer disease. Mechanisms to avoid elimination and persistence in the host include:** expression of low heterogenic Osp A, B replaced by polymorphic in sequence and antigenic reactivity OspC, the hindrance of access to some membrane proteins by other proteins on the spirochete's surface, effects of tick saliva proteins action.**Hiding of spirochetes is possible by invagination into fibrocytes membrane** as well as, coating by antigens derived from lymphocytes B. Distribution of spirochetes is facilitated by binding to platelets through integrin

aIIb b3, and to the endothelial cells through integrins av b3 i a5b1, recognition of decorin by lipoproteins DbpA i DbpB, receptor for NAG, N-acetyl glucosamina. Endothelial cells, toxic products of granulocytes, monocytes, macrophages as well as phagocytosis counterpart in pathogenesis. Induced cytokines are connected with activation subsets of T lymphocytes involved in inflammatory response. Cytokines produced by Th1 as cytotoxic CD8 accompany the disease. Important are also dendritic cells regarded as initiators of Th1 response with participation of IL-12. In pathogenesis of Lyme disease participation of autoimmunity is notified, e specially molecular similarities between OspA and human lymphocytic antigen, hLFA-1. Neurotoxin, produced by B. burgdorferi Bbtox1 was identified. Encephalopathy signs in Lyme borreliosis could be result of releasing toxico-metabolic products, **ability of spirochetes to pass the blood-brain barrier as well as, effect of lymphocytes migration. Active invasion of brain endothelium** as ability to adherence to endothelial wall could be the source of focused or disseminated inflammation of brain vessels. Antiaxonal antibodies could disturb axon conduction without damaging. But damage of white matter could be connected with damage of mielin production cells, probably by antibodies, induced in cross reaction.

91: Repeat

92: Neurology. 2003 Jun 24;60,12:1923-30. Comment in: Neurology. 2003 Jun 24;60,12:1888-9.**Study and treatment of post Lyme di sease, STOP-LD: a randomized double masked clinical trial.** Krupp LB, Hyman LG, Grimson R, Coyle PK, Melville P, Ahnn S, Dattwyler R, Chandler B.

Department of Neurology, Stony Brook University Medical Center, Stony Brook, NY 11794-8121, USA. lkrupp@notes.cc.sunysb.edu

OBJECTIVE: **To determine whether post Lyme syndrome, PLS, is antibiotic responsive.** METHODS: The authors conducted a single-center randomized double-masked placebo-controlled trial on 55 patients with Lyme disease with persistent severe fatigue at least 6 or more months after antibiotic therapy. Patients were randomly assigned to receive 28 days of IV ceftriaxone or placebo. The primary clinical outcomes were improvement in fatigue, defined by a change of 0.7 points or more on an 11-item fatigue questionnaire, and improvement in cognitive function, mental speed, defined by a change of 25% or more on a test of reaction time. The primary laboratory outcome was an experimental measure of CSF infection, outer surface protein A, OspA. Outcome data were collected at the 6-month visit. RESULTS: **Patients assigned to ceftriaxone showed improvement in disabling fatigue compared to the placebo group, rate ratio, 3.5; 95% CI, 1.50 to 8.03; p = 0.001.** No beneficial treatment effect was observed for cognitive function or the laboratory measure of persistent infection. Four patients, three of whom were on placebo, had adverse events associated with treatment, which required hospitalization.

CONCLUSIONS: **Ceftriaxone therapy in patients with PLS with severe fat igue was associated with an improvement in fatigue but not with cognitive function or an experimental laboratory measure of infection in this study.** Because fatigue, a nonspecific symptom, was the only outcome that improved and because treatment was associated with adverse events, **this study does not support the use of additional antibiotic therapy with parenteral ceftriaxone in post-treatment, persistently fatigued patients with PLS.**

93: Med Sci Monit. 2003 Nov;9,11:PI136-42. **Macrolide therapy of chronic Lyme Disease.** Donta ST.

Boston University Medical Center, 650 Albany Street-8th Floor, Boston, MA 02118, U.S.A. sam.donta@bmc.org

BACKGROUND: Macrolide antibiotics are highly active in vitro against B.burgdorferi, but have limited efficacy in the treatment of patients with Lyme Disease. As macrolides are less active at a low pH, their poor clinical activity might be due to localization of borrelia to an acidic endosome, and their activity improved by alkalinization of that compartment with hydroxychloroquine. MATERIAL/METHODS: 235 patients with a multi-symptom complex typical of chronic Lyme disease, ie fatigue, musculoskeletal pain, and neurocognitive dysfunction and with serologic reactivity against B.burgdorferi were treated with a macrolide antibiotic, eg clarithromycin, and hydroxychloroquine. RESULTS: **Eighty% of patients had self-reported improvement of 50% or more at the end of 3 months. After 2 months of treatment, 20% of patients felt markedly improved, 75-100% of normal; after 3 months of treatment, 45% were markedly improved. Improvement frequently did not begin until after several weeks of therapy.** There were no differences among the three macrolide antibiotics used. **Patients who had been on hydroxychloroquine or macrolide antibiotic alone had experienced little or no improvement. Compared to patients ill for less than 3 years, the onset of improvement was slower, and the failure rate higher in patients who were ill for longer time periods.** CONCLUSIONS: These results support the hypothesis that the Lyme borrelia reside in an acidic endosome and that the use of a lysosomotropic agent augments the clinical activity of macrolide antibiotics in the treatment of patients with chronic Lyme Disease. In contrast, the efficacy of tetracycline in such patients is not affected by hydroxychloroquine.

93.5: 1: Vet Microbiol. 2005 May 20;107(3-4):285-94 **Antibiotic treatment of experimentally Borrelia burgdorferi-infected ponies.** Chang YF, Ku YW, Chang CF, Chang CD, McDonough SP, Divers T, Pough M, Torres A. College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, USA. yc42@cornell.edu

The objective of this study is to determine whether doxycycline, ceftiofur or tetracycline could be

effectively used to treat equine Lyme disease. Ponies experimentally infected with Borrelia burgdorferi by tick exposure were **treated with doxycycline, ceftiofur or tetracycline for 4 weeks, 28 days. Doxycyline and ceftiofur treatment were inconsistent in eliminating persistent infection in this experimental model. However, tetracycline treatment seems to eliminate persistent infection.** Although serum antibody levels to B. burgdorferi in all ponies declined gradually after antibiotic treatment, three out of four ponies treated with doxycline and two out of four ponies treated with ceftiofur, serum KELA titers were raised again 3 month after treatment was discontinued.**Five months after antibiotic treatment, tissues aseptically collected at necropsy from ponies with increased antibody levels after antibiotic treatment also showed culture positive to B. burgdorferi in various post-mortem tissues.**However, all four-tetracycline treatment ponies showed a negative antibody level and culture negative from post-mortem tissues. Untreated infected ponies maintained high KELA titers throughout the study and were tissue culture positive.

94: Int J Antimicrob Agents. 2005 Jun;25,6:474-8. **Susceptibility of Borrelia afzelii strains to antimicrobial agents.** Ruzi·-Sablji· E, Podreka T, Maraspin V, Strle F.

Institute of Microbiology and Immunology, Medical Faculty Ljubljana, Slovenia. eva.ruzic-sabljic@mf.uni-lj.si

The aim of the present study was to determine the susceptibility of Borrelia afzelii strains to antibiotics, and to test the hypothesis that persistence of borrelia in skin, after therapy, is a consequence of resistance to the antibiotic used for treatment. Ten B. afzelii strains isolated from skin of seven adult patients, two with acrodermatitis chronica atrophicans, five with erythema migrans, were studied. In three patients B. afzelii was isolated from erythema migrans lesion before antibiotic therapy and 2-3 months after treatment with cefuroxime axetil, two patients, or with ceftriaxone, one patient. MICs and MBCs for amoxicillin, azithromycin, ceftriaxone, cefuroxime, doxycycline and amikacin were measured. There was total resistance to amikacin but isolates were susceptible to all other antibiotics except one isolate that was resistant to cefuroxime, MIC > 4 mg/L. Comparison of MBC values after 3 and 6 weeks' incubation revealed comparable results for azithromycin and ceftriaxone while for amoxicillin, cefuroxime and doxycycline, some differences were found. In one of the patients from whom there were **borrelia isolated before and after treatment with cefuroxime axetil, both isolates were resistant to cefuroxime.** In the other two patients, the paired isolates were susceptible to the antibiotic used for therapy.

95: Int J Med Microbiol. 2006 May;296 Suppl 40:233-41. Epub 2006 Mar 10.**Risk of culture-confirmed borrelial persistence in patients treated for erythema migrans and possible mechanisms of resistance.** Hunfeld KP, Ruzi·-Sablji· E, Norris DE, Kraiczy P, Strle F. Institute of

Medical Microbiology, University Hospital of Frankfurt, Paul-Ehrlich Str. 40, D-60596 Frankfurt/Main, Germany. K.Hunfeld@em.uni-frankfurt.de

Erythema migrans, EM, develops at the site of the tick bite in 77-90% of Lyme borreliosis, LB, patients and is therefore a common manifestation of early disease.**Clinical treatment failures have been reported in early LB cases for almost every suitable antimicrobial agent. The exact risk of resistance to antibiotic treatment in patients with EM, however, is not known and there are few published cases of culture-proven treatment failure.** Moreover, currently available diagnostic techniques cannot reliably discriminate between possible reinfection, true endogenous relapse and co-infection with other tick-borne pathogens. These drawbacks together with the phenomenon of r esistance to therapy in individual patients undoubtedly contribute to the inconsistencies surrounding the optimal treatment regimens for LB and are often misinterpreted and misused to support prolonged antibiotic treatment regimens. The question for the underlying mechanisms of possible antimicrobial resistance in Borrelia burgdorferi sensu lato remains unresolved but a better understanding of such genetic or phenotypic mechanisms would be helpful for the treatment of LB and other spirochetal diseases. Investigations on this issue, at best, should start with borrelial isolates cultured from patients before the start of antibiotic therapy and again after the conclusion of treatment. This task, however, remains challenging insofar, as culture is rarely successful under routine laboratory conditions after antimicrobial therapy.**Here, we review recent clinical and experimental data on treatment resistance in EM patients suggesting that, although rare, borrelial persistence does occur at the site of the infectious lesion after antibiotic treatment.** Borrelial persistence, however, is unlikely to result from acquired resistance against antimicrobial agents that were used for initial specific chemotherapy.

96: Eur J Pediatr. 2006 Jun;165,6:420-1. Epub 2006 Mar 4. **Persistent synovitis in two children with Lyme arthritis linked with HLA-DRB1*1104.** Hendrickx G, Demanet C, Vandenplas Y.

Department of Paediatrics, Paediatric Orthopaedic and Rheumatology Unit, Academisch Ziekenhuis -Vrije Universiteit Brussel, Laarbeeklaan 101, 1090 Brussels, Belgium. g.hendrickx@st-anna.nl

We report on two patients with a persistent Lyme arthritis. In addition both had a peculiar disease history. The first patient had oligoarticular juvenile idiopathic arthritis in remission. Five months after an infected tick bite, she developed a relapse of arthritis in the same knee. We considered Lyme borreliosis as the possible trigger for this reactivation. **The disease history of the second patient was that of a classical non-responder. After extensive antibiotic treatment osteolytic lesions became visible.** MRI images suggested an erosive arthropathy and arthroscopy was used to investigate possible erosive arthritis. **Studies on collected material made us consider**

the following hypothesis. Despite demonstration of a spirochete fragment in a synovial biopsy, the patient recovered without additional antibiotic treatment. *Conclusion*: delay of antibiotic treatment after appearance of erythema migrans may cause systemic spread of the antigen and predispose to Lyme arthritis. If intra-articular steroids are considered when spontaneous resolution of Lyme arthritis does not occur, magnetic resonance imaging of the affected joint, prior to administration, may provide additional information. The success of synovectomy may be related to removal of undegraded antigenic material which may prolong the inflammation.

97: Int J Immunopathol Pharmacol. 2006 Jul-Sep;19,3:545-9. **In vitro susceptibility of isolates of Borrelia burgdorferi s.l. to antimicrobial agents.** Santino I, Scazzocchio F, Ciceroni L, Ciarrocchi S, Sessa R, Del Piano M. Department of Public Health Sciences, La Sapienza University, Rome, Italy. iolanda.santino@uniroma1.it

In the present study, we investigate the in vitro antimicrobial activity of macrolides, beta-lactams and tetracycline against Borrelia burgdorferi s.l. clinical and tick isolates. Minimal inhibitory concentrations, MICs, were determined in normal growth condition and after pre-exposure of the strains to sub-MIC of the founder of each drug family. All the classes of tested antibiotics showed good antibacterial activity against all the borreliae isolates and there were no significant susceptibility differences among clinical and tick isolates. **After pre-exposure of the strains to sub-MIC of erythromycin, cefoxitin and tetracycline, we observed that some strains of B. burgdorferi s.l. showed higher MIC values to both the pre-exposed drug and drugs of the same family. The less susceptibility of borreliae, in the last growth condition in vitro, could be one of the justifications of clinical results indicating the limited efficacy of these antibiotics in treatment of B. burgdoferi infections.**

98: 1: Microbes Infect. 2006 Nov-Dec; 8,14-15:2832-40. Epub 2006 Sep 22.**Invasion of human neuronal and glial cells by an infectious strain of Borrelia burgdorferi.** Livengood JA, Gilmore RD Jr.

Centers for Disease Control and Prevention, Divi sion of Vector-borne Infectious Diseases, 3150 Rampart Road, CSU Foothills Campus, Fort Collins, CO 80522, USA.

Human infection by Borrelia burgdorferi, the etiological agent for Lyme disease, can result in serious acute and late-term disorders including neuroborreliosis, a degenerative condition of the peripheral and central nervous systems.**To examine the mechanisms involved in the cellular pathogenesis of neuroborreliosis, we investigated the ability of B. burgdorferi to attach to and/or invade a panel of human neuroglial and cortical neuronal cells.** In all neural cells tested, we observed B. burgdorferi in association with the cell by confocal microscopy. Further analysis

by differential immunofluorescent staining of external and internal organisms, and a **gentamicin protection assay demonstrated an intracellular localization of B. burgdorferi. A non-infectious strain of B. burgdorferi** was attenuated in its ability to associate with these neural cells, suggesting that a specific borrelial factor related to cellular infectivity was responsible for the association. Cytopathic effects were not observed following infection of these cell lines with B. burgdorferi, and **internalized spirochetes were found to be viable. Invasion of neural cells by B. burgdorferi provides a putative mechanism for the organism to avoid the host's immune response** while potentially causing functional damage to neural cells during infection of the CNS.

98.5: Acta Radiologica, Volume 48, Issue 7 2007 , pages 755 - 762 Brain Magnetic Resonance Imaging Does Not Contribute to the Diagnosis of Chronic Neuroborreliosis. Aalto A, Sjöwall J, Davidsson L, Forsberg P, Smedby O. Division of Radiology, Department of Medicine and Care, Faculty of Health Sciences, Linköping University, Linköping, Sweden. anne.aalto@imv.liu.se

BACKGROUND: **Borrelia infections, especially chronic neuroborreliosis, NB, may cause considerable diagnostic problems.** This diagnosis is based on symptoms and findings in the cerebrospinal fluid but is not always conclusive. PURPOSE: To evaluate brain magnetic resonance imaging, MRI, in chronic NB, to compare the findings with healthy controls, and to correlate MRI findings with disease duration. MATERIAL AND METHODS: Sixteen well-characterized patients with chronic NB and 16 matched controls were examined in a 1.5T scanner with a standard head coil. T1-, with and without gadolinium, T2-, and diffusion-weighted imaging plus fluid-attenuated inversion recovery, FLAIR, imaging were used. RESULTS: White matter lesions and lesions in the basal ganglia were seen in 12 patients and 10 controls, no significant difference. Subependymal lesions were detected in patients down to the age of 25 and in the controls down to the age of 43. The number of lesions was correlated to age both in patients, rho = 0.83, $P<0.01$, and in controls, rho = 0.61, $P<0.05$, but not to the duration of disease. Most lesions were detected with FLAIR, but many also with T2-weighted imaging. CONCLUSION: **A number of MRI findings were detected in patients with chronic NB, although the findings were unspecific** when compared with matched controls and did not correlate with disease duration.

99: Pol Merkur Lekarski. 2007 Apr;22,130:275-9. Related Articles, **Concentrations of pro-inflammatory cytokines IFN-gamma, IL-6, IL-12 and IL-15 in serum and cerebrospinal fluid in patients with neuroborreliosis undergoing antibiotic treatment.** Article in Polish. Pancewicz SA, Kondrusik M, Zajkowska J, Grygorczuk S. Akademia Medyczna w Bialymstoku, Klinika ChorÃ³b Zakaznych i Neuroinfekcji.20spancewicz@interia.pl

Pathogenesis of Lyme disease, including neuroborreliosis, remains unclear. However,

pro-inflammatory cytokines seem to be involved and might be used to monitor the course of the disease. It has been also shown that B. burgdorferi protects itself from elimination by modulating function of the host's immune system. THE AIM OF THIS STUDY: The purpose of this study was to evaluate the serum and cerebrospinal fluid, CSF, concentrations of selected cytokines in patients with neuroborreliosis and their change during antibiotic treatment. MATERIAL AND METHODS: The group of 25 patients was examined, all undergoing antibiotic therapy due to meningitis caused by Borrelia burgdorferi infection. The group included 10, 40%, females and 15, 60%, males in the mean age x = 42,3 years. The control group for serum measurements consisted of 25 healthy individuals, mean age x =43, 1, while control group for CSF study included 10 patients, aged x = 53,5 years, from whom CSF with normal parameters was taken during diagnostic procedures neurosurgical. We examined serum and CSF before and after antibiotics for concentrations of interferon-gamma, INF-gamma, interleukin-6, IL-6, interleukin-12, IL-12, and interleukin-15, IL-15. RESULTS: In the first examination the significant increase of IFN-gamma, IL-6, IL-2, IL-15 serum and CSF concentration was detected in comparison to control group. After 4-weeks antibiotic treatment the concentrations of studied cytokines decreased significantly in serum as well as in CSF but remained increased in compa rison with controls. CONCLUSIONS: **Although antibiotic treatment leads to withdrawal of clinical symptoms of neuroborreliosis** and normalization of CSF general parameters, pro-inflammatory cytokines' concentrations in serum and CSF remain elevated.**It may be explained by the persistence of inflammatory conditions, perhaps related to surviving of a fraction of Borrelia burgdorferi spirochetes within CNS tissue.** This phenomenon might lead to development of chronic CNS lesions.

100: 1: J Infect Dis. 2007 May 15;195,10:1489-96. Epub 2007 Apr 6.**Anti-tumor necrosis factor-alpha treatment activates Borrelia burgdorferi spirochetes 4 weeks after ceftriaxone treatment in C3H/He mice.** Yrjänäinen H, Hytönen J, Song XY, Oksi J, Hartiala K, Viljanen MK. Department of Medical Microbiology, University of Turku, Turku, 20520, Finland. heta.yrjanainen@utu.fi

BACKGROUND: The effect of anti-tumor necrosis factor, TNF,-alpha treatment in Borrelia burgdorferi-infected and ceftriaxone-treated C3H/He mice was evaluated. METHODS: Mice were infected with B. garinii A218 or B. burgdorferi sensu stricto N40. At 2 weeks of infection, **one group was treated simultaneously with ceftriaxone** and anti-TNF-alpha, whereas another received ceftriaxone at 2 weeks and anti-TNF-alpha 4 weeks later. One group received ceftri axone treatment only. Infected and noninfected control groups were sham treated. RESULTS: At 14 weeks of infection, B. burgdorferi could not be detected by cultivation or by polymerase chain reaction in tissue samples of any mouse treated with ceftriaxone only. However, **spirochetes grew from the tissue samples of one-third of the mice treated with anti-TNF-alpha simultaneously or 4 weeks after ceftriaxone.** These activated spirochetes showed ceftriaxone sensitivity rates,

plasmid profiles, and virulence rates similar to those of bacteria used to infect the mice. All infected control mice and mice given anti-TNF-alpha only were culture positive. CONCLUSIONS: This report shows that, after **ceftriaxone treatment for 5 days, a portion of B. burgdorferi-infected mice still have live spirochetes in their body**, which are activated by anti-TNF-alpha treatment.

101: Adv Med Sci. 2007;52:174-8. **Concentration of TGF-beta1 in the supernatant of peripheral blood mononuclear cells cultures from patients with early disseminated and chronic lyme borreliosis.** Grygorczuk S, Chmielewski T, Zajkowska J, Swierzbi·ska R, Pancewicz S, Kondrusik M, Tylewska-Wierzbanowska S, Hermanowska-Szpakowicz T. Department of Infectious Diseases and Neuroinfections, Medical University of Bia·ystok, ul. Zurawia 14, 15-540 Bia·ystok, Poland. neuroin@amb.edu.pl

PURPOSE: The aberrant inflammatory response is probably involved in the pathogenesis of chronic Lyme borreliosis, including chronic Lyme arthritis and neuroborreliosis. Transforming growth factor-beta 1, TGF-beta1, is an important anti-inflammatory and immunomodulatory cytokine and its deficient synthesis is linked to exaggerated inflammation and immune response. MATERIAL AND METHODS: Peripheral blood mononuclear cells, PBMC, from 25 patients with Lyme borreliosis and 6 controls were incubated for 7 days with suspension of Borrelia afzeli, B. garinii and B. burgdorferi sensu stricto spirochetes. TGF-beta1 concentration in culture supernatants was measured with ELISA. Results were analyzed according to disease duration, group I--chronic borreliosis, n=20; group II--early borreliosis, n=5, and clinical form, LA--arthritis, NB--neuroborreliosis. RESULTS: TGF-beta1 concentration was increased in supernatants of PBMC cultures of patients with early neuroborreliosis, in comparison with chronic borreliosis and controls. **In chronic, but not in early borreliosis, there was a tendency for decrease of TGF-beta1 synthesis under stimulation with B. burgdorferi spirochetes. CONCLUSIONS: Impaired synthesis of TGF-beta1 by mononuclear cells seems to be present in patients with chronic forms of Lyme borreliosis when compared to those with early stage of the disease. It may be a factor contributing to the persistence of inadequate inflammatory response in patients in whom chronic form of the disease develops.**

102: 1: Rheumatol Int. 2007 Sep;27,11:1091-3. Epub 2007 Apr 4. **Seronegative Lyme arthritis.** Holl-Wieden A, Suerbaum S, Girschick HJ. Children's hospital, Section of Pediatric Rheumatology, Immunology and Infectious diseases, University of Wuerzburg, Josef-Schneider-Str. 2, 97090 Wuerzburg, Germany.

We present a 10-year-old girl who had been diagnosed with juvenile idiopathic arthritis 5 years before and who experienced a flare of arthritis affecting one knee while **she was off medication for almost 3 years. Seronegative Lyme arthritis had to be diagnosed based on the detection of**

Borrelia burgdorferi DNA in synovial fluid. No humoral immune response to Borrelia burgdorferi was detectable before, at the time of diagnosis and up to 3 years later.

103: Pol Merkur Lekarski. 2007 Sep;23,135:174-8. **Concentration of soluble forms of selectins in serum and in cerebrospinal fluid in group of patients with neuroborreliosis--a preliminary study** Moniuszko AM, Pancewicz SA, Ko ndrusik M, Zajkowska J, Grygorczuk S, Swierzbi·ska R. Akademia Medyczna w Bia·ymstoku, Klinika Chorób Zaka·nych i Neuroinfekcji.

The results of the research already done, suggest an important role of selectins in inflammatory process of various etiology. Lack of selectins or their ligands causes severe complications, such as chronic inflammatory processes. The aim of this study was to analyze the role of selectins sL, sE and sP in the development and course of neuroborreliosis in the form of meningitis. We have also analyzed the influence of treatment on changes of selectins' concentration in serum and cerebrospinal fluid. MATERIAL AND METHODS: We have analyzed 17 patients with neuroborreliosis presenting as meningitis, in whom we measured by immunoenzymatic method concentration of selectins sL, sP and sE in blood and cerebrospinal fluid before and after 4-week therapy with cefotaxim. We used Human sL-selectin, Human sE-selectin and Human sP-selectin kits produced by Bender Med. Systems, Austria. Control group for measurement of concentration of selectins in serum consisted of 8 healthy patients. Control group for measurement of concentration of selectins in cerebrospinal fluid consisted of 8 patients, in whom lumbar puncture excluded inflammatory disease of the central nervous system. RESULTS: In serum concentration of selectins sL and sP was significantly higher comparing to control group. After treatment concentration of these selectins decreased, but still was significantly higher than in control group. Only con centration of selectin sE was significantly lower than in control group and after treatment decreased further remaining lower comparing to control group. In cerebrospinal fluid concentration of selectin sL was significantly higher comparing to control group and increased after treatment. Concentration of selectins sE and sP increased before treatment and decreased after treatment, but still remained elevated comparing to control group. CONCLUSIONS: **Persistence of increased concentration of selectins sP and sL in serum and also of selectin sE in cerebrospinal fluid in patients with neuroborreliosis after completed antibiotic therapy and regression of clinical symptoms can suggest permanence of chronic inflammatory state in consequence of survival of B. burgdorferi spirochetes in affected tissues.**

104: Volume 358:428-431 January 24, 2008 Number An Appraisal of "Chronic Lyme Disease" To the Editor: Feder et al., Oct. 4 issue,1 review the great controversy surrounding "chronic Lyme disease."v For most patients with this diagnosis, the authors advocate against the use of antibiotics. **But before the decision is made not to use antibiotics for patients with post–tick-bite symptoms, anaplasma, babesia, bartonella,** 2 **and ehrlichia must be ruled out.**

These tick-borne 2 **intracellular pathogens are difficult to diagnose and can establish long-term, persistent infection.** 3,4,5 Anaplasma, babesia, and bartonella are underdiagnosed: the nonspecific symptoms of infections with these organisms tend to be ascribed to the more easily identifiable Lyme disease, which often accompanies them.2,3,4,5,6 Indeed, when studied prospectively, 65 of 161 patients with Lyme disease, 40%, were coinfected with babesia, and 11 of 161, 7%, with anaplasma.6 **Accurate diagnosis of these infections helps steer successful treatment: babesia 3 and bartonella 5 are especially difficult to eradicate.** Accurate diagnosis is also important, since babesia 3 and anaplasma 4 can spread through blood transfusion. As Feder et al. note, "chronic Lyme disease" is often unrelated to borrelia. **If symptoms occur after a tick bite in the absence of evidence of active borrelia infection or if they persist despite anti-borrelia treatment, another tick-borne infection should be suspected. If such an infection is found, the patient may indeed benefit from appropriate antibiotics.**

1 Feder HM Jr, Johnson BJB, O'Connell S, et al. A critical appraisal of "chronic Lyme disease." N Engl J Med 2007;357:1422-1431.[Free Full Text]
2 Adelson ME, Rao RV, Tilton RC, et al. Prevalence of Borrelia burgdorferi, Bartonella spp., Babesia microti, and Anaplasma phagocytophila in Ixodes scapularis ticks collected in northern New Jersey. J Clin Microbiol 2004;42:2799-2801.[Free Full Text]
3 Krause PJ, Spielman A, Telford SR III, et al. Persistent parasitemia after acute babesiosis. N Engl J Med 1998;339:160-165.[Free Full Text]
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105: To the Editor: Feder et al. fail to adequately inform readers about the science underlying the "chronicity" debate. Multiple researchers have documented Borrelia burgdorferi's ability to penetrate human cells. In demonstrating the presence of the organism inside neurons and glial cells, **Livengood and Gilmore established that it can exist in an intracellular state within a protected site,** 1 **characteristics favoring persistence and necessitating longer courses of antibiotics. B. burgdorferi's pleomorphic abilities also favor persistence.** One study suggested that penicillin, ceftriaxone, and doxycycline are ineffective against the bacteria in its cystic form.2 **The study by Yrjänäinen et al. revealed that B. burgdorferi can survive standard therapy**, lending further credence to the theory of bacterial persistence.3 Krupp et al. found that, as

compared with 23% of the placebo group, had significant improvement in fatigue.4 "Clinical assessment remains the most important method for determining the efficacy of treatment."5 **Persistent symptoms in patients with late Lyme disease suggest treatment failure and the need for a new approach.**

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106: To the Editor: The article by Feder et al. on the proper therapy of chronic Lyme disease addresses a very timely concern. **Unfortunately, the authors' statement that there are no "scientific data" that support persistent B. burgdorferi infection in the face of negative serologic test results is erroneous. In 1988, we reported on 17 patients who had all had erythema migrans, received inadequate antibiotic therapy, had vigorous T-cell blastogenesis to borrelia antigens, and were seronegative on the basis of enzyme-linked immunoassay.** 1,2 **The majority of these patients had improvement after definitive antibiotic therapy. Seronegative infection was confirmed by other laboratories using polymerase-chain-reaction, PCR, assays to document the presence of microbes in seronegative patients.** 3,4 Abrogation of a humoral response by removal of the bulk of microbial antigens has been seen in other settings, including infection with Treponema pallidum. Although the use of repeated courses of antibiotics for a putative borrelia infection is unsupported and may cause serious morbidity,5 persons with evidence of previously inadequately treated Lyme disease may be seronegative and may benefit from adequate antibiotic therapy. Fortunately, erythema migrans is now more readily recognized,

and occult Lyme disease is rarer. In the absence of antibiotic treatment, most persons become seropositive.

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107: **Persistence of Borrelia burgdorferi Following Antibiotic Treatment in Mice Antimicrobial Agents and Chemotherapy**, published online ahead of print on 3 March 2008
Emir Hodzic, Sunlian Feng, Kevin Holden, Kimberly J. Freet, and Stephen W. Barthold

The effectiveness of antibiotic treatment was examined in a mouse model of Lyme borreliosis.

Mice were treated with ceftriaxone or saline for one month, commencing during the early, 3 weeks, or chronic, 4 months, stages of infectionwith Borrelia burgdorferi. Tissues from mice were tested for infection by culture, polymerase chain reaction, PCR, xenodiagnosis, and transplantation of allografts at 1 and 3 months after completion of treatment. In addition, tissues were examined for spirochetes by immunohistochemistry.

In contrast to saline-treated mice, **mice treated with antibiotic were consistently culture-negative, but tissues from some of the mice remained PCR-positive, and spirochetes**

could be visualized in collagen-rich tissues. Furthermore, when some of the antibiotic treated mice were fed upon by Ixodes scapularis ticks, xenodiagnosis, spirochetes were acquired by the ticks, based upon PCR, and ticks from those cohorts transmitted spirochetes to naïve SCID mice, which became PCR-positive, but culture-negative.

Results indicated that **following antibiotic treatment, mice remained infected with non-dividing but infectious spirochetes**, particularly when antibiotic treatment was commenced during the chronic stage of infection.

108: Antimicrobial Agents and Chemotherapy, May 2008, p. 1728-1736, Vol. 52, No. 50066-4804 **Persistence of Borrelia burgdorferi following Antibiotic Treatment in Mice** Emir Hodzic, Sunlian Feng, Kevin Holden, Kimberly J. Freet, and Stephen W. Barthold*

Center for Comparative Medicine, Schools of Medicine and Veterinary Medicine, University of California at Davis, One Shields Avenue, Davis, California 95616 Received 9 August 2007/ Returned for modification 1 November 2007/ Accepted 26 December 2007

The effectiveness of antibiotic treatment was examined in a mouse model of Lyme borreliosis. Mice were treated with ceftriaxone or saline solution for 1 month, commencing during the early, 3 weeks, or chronic, 4 months, stages of infection with Borrelia burgdorferi. Tissues from mice were tested for infection by culture, PCR, xenodiagnosis, and transplantation of allografts at 1 and 3 months after completion of treatment. In addition, tissues were examined for the presence of spirochetes by immunohistochemistry. In contrast to saline solution-treated mice, **mice treated with antibiotic were consistently culture negative, but tissues from some of the mice remained PCR positive, and spirochetes could be visualized in collagen-rich tissues.** Furthermore, when some of the antibiotic-treated mice were fed on by Ixodes scapularis ticks, xenodiagnosis, spirochetes were acquired by the ticks, as determined based upon PCR results, and ticks from those cohorts transmitted spirochetes to naïve SCID mice, which became PCR positive but cult ure negative. **Results indicated that following antibiotic treatment, mice remained infected with nondividing but infectious spirochetes, particularly when antibiotic treatment was commenced during the chronic stage of infection.**

109: Pol Arch Med Wewn. 2008 May;118 5:314-7. : Neuroborreliosis with extrapyramidal symptoms: a case report. Biesiada G, Czapiel J, Sobczyk-Krupiarz I, Garlicki A, Mach T.

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The disease of Lyme is a tick-borne infection. It involves skin, the nervous system, joints and the

heart. Spirochaeta Borrelia burgdorferi is the etiologic agent of the disease. In the majority of cases, clinical symptoms, like migrating erythema, occur from 3 to 30 days, sometimes to 3 months after a bite from a tick. The early disseminated infection involves multiple migrating erythema, neuroborreliosis, arthritis, myocarditis and other organ-related symptoms. The late stage of chronic infection involves chronic atrophic leg dermatitis, neurological and rheumatological symptoms, and other organ-related symptoms which persist for above 12 months. The diagnosis of the disease of Lyme is based upon specific clinical symptoms confirmed by serologic tests. The two-step diagnostic protocol including the ELISA method, confirmed by the Western-blot test, is optimal. **The present article describes a case of a 59-year-old man, a computer specialist, who often spends his free time walking in woods for recreation, and who was bitten by a tick 3 years before hospitalization**. The bite resulted in migrating erythema that subsided without antimicrobial treatment. **In spite of this, the man had not changed his hobby exposing himself to bites from ticks. One year later, multiple migrating erythema and extrapyramidalis symptoms appeared without any other organ malfunctions.** In the current year, the patient was admitted to the Infectious Diseases Hospital, and **received antibiotics ceftriaxon with following neurological improvement. Several months later, extrapyramidal symptoms increased.** On the day of admission to the hospital, the neurologic examination showed abnormalities of upper and lower limbs movements propulsive walking and the right lower leg traction), the right hand tremor, pouts of the face, and sleepiness.

109: 1: J Neuroinflammation. Sep 25;5:40. **Persisting atypical and cystic forms of Borrelia burgdorferi and local inflammation in Lyme neuroborreliosis.** Miklossy J, Kasas S, Zurn AD, McCall S, Yu S, McGeer PL.

Kinsmen Laboratory of Neurological Research, University of British Columbia, Vancouver, BC, Canada. judithmiklossy@bluewin.ch

BACKGROUND: The long latent stage seen in syphilis, followed by chronic central nervous system infection and inflammation, can be explained by the persistence of atypical cystic and granular forms of Treponema pallidum. We investigated whether a similar situation may occur in Lyme neuroborreliosis. METHOD: Atypical forms of Borrelia burgdorferi spirochetes were induced exposing cultures of Borrelia burgdorferi, strains B31 and ADB1, to such unfavorable conditions as osmotic and heat shock, and exposure to the binding agents Thioflavin S and Congo red. We also analyzed whether these forms may be induced in vitro, following infection of primary chicken and rat neurons, as well as rat and human astrocytes. We further analyzed whether atypical forms similar to those induced in vitro may also occur in vivo, in brains of three patients with Lyme neuroborreliosis. We used immunohistochemical methods to detect evidence of neuroinflammation in the form of reactive microglia and astrocytes. RESULTS: Under these conditions we observed atypical cystic, rolled and granular forms of these spirochetes. We

characterized these abnormal forms by histochemical, immunohistochemical, dark field and atomic force microscopy, AFM, methods. The atypical and cystic forms found in the brains of three patients with neuropathologically confirmed Lyme neuroborreliosis were identical to those induced in vitro. We also observed nuclear fragmentation of the infected astrocytes using the TUNEL metho d. Abundant HLA-DR positive microglia and GFAP positive reactive astrocytes were present in the cerebral cortex. CONCLUSION: **The results indicate that atypical extra- and intracellular pleomorphic and cystic forms of Borrelia burgdorferi and local neuroinflammation occur in the brain in chronic Lyme neuroborreliosis. The persistence of these more resistant spirochete forms, and their intracellular location in neurons and glial cells, may explain the long latent stage and persistence of Borrelia infection.** The results also suggest that Borrelia burgdorferi may induce cellular dysfunction and apoptosis. The detection and recognition of atypical, cystic and granular forms in infected tissues is essential for the diagnosis and the treatment as they can occur in the absence of the typical spiral Borrelia form.

110: Microb Pathog. 2008 Sep 20. **Borrelia burgdorferi expression of the bba64, bba65, bba66, and bba73 genes in tissues during persistent infection in mice.** Gilmore RD Jr, Howison RR, Schmit VL, Carroll JA.

Division of Vector-Borne Infectious Diseases, Centers for Disease Control and Prevention, 3150 Rampart Rd, Fort Collins, CO 80521, USA.

Borrelia burgdorferi, the etiological agent of Lyme disease in humans, is vectored between mammalian hosts in nature by Ixodes ticks. **The organism adapts to diverse environments encountered throughout the enzootic cycle by differentially expressing essential gene products to survive the specialized conditions, whether in ticks or warm-blooded hosts.** Ho wever, little is known regarding the identity and/or function of B. burgdorferi genes expressed during colonization of tissues during mammalian infection. Experimental evidence has shown that a group of genes, formerly classified as paralogous gene family 54, contiguously localized on the 54-kilobase linear plasmid of B. burgdorferi, are among the most highly regulated by in vitro conditions resembling mammalian infection. In this study, we employed quantitative reverse transcription-PCR to measure temporal gene expression of a subset of this B. burgdorferi gene family, bba64, bba65, bba66, and bba73, in tissues during chronic murine infection. The goal was to gain insight into the role of these genes in infectivity and pathogenesis by identifying when the genes are induced and whether they are expressed in specific target tissues. B. burgdorferi bba64, bba65, bba66, and bba73 expression was measured from infected mouse tissues relative to expression in in vitro culture conditions at specific times post-infection. bba64 expression was highly upregulated in bladder, heart, and spleen tissues throughout the infection period, contrasting with the sharp downregulation previously observed in ear tissues. **bba65, bba66, and bba73 demonstrated upregulated differential expression in various tissues over 1year**

post-infection. These results suggest an essential role for these genes in borrelial survival, persistence, and/or pathogenesis.

111: Med Hypotheses. 2008;70,5:967-74. Epub 2007 Nov 5. **The association between tick-borne infections, Lyme borreliosis and autism spectrum disorders.** Bransfield RC, Wulfman JS, Harvey WT, Usman AI.

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Chronic infectious diseases, including tick-borne infections such as Borrelia burgdorferi may have direct effects, promote other infections and create a weakened, sensitized and immunologically vulnerable state during fetal development and infancy leading to increased vulnerability for developing autism spectrum disorders. A dysfunctional synergism with other predisposing and contributing factors may contribute to autism spectrum disorders by provoking innate and adaptive immune reactions to cause and pe rpetuate effects in susceptible individuals that result in inflammation, molecular mimicry, kynurenine pathway changes, increased quinolinic acid and decreased serotonin, oxidative stress, mitochondrial dysfunction and excitotoxicity that impair the development of the amygdala and other neural structures and neural networks resulting in a partial Klüver-Bucy Syndrome and other deficits resulting in autism spectrum disorders and/or exacerbating autism spectrum disorders from other causes throughout life. **Support for this hypothesis includes multiple cases of mothers with Lyme disease and children with autism spectrum disorders;** fetal neurological abnormalities associated with tick-borne diseases; similarities between tick-borne diseases and autism spectrum disorder regarding symptoms, pathophysiology, immune reactivity, temporal lobe pathology, and brain imaging data; **positive reactivity in several studies with autistic spectrum disorder patients for Borrelia burgdorferi, 22%, 26% and 20-30%, and 58% for mycoplasma; similar geographic distribution and improvement in autistic symptoms from antibiotic treatment.** It is imperative to research these and all possible causes of autism spectrum disorders in order to prevent every preventable case and treat every treatable case until this disease has been eliminated from humanity.

111.5: Journal of Veterinary Diagnostic Investigation Vol. 20 Issue 3, 321-324

Validation of an in-clinic enzyme-linked immunosorbent assay kit for diagnosis of Borrelia burgdorferi infection in horses. Amy L. Johnson1, Thomas J. Divers and Yung-Fu Chang

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Confirmation of Borrelia burgdorferi infection in horses has required enzyme-linked immunosorbent assay (ELISA) or Western blot tests performed by reference laboratories. An in-clinic C6 ELISA SNAP kit has been marketed for dogs. This canine kit was evaluated for horses using serum from experimentally infected ponies. Serum samples originated from 2 previous studies. In the first study, 7 ponies were exposed to B. burgdorferi–infected ticks; 4 ponies served as uninfected controls. Serum samples were obtained bimonthly for 9 months. In the second study, 16 ponies were exposed to B. burgdorferi–infected ticks. After confirmation of infection by skin culture, polymerase chain reaction (PCR), and serology, the ponies were allocated to 4 groups that received tetracycline, doxycycline, ceftiofur, or no treatment. Serum samples were obtained monthly, both before and after antibiotic treatments, for 11 months. For the current study, selected samples (n = 220) from both studies were tested with IDEXX SNAP Heartworm Ab/Borrelia burgdorferi Ab/Ehrlichia canis Ab Test Kits. Tested samples included samples taken before infection, from various times postinfection, and after antibiotic treatments. Results from confirmed positive or negative samples were used to determine sensitivity and specificity of the assay. Results indicate that the test kits have fair sensitivity (63%) and very high specificity (100%) for horses recently infected with B. burgdorferi. Validation of this test provides equine practitioners with an inexpensive, in-clinic method to confirm infection, although its moderate sensitivity may result in a moderate chance of a false negative test.

Finally, recent reports 14,15 indicate that C6 technology may allow evaluation of successful antibiotic treatment of Lyme disease based on a decreasing titer. **Human studies indicate that a decreasing titer is usually seen with successful treatment of early infection but not in cases of chronic infection despite extensive antibiotic treatment.**7 SNAP testing of experimentally infected ponies successfully treated with antibiotics (based on negative culture and PCR results by the end of the study) revealed that all ponies became negative on SNAP test. **Practitioners in Lyme-endemic areas often see horses with persistently positive ELISA results despite long-term antibiotic treatment. It is not known whether those cases represent failure of antibiotic therapy to eliminate the organism, reinfection with B. burgdorferi, or a persistent immune response despite successful treatment.** Further research is needed to determine whether C6 technology will better define the infection status of these horses

112: J Antimicrob Chemother. 2009 Jun;63 6:1163-72. Epub 2009 Apr 17. Assessment of methylthioadenosine/S-adenosylhomocysteine nucleosidases of Borrelia burgdorferi as targets for novel antimicrobials using a novel high-throughput method. Cornell KA, Primus S, Martinez JA, Parveen N.

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Abstract

BACKGROUND: Lyme disease is the most prevalent tick-borne disease in the USA with the highest number of cases (27 444 patients) reported by CDC in the year 2007, representing an unprecedented 37% increase from the previous year. The haematogenous spread of Borrelia burgdorferi to various tissues results in multisystemic disease affecting the heart, joints, skin, musculoskeletal and nervous system of the patients. OBJECTIVES: **Although Lyme disease can be effectively treated with doxycycline, amoxicillin and cefuroxime axetil, discovery of novel drugs will benefit the patients intolerant to these drugs and potentially those suffering from chronic Lyme disease that is refractory to these agents and to macrolides.** In this study, we have explored 5'-methylthioadenosine/S-adenosylhomocysteine nucleosidase as a drug target for B. burgdorferi, which uniquely possesses three genes expressing homologous enzymes with two of these proteins apparently exported. METHODS: The recombinant B. burgdorferi Bgp and Pfs proteins were first used for the kinetic analysis of enzymatic activity with both substrates and with four inhibitors. We then determined the antispirochaetal activity of these compounds using a novel technique. The method involved detection of the live-dead B. burgdorferi by fluorometric analysis after staining with a fluorescent nucleic acids stain mixture containing Hoechst 33342 and Sytox Green. RESULTS: Our results indicate that this method can be used for high-throughput screening of novel antimicrobials against bacteria. The inhibitors formycin A and 5'-p-nitrophenythioadenosine particularly affected B. burgdorferi adversely on prolonged treatment. CONCLUSIONS: On the basis of our analysis, we expect that structure-based modification of the inhibitors can be employed to develop highly effective novel antibiotics against Lyme spirochaetes.

113: Proc Natl Acad Sci U S A. 2009 Nov 3;106(44):18656-61. Epub 2009 Oct 20. Destruction of spirochete Borrelia burgdorferi round-body propagules (RBs) by the antibiotic tigecycline. Brorson Ø, Brorson SH, Scythes J, MacAllister J, Wier A, Margulis L.

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Abstract

Persistence of tissue spirochetes of Borrelia burgdorferi as helices and round bodies (RBs) explains many erythema-Lyme disease symptoms. Spirochete RBs (reproductive propagules also called coccoid bodies, globular bodies, spherical bodies, granules, cysts, L-forms, sphaeroplasts, or vesicles) are induced by environmental conditions unfavorable for growth. Viable, they grow, move and reversibly convert into motile helices. Reversible pleiomorphy was recorded in at least six spirochete genera (>12 species). Penicillin solution is one unfavorable condition that induces RBs. **This antibiotic that inhibits bacterial cell wall synthesis cures neither the second "Great Imitator" (Lyme borreliosis)** nor the first: syphilis. Molecular-microscopic techniques, in

principle, can detect in animals (insects, ticks, and mammals, including patients) helices and RBs of live spirochetes. Genome sequences of B. burgdorferi and Treponema pallidum spirochetes show absence of >75% of genes in comparison with their free-living relatives. Irreversible integration of spirochetes at behavioral, metabolic, gene product and genetic levels into animal tissue has been documented. Irreversible integration of spirochetes may severely impair immunological response such that they persist undetected in tissue. We report in vitro inhibition and destruction of B. burgdorferi (helices, RBs = "cysts") by the antibiotic Tigecycline (TG; Wyeth), a glycylcycline protein-synthesis inhibitor (of both 30S and 70S ribosome subunits). Studies of the pleiomorphic life history stages in response to TG of both B. burgdorferi and Treponema pallidum in vivo and in vitro are strongly encouraged.

114: APMIS. 2010 Sep 1;118 (9):665-73 20718718: **Persistence of borrelial DNA in the joints of Borrelia burgdorferi-infected mice after ceftriaxone treatment** HETA YRJÄNÄInen 1 , JUKKA HYTÖNen 1 , PAULIINA HARTIALA 1 , JARMO OKSI 2 and MATTI K. VILJANEN Departments of 1Medical Microbiology and Immunology and 2 Medicine, University of Turku, Turku, Finland

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We have earlier shown that Borrelia burgdorferi-infected and ceftriaxone-treated mice have viable spirochetes in their body, since immunosuppressive treatment allows B. burgdorferi to be detected by culture. However, the **niche of the persisting spirochetes remained unknown**. In the present study, we analyzed the tissues of B. burgdorferi-infected and ceftriaxone-treated mice by culture and PCR to reveal the foci of persisting spirochetes. C3H/HeN mice were infected via intradermal needle injection with B. burgdorferi s.s. N40. The mice were treated as follows: (i) short (5 days) and (ii) long (18 days) course of ceftriaxone at 2 weeks of infection and killed after either 10 or 30 weeks, or (iii) the mice received ceftriaxone for 5 days at 18 weeks of infection and were killed 21 weeks after the treatment. All samples of ceftriaxone-treated mice were culture negative, whereas all untreated controls were culture positive. Importantly, B. burgdorferi DNA was detected in the joints of 30–100% of the treated mice. **In conclusion, these results combined with earlier results suggest that the joint or a tissue adjacent to the joint is the niche of persisting B. burgdorferi in ceftriaxone-treated mice.**

115: **Ineffectiveness of Tigecycline against Persistent Borrelia burgdorferi :** Stephen W. Barthold,1* Emir Hodzic,1 Denise M. Imai,1 Sunlian Feng,1 Xiaohua Yang,2 and Benjamin J. Luft

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The effectiveness of a new first-in-class antibiotic, tigecycline (glycylcycline), was evaluated during the early dissemination (1 week), early immune (3 weeks), or late persistent (4 months) phases of Borrelia burgdorferi infection in C3H mice. Mice were treated with high or low doses of tigecycline, saline (negative-effect controls), or a previously published regimen of ceftriaxone (positive-effect controls). **Infection status was assessed at 3 months after treatment by culture, quantitative ospA real-time PCR, and subcutaneous transplantation of joint and heart tissue into SCID mice**. Tissues from all saline-treated mice were culture and ospA PCR positive, tissues from all antibiotic-treated mice were culture negative, and some of the tissues from most of the mice treated with antibiotics were ospA PCR positive, although the DNA marker load was markedly decreased compared to that in saline-treated mice. Antibiotic treatment during the early stage of infection appeared to be more effective than treatment that began during later stages of infection. The viability of noncultivable spirochetes in antibiotic-treated mice (demonstrable by PCR) was confirmed by transplantation of tissue allografts from treated mice into SCID mice, with dissemination of spirochetal DNA to multiple recipient tissues, and by xenodiagnosis, including acquisition by ticks, transmission by ticks to SCID mice, and survival through molting into nymphs and then into adults. Furthermore, PCR-positive heart base tissue from antibiotic-treated mice revealed RNA transcription of several B. burgdorferi genes. **These results extended previous studies with ceftriaxone, indicating that antibiotic treatment is unable to clear persisting spirochetes, which remain viable and infectious, but are nondividing or slowly dividing.**

116: Journal of Basic Microbiology, Medical Microbiology. Volume 50, Issue Supplement 1, pages S5–S17, December 2010. **Metamorphosis of Borrelia burgdorferi organisms – RNA, lipid and protein composition in context with the spirochetes' shape**: Article first published online: 21 OCT 2010

1. Samiya Al-Robaiy1,2,†,2. Hassan Dihazi3,†,3. Johannes Kacza4, 4. Johannes Seeger4, 5. Jürgen Schiller5,6. Daniel Huster5, 7. Jens Knauer1,6, 8. Prof. Dr. Reinhard K. Straubinger1,7,*

Abstract

Borrelia burgdorferi, the agent of Lyme borreliosis, has the ability to undergo morphological transformation from a motile spirochetal to non-motile spherical shape when it encounters unfavorable conditions. However, little information is available on the mechanism that enables the bacterium to change its shape and whether major components of the cells – nucleic acids, proteins, lipids – are possibly modified during the process. Deducing from investigations utilizing electron microscopy, it seems that shape alteration begins with membrane budding followed by folding of the protoplasmatic cylinder inside the outer surface membrane. Scanning electron microscopy confirmed that a deficiency in producing functioning periplasmic flagella did not hinder sphere formation. Further, it was shown that the spirochetes' and spheres' lipid compositions were indistinguishable. Neither phosphatidylcholine nor phosphatidylglycerol were altered by the structural transformation. In addition, no changes in differential protein expression were detected during this process. However, minimal degradation of RNA and a reduced antigen-antibody binding activity were observed with advanced age of the spheres. The results of our comparisons and the failure to generate mutants lacking the ability to convert to spheres suggest that the metamorphosis of B. burgdorferi results in a conditional reconstruction of the outer membrane. **The spheres, which appear to be more resistant to unfavorable conditions and exhibit reduced immune reactivity when compared to spirochetes, might allow the B. burgdorferi to escape complete clearance and possibly ensure long-term survival in the host.**

117: Evaluation of in-vitro antibiotic susceptibility of different morphological forms of Borrelia burgdorferi Authors: Sapi E, Kaur N, Anyanwu S, Luecke DF, Datar A, Patel S, Rossi M, Stricker RB

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Background: Lyme disease is a tick-borne illness caused by the spirochete Borrelia burgdorferi. Although antibiotic therapy is usually effective early in the disease, relapse may occur when administration of antibiotics is discontinued. **Studies have suggested that resistance and recurrence of Lyme disease might be due to formation of different morphological forms of B. burgdorferi, namely round bodies (cysts) and biofilm-like colonies.** Better understanding of the effect of antibiotics on all morphological forms of B. burgdorferi is therefore crucial to provide effective therapy for Lyme disease.

Methods: Three morphological forms of B. burgdorferi (spirochetes, round bodies, and biofilm-like colonies) were generated using novel culture methods. Minimum inhibitory concentration and minimum bactericidal concentration of five antimicrobial agents (doxycycline, amoxicillin, tigecycline, metronidazole, and tinidazole) against spirochetal forms of B. burgdorferi were evaluated using the standard published microdilution technique. The susceptibility of spirochetal and round body forms to the antibiotics was then tested using fluorescent microscopy (BacLight™ viability staining) and dark field microscopy (direct cell counting), and these results were compared with the microdilution technique. Qualitative and quantitative effects of the antibiotics against biofilm-like colonies were assessed using fluorescent microscopy and dark field microscopy, respectively.

Results: Doxycycline reduced spirochetal structures ~90% but increased the number of round body forms about twofold. Amoxicillin reduced spirochetal forms by ~85%–90% and round body forms by ~68%, while treatment with metronidazole led to reduction of spirochetal structures by ~90% and round body forms by ~80%. Tigecycline and tinidazole treatment reduced both spirochetal and round body forms by ~80%–90%. When quantitative effects on biofilm-like colonies were evaluated, the five antibiotics reduced formation of these colonies by only 30%–55%. In terms of qualitative effects, only tinidazole reduced viable organisms by ~90%. Following treatment with the other antibiotics, viable organisms were detected in 70%–85% of the biofilm-like colonies.

Conclusion: **Antibiotics have varying effects on the different morphological forms of B. burgdorferi. Persistence of viable organisms in round body forms and biofilm-like colonies may explain treatment failure and persistent symptoms following antibiotic therapy of Lyme disease.**

118: **Benefit of intravenous antibiotic therapy in patients referred for treatment of neurologic Lyme disease:** Authors: Stricker RB, DeLong AK, Green CL, Savely VR, Chamallas SN, Johnson L. Published Date September 2011 Volume 2011:4 Pages 639 - 646

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Background: We have shown previously that extended intravenous antibiotic therapy is

associated with low morbidity and no mortality in patients referred for treatment of neurologic Lyme disease. In this study, **we evaluated the benefit of extended intravenous antibiotic therapy in patients with symptoms of neurologic Lyme disease.**

Methods: Patients with significant neurologic symptoms and positive testing for Borrelia burgdorferi were treated with intravenous antibiotics, and biweekly evaluation of symptom severity was performed using a six-level ordinal scale. Four symptoms were selected a priori as primary outcome measures in the study, ie, fatigue, cognition, myalgias, and arthralgias. Patients were placed into five groups according to time on treatment (1–4, 5–8, 9–12, 13–24, and 25–52 weeks), and changes in the primary symptoms as a function of time on treatment were analyzed using a mixed-effects proportional odds model.

Results: **Among 158 patients with more than one follow-up visit who were monitored for up to 1 year, there were on average 6.7 visits per person** (median 5, range 2–24). The last follow-up day was on average 96 days after enrollment (median 69, range 7–354 days), corresponding to the length of antibiotic therapy. Each primary symptom was significantly improved at one or more time points during the study. **For cognition, fatigue, and myalgias, the greatest improvement occurred in patients on the longest courses of treatment (25–52 weeks) with odds ratios (OR) for improvement of 1.97 (P = 0.02), 2.22 (P < 0.01), and 2.08 (P = 0.01), respectively.** In contrast, arthralgias were only significantly improved during the initial 1–4 weeks of therapy (OR: 1.57, P = 0.04), and the beneficial effect of longer treatment did not reach statistical significance for this symptom.

Conclusion: **Prolonged intravenous antibiotic therapy is associated with improved cognition, fatigue, and myalgias in patients referred for treatment of neurologic Lyme disease. Treatment for 25–52 weeks may be necessary to obtain symptomatic improvement in these patients.**

119: Embers ME, Barthold SW, Borda JT, Bowers L, Doyle L, et al. (2012) Persistence of Borrelia burgdorferi in Rhesus Macaques following Antibiotic Treatment of Disseminated Infection. PLoS ONE 7(1): e29914. doi:10.1371/journal.pone.0029914

The persistence of symptoms in Lyme disease patients following antibiotic therapy, and their causes, continue to be a matter of intense controversy. The studies presented here explore antibiotic efficacy using nonhuman primates. Rhesus macaques were infected with B. burgdorferi and a portion received aggressive antibiotic therapy 4–6 months later. Multiple methods were utilized for detection of residual organisms, including the feeding of lab-reared ticks on monkeys (xenodiagnosis), culture, immunofluorescence and PCR. Antibody responses to the B. burgdorferi-specific C6 diagnostic peptide were measured longitudinally and declined in all

treated animals. B. burgdorferi antigen, DNA and RNA were detected in the tissues of treated animals. Finally, small numbers of intact spirochetes were recovered by xenodiagnosis from treated monkeys. These results demonstrate that B. burgdorferi can withstand antibiotic treatment, administered post-dissemination, in a primate host. **Though B. burgdorferi is not known to possess resistance mechanisms and is susceptible to the standard antibiotics (doxycycline, ceftriaxone) in vitro, it appears to become tolerant post-dissemination in the primate host. This finding raises important questions about the pathogenicity of antibiotic-tolerant persisters and whether or not they can contribute to symptoms post-treatment.** "Our studies do however offer proof of the principle that intact spirochetes can persist in an incidental host comparable to humans, following antibiotic therapy. Additionally, our experiments uncover residual antigen associated with inflammatory foci."

1 "We conclude that the treatment of Borrelia burgdorferi (**Lyme Disease**) with appropriate **antibiotics for even more than 3 months may not always eradicate the spirochete**." - Ann Med. 1999 Jun; 3,3:225-32.

Michael Parent Sept 1, 2010 12:10 PM

2 "The Lyme disease spirochete, can be recovered long after initial infection, even from antibiotic-treated patients, indicating that it **resists eradication by host defense mechanisms and antibiotics**".- 1:20 J Infect Dis. 1992 Aug;166,2:440-4

Michael Parent Sept 1, 2010 12:15 PM

3 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.**" - Infection. 1998 Nov-Dec; 26,6:364-7

Michael Parent Sept 1, 2010 12:18 PM

4 "These data demonstrate that **Lyme neuroborreliosis is a persistent infection**."- Ann Neurol. 2001 Sep;50,3, :330-8

Michael Parent Sept 1, 2010 12:21 PM

5 "In one of the six analysed brain tissue specimens [from a patient that received more than **six months of antibiotic treatment prior to death**, including two 3-week courses of IV ceftriaxone], **B. burgdorferi DNA was detected by PCR.**" - Brain. 1996 Dec;119, Pt 6:2143-54

Michael Parent Sept 1, 2010 12:43 PM

6 "Two hundred seventy-seven patients with chronic Lyme disease were treated with tetracycline for 1 to 11 months, **These results support the use of longer courses of treatment in the management of patients with chronic Lyme disease."** - Clin Infect Dis. 1997 Jul;25 Suppl 1:S52-6.

Michael Parent Sept 1, 2010 12:12 PM

7 "**Prolonged intravenous antibiotic therapy is associated with improved cognition, fatigue, and myalgia**s in patients referred for treatment of **neurologic Lyme** disease. **Treatment for 25–52 weeks** may be necessary **to obtain symptomatic improvement** in these patients. International Journal of General Medicine Published Date September 2011 Volume 2011:4 Pages 639 - 646

Anonymous 1 Sept 8, 2011 1:18 PM