



# Chronic Lyme borreliosis at the root of multiple sclerosis – is a cure with antibiotics attainable?

Markus Fritzsche\*

*Clinic for Internal and Geographical Medicine, Soodstrasse 13, 8134 Adliswil, Switzerland*

Received 15 September 2004; accepted 17 September 2004

**Summary** Apart from its devastating impact on individuals and their families, multiple sclerosis (MS) creates a huge economic burden for society by mainly afflicting young adults in their most productive years. Although effective strategies for symptom management and disease modifying therapies have evolved, there exists no curative treatment yet. Worldwide, MS prevalence parallels the distribution of the Lyme disease pathogen *Borrelia* (*B.*) *burgdorferi*, and in America and Europe, the birth excesses of those individuals who later in life develop MS exactly mirror the seasonal distributions of *Borrelia* transmitting *Ixodes* ticks. In addition to known acute infections, no other disease exhibits equally marked epidemiological clusters by season and locality, nurturing the hope that prevention might ultimately be attainable. As minocycline, tinidazole and hydroxychloroquine are reportedly capable of destroying both the spirochaetal and cystic L-form of *B. burgdorferi* found in MS brains, there emerges also new hope for those already afflicted. The immunomodulating anti-inflammatory potential of minocycline and hydroxychloroquine may furthermore reduce the Jarisch Herxheimer reaction triggered by decaying *Borrelia* at treatment initiation. Even in those cases unrelated to *B. burgdorferi*, minocycline is known for its beneficial effect on several factors considered to be detrimental in MS. Patients receiving a combination of these pharmaceuticals are thus expected to be cured or to have a longer period of remission compared to untreated controls. Although the goal of this rational, cost-effective and potentially curative treatment seems simple enough, the importance of a scientifically sound approach cannot be overemphasised. A randomised, prospective, double blinded trial is necessary in patients from *B. burgdorferi* endemic areas with established MS and/or *Borrelia* L-forms in their cerebrospinal fluid, and to yield reasonable significance within due time, the groups must be large enough and preferably taken together in a multi-centre study.

© 2004 Elsevier Ltd. All rights reserved.

## Introduction

Multiple sclerosis (MS) manifests as an acute inflammatory demyelination of the central nervous system (CNS) culminating in the multifocal sclerotic plaques from which the disease gets its name.

Afflicting as many as one million young adults in their most productive years worldwide, the chronic disease often takes a severe, disabling course. Worse still, although effective strategies for symptom management and disease modifying therapies have evolved, there exists neither prevention nor a cure for MS yet. If caused by an infection as presupposed by Pierre Marie in 1884 [1], what particular germ could lie at its root and what kind of treatment would be effective?

\* Tel.: +41 1 710 93 43; fax: +41 1 710 93 44.  
E-mail address: [markus.fritzsche@gmx.ch](mailto:markus.fritzsche@gmx.ch).

In 1901, Robert Koch postulated that for determining the cause of a disease the following conditions must be met: (i) the infectious agent must be found in every case in a logical pathological relationship to the disease and its symptoms, (ii) the agent should occur in no other situation, (iii) it must be isolated and obtained in pure culture, and (iv) when transferred to a susceptible host, preferably an animal from which it can subsequently be recovered, the organism should adequately reproduce the disease process. With respect to the fourth postulate, Koch was aware of the natural constraints and limitations of the experimental approach [2].

## Spirochaetal aetiology

When in 1925 Adams et al. [3] inoculated rhesus monkeys with material from patients with MS, spirochaetes emerged in their cerebrospinal fluid after several months. Stained films showed “several spirochaetes with rather irregular open spirals and varying from 15 to 20µm in length and about 1 µm in thickness” in one animal. In the other “on examination of the fluid from the lateral ventricle immediately after death, a single actively motile spirochaete, similar to those already noted in the first animal, was found on dark-ground examination”.

In contrast to infection with *Treponema (T.) pallidum*, which is an obligate human parasite and for which no animal models exist, there are many animal models of Lyme borreliosis under investigation. In some animals, infection with *Borrelia* spontaneously clears, in others infection clears with antibiotics, while still in others antibiotics contain but never clear the infection. The best animal model for neuroborreliosis is the rhesus monkey (*Macaca mulatta*). However, for as yet unclear reasons CNS tissues of the rhesus monkey inoculated with *B. burgdorferi* have all remained culture negative [4]. Many bacteria including *T. pallidum* can be visualised by electron and dark-field microscopy, but cannot be grown in culture. Koch’s postulates cannot be fulfilled, because it is impossible to experimentally duplicate all the variables that are involved in the disease expression of persistent, difficult- or impossible-to-culture bacteria.

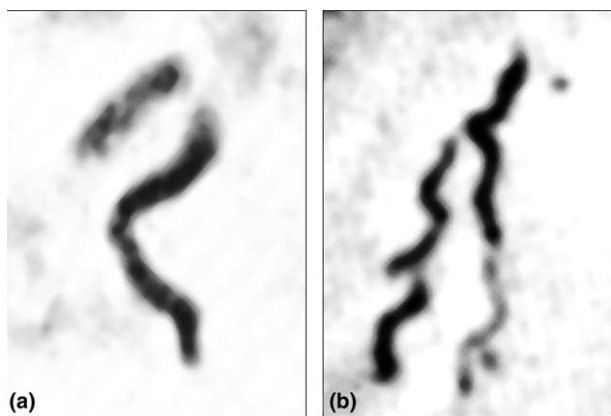
Since the first experimental studies with MS in animals, dark-field microscopy studies of human brains pointed to an aetiological involvement of spirochaetes in MS. As early as 1928, Gabriel Steiner [5] demonstrated in the periphery of MS pla-

ques numerous argyrophilic granules. Their polymorphic arrangement was highly reminiscent of neurosyphilis and leptospirosis. “After an extremely fatiguing inspection of countless slides it was possible to find well-preserved forms, which did not lie in cells and the morphological feature of which had to be specified as nothing else than the one of a spirochaete” [5]. These findings were replicated and published by Steiner [6,7] on several occasions and by 1952 “no structures similar to or identical with the spirochaetes have been found in over 250 control cases of diversified diseases other than multiple sclerosis and of normal brains”.

The reported spirochaetes were dismissed by his critics as “spirochaete-like structures of the tissue proper, such as reticulin fibrils” – an interesting historical parallel to the first histopathological demonstration of *T. pallidum*, which met the same “objection of being reticulin fibres and not spirochaetes” [7]. Due to damage to the brain barrier and subsequent invasion into the CNS, or due to heightened susceptibility to infection, it was also contended, the organisms could be contaminants in chronic MS patients with bedsores. A caveat that applies to all infectious pathogens possibly associated with MS, irrespective of whether we regard ‘MS’ as a disease entity or a set of different aetiologies. The criticism, however, did not account for the limitation of the spirochaetes to acute MS lesions which Steiner obtained from patients who had just died of MS before the autopsy. Nor did it account for the fact that he observed in their brains actively reproducing spirochaetes (Fig. 1).

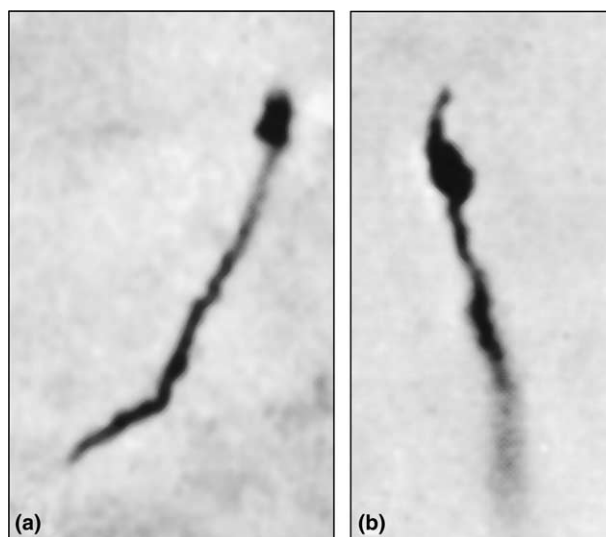
In addition, the so-called myelin sheath destroying spirochaete *Spirochaeta myelophtora* [7] exhibited distinct blebs in the peripheral part of the bacterium (Fig. 2, see also Fig. 3(b)). The argyrophilic granules were in intimate pathogenic relation to the spirochaetal form. Breaking up started with the appearance of a partial thickening and finally the formation of cystic granules of different size. According to Steiner, this sequence of events represented the possible transitional phases from the spirochaete to cyst formation – later known as Lister or L-forms.

Although still clouded with controversy, a considerable body of clinical evidence supports the concept that these cystic L-forms [8] of *B. burgdorferi* (Fig. 3(a)) may cause chronic, persistent disease including MS. Even in the absence of anti-borrelia antibodies, exposure to stress in the mammalian milieu may trigger the production of membrane bound and possibly secreted L-forms. These cystic forms typically present as argyrophilic granules and relate to the unpredictable appearance of



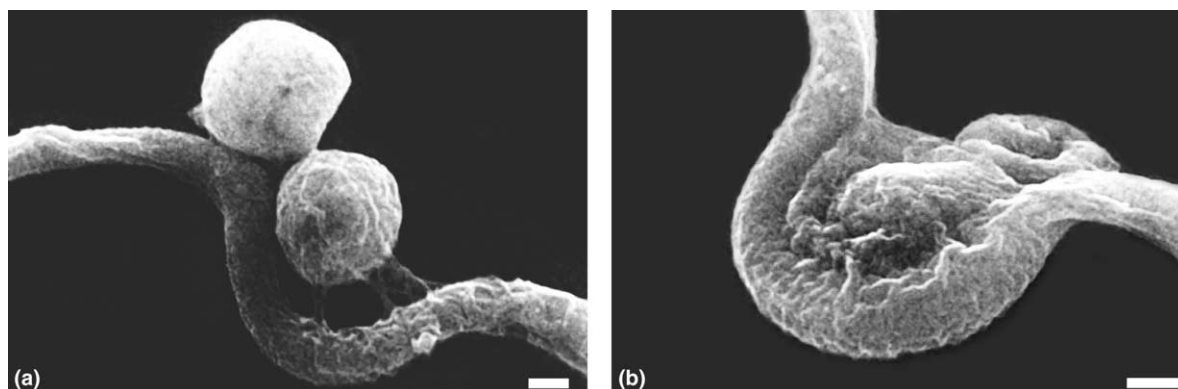
**Figure 1** (a) and (b) Reproducing spirochaetes from MS plaques. As spirochaetes reproduce by transverse fission, the division is preceded by a longitudinal growth of the individual spirochaete. The elongated spirochaetes Steiner observed were mature for fission. Sometimes a very delicate fine filament disclosed the place of final separation, or the two spirochaetes tapered off at their adjacent ends leaving only a short distance between them. Another indication of fission was, according to Steiner, the appearance of two spirochaetes, with their longitudinal axis arranged in nearly parallel direction to each other. In such a case the division may have occurred at a point where both portions of the parent spirochaete were bent to an inclination of nearly 180°. (Reproduced with permission from Der Nervenarzt [5] and the Journal of Neuropathology and Experimental Neurology [7].)

spirochaetes in host tissues [9]. The blebs, which are often located to the spirochaetal ends, contain plasmid DNA and virulence factors capable of adhering to human endothelial cells [10]. Once separated from the parent spirochaete, the encysted forms are of low metabolic states as a starvation response retaining the capacity of retransforming into regular, mobile bacteria in better times. In



**Figure 2** (a) and (b) Spirochaetes from MS plaques exhibiting blebs. (Reproduced with permission from the Journal of Neuropathology and Experimental Neurology [7].)

the first half of the 20th century, an alternative viewpoint was that these blebs or ‘gemmae’ represented another stage of the life cycle of spirochaetes. This actually encouraged the early classification of borreliae as protozoans [10]. However, as Steiner noted [7], the specific argyrophilia of the spirochaetal surface is common to bacterial but not to protozoan surfaces, and in addition to immunohistological methods, silver impregnation is still a routinely employed technique for demonstrating spirochaetes in host tissues. More recently, neuropathologists documented the presence of cystic structures suggesting that MS patients are chronically infected with a spirochaete, most likely *B. burgdorferi* [11], the causative agent in Lyme disease (Fig. 4).

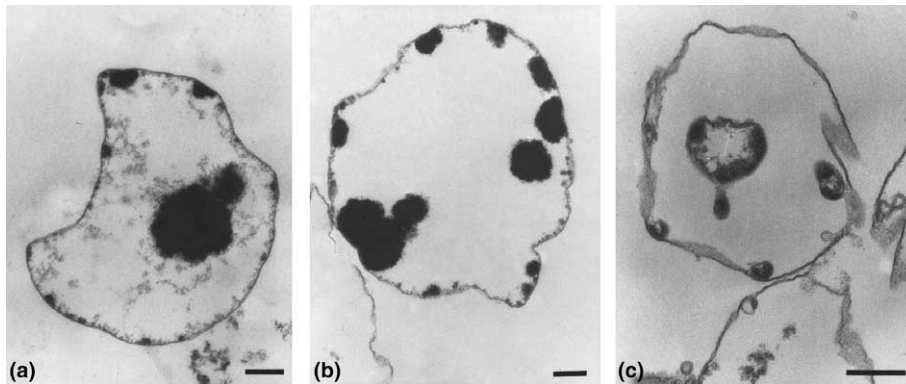


**Figure 3** Spirochaetal and L-forms of *B. burgdorferi*. Note the cystic structures representing L-forms (a) and blebs (b) adherent to a spirochaetal form of *B. burgdorferi*. The bar represents 200 nm. (Reproduced with permission from Infection [8].)

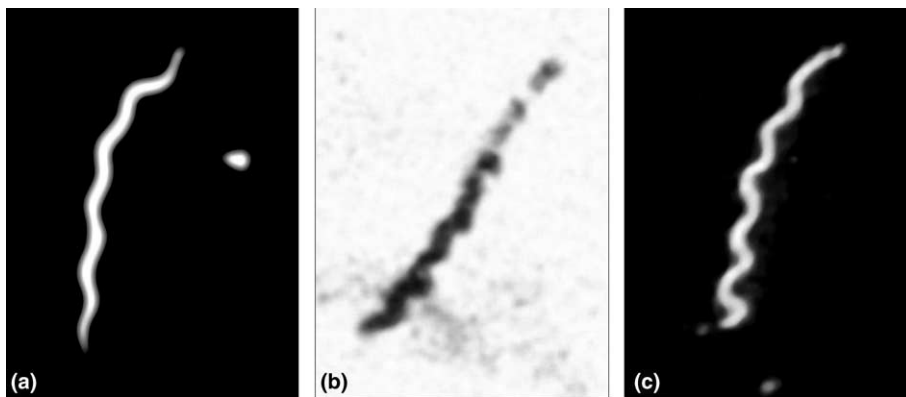
Despite oscillating ‘‘spirochaetes of the *Borrelia* type’’ (Fig. 5(a)) independently and exclusively documented in the cerebrospinal fluid of MS patients [12], scepticism prevailed amongst neurologists, and seroepidemiological studies relating *B. burgdorferi* to MS have produced conflicting results at best. These, however, come as no surprise. When entering their hosts, spirochaetes including *B. burgdorferi* often undergo extensive antigenic and metabolic changes, which appear to prevent them from being recognised by the host’s immune system. For this reason it is often difficult, if not impossible, to reach a conclusive diagnosis for several reasons even with respect to clinical Lyme borreliosis [8,13].

Although prone to be dismissed as ‘anecdotal’, a case report makes perfect sense with regard to reconversion of L-forms to *B. burgdorferi* during an acute attack of MS. In a retrospective study on Lyme borreliosis in Italy [14], an immunofluorescence-absorbition titre of 1/64 was found in a serum sample of a patient diagnosed with MS. A serum sample taken from the same patient during an exacerbation of MS converted to a titre of 1/256, which suggested the acute attack was directly related to an altered exposure to *Borrelia* antigens.

The truth resurfaced in 2001 [11]. Cystic structures originating from spirochaetes were isolated in 8 out of 10 Norwegian MS patients by means



**Figure 4** Cystic L-forms of *B. burgdorferi*. Note the fascinating similarity between (a) the cyst collected from the cerebrospinal fluid of an MS patient, (b) the cyst from the cerebrospinal fluid of a Lyme borreliosis patient with a migrating rash and (c) the cysts of *B. burgdorferi* grown in vitro. The bar represents 500 nm (EM photographs kindly presented by Øystein Brorson and Sverre-Henning Brorson, 2003). Reproduced with permission (from Fritzsche M. Epidemiological correlation of sporadic schizophrenia to Lyme borreliosis. In: Fatemi SH, editor. Infectious Disease and Neuropsychiatric Disorders, 2005. London: Taylor & Francis, in press).



**Figure 5** Spirochaetal forms of *B. burgdorferi*. (a) Living, partly despiralised spirochaete of approximately 15  $\mu\text{m}$  in length collected from the cerebrospinal fluid of an MS patient, (b) ‘decaying’ spirochaete obtained from an MS plaque, and (c) *B. burgdorferi* s.s. grown in culture. (Reproduced with permission from Schweizerische Medizinische Wochenschrift [12], Der Nervenarzt [5] and Virion Laboratories, Zurich/Switzerland, respectively.)

of immunofluorescence and in all 10 MS patients by transmission electron microscopy (see Fig. 4(a)) and staining after culture. No such cysts could be observed in the five controls with either method, but the investigators noted a similarity between those found in the MS patients and the cystic forms characteristic of chronic *B. burgdorferi* infection (Fig. 4(b)). More significantly, the cysts of the MS patients exhibited positive reactions to anti-borrelia antiserum and after culturing (Fig. 4(c)), curved spirochaete-like bacteria emerged and these structures could be propagated [11]. Albeit limited in the number of patients, the first, second and third of Koch's postulates were thus fulfilled and the microbiological evidence based on solid ground. The authors also observed that transformation of the *B. burgdorferi* to cystic forms occurred invariably and rapidly after incubation in cerebrospinal fluid (CSF) and that they could be reconverted to spirochaetes if the conditions became favourable. The MS patients in this Norwegian study in fact originated from a well-defined area where Lyme borreliosis as well as MS is endemic. Clinically, all of them had relapsing remitting MS according to Poser's criteria [11].

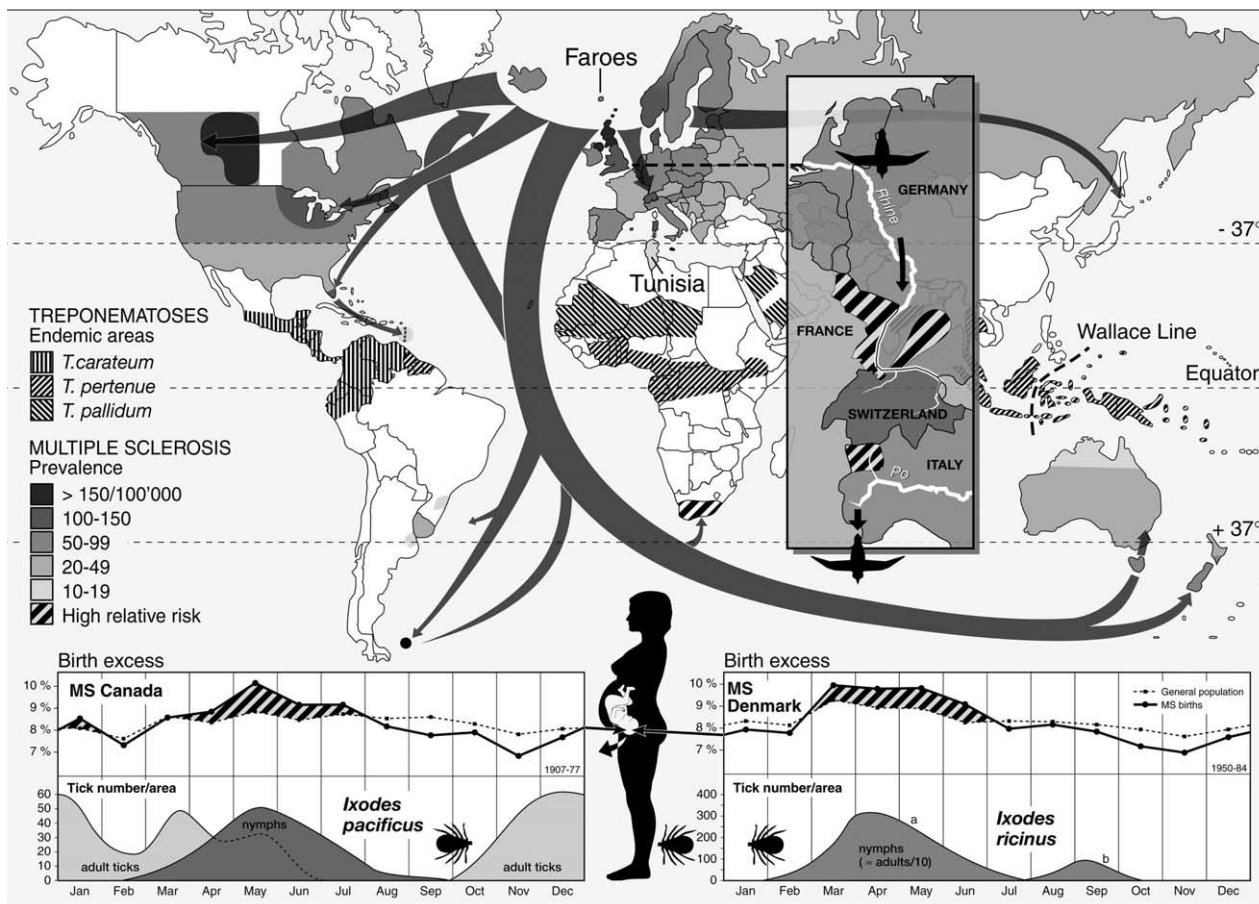
In spite of numerous reports about a possible association between MS and chronic infection with *B. burgdorferi*, which in its chronic form is supposed to be an autoimmune disease triggered by these spirochaetes [15–18], the same counter-arguments re-emerged. "Whether this infection really was *B. burgdorferi* and whether it occurred before or after the onset of multiple sclerosis cannot be determined from this study and indeed, given current methodology, it is difficult to imagine how this could be determined" [19]. In such a situation, technology plays a lesser role and the art of epidemiology prevails.

## Epidemiological correlation

### *Ixodes* ticks, *Borrelia burgdorferi* and MS

Additional constraints usually determined by probabilistic approaches are known as risk factors, and these we assume – with a leap of faith – to be causative. Worldwide, MS prevalence parallels *B. burgdorferi sensu lato* (*s.l.*) endemicity, and in America and Europe, the birth excesses of those individuals who later in life develop MS exactly mirror the seasonal distributions of *B. burgdorferi* transmitting *Ixodes* ticks at the time of birth [20] (Fig. 6).

The arrows on Fig. 6 represent the migratory routes of seabirds, if a number of species such as puffins and seagulls are taken together. To avoid very cold and hot weather, seagulls usually move parallel to latitude or they simply disperse over comparatively short distances along rivers. Although epidemics of MS have been attributed to changes in ascertainment or better diagnosis, particularly of more benign cases in the post-war era, another common setting for MS is proximity to islands or coastal areas where seabirds nest. At a site near three major seabird colonies in south-eastern Alaska, for example, MS was unknown until its first outbreak occurred in 1965. Tunisia, which is reached by European migratory birds introducing *Ixodes* ticks and *B. burgdorferi*, scores the highest rate of MS in Africa. And on the Faroes, where *Ixodes* ticks have reportedly transmitted Lyme borreliosis from seabirds to human bird catchers, MS unfolded after an annulled ban on fowling seabirds during a food shortage in World War II. Mainly responsible for the transhemispheric exchange of *B. burgdorferi* are puffins or shearwaters. Between September and December, these birds spend their time along the American coast from Rio de Janeiro in the north to the Rio de la Plata in the south. By March and April, the puffins leave their breeding colonies on the Falklands and other islands in the South Atlantic heading northwest across the equator to the rich fishing waters off Newfoundland. By the end of July, they gradually move back across the North Atlantic, where they are often seen around Scotland, Ireland and the Faroes during the traditional puffin-hunting season. In the southern oceans, where the winds blow almost continuously eastwards in the roaring forties and furious fifties, a ringed great puffin has even been found in Australia. Short-tailed puffins are limited to this part of the southern hemisphere, where the birds breed on islands off the coast of New Zealand and Australia, and in Tasmania, as on the Faroes, their so-called mutton-bird chicks are fowled regularly. Although of hitherto unexplained low prevalence, Lyme borreliosis as well as MS can be found in South East Asia, namely in Japan and Taiwan down to the Philippines, where the Wallace Line limits the southward spread of *Borrelia* harbouring *Ixodes* ticks. Southern Australia and New Zealand, by contrast, which can be reached directly by polar seabirds carrying *B. burgdorferi* via the Antarctic, score relatively high rates of MS. Yet even the highest prevalence rate for PR in these communities, largely originating from the UK, is not much more than half the rate in most parts of the British Isles. This difference in relative risk is hard to understand from a purely genetic point of view. But



**Figure 6** Geographical and seasonal correlation of multiple sclerosis to *Borrelia burgdorferi* transmitting *Ixodes* ticks (adapted from [20]).

there is not much room for pure environmentalists either. For New Zealand scores lower MS rates than places in Australia on a comparable southerly latitude, and migratory seabirds introducing *B. garinii* from the northern hemisphere reach Australia before they reach New Zealand. Likewise, in the US south of the 37° latitude, the prevalence rate of both Lyme borreliosis and MS is significantly lower, as ticks lose their infectious potential for human beings when feeding on endemic lizards. The only notable exception is Florida where migratory seabirds stop over for nesting. In America and Europe, the birth excesses of those individuals who later in life develop MS exactly mirror the seasonal distributions of *Ixodes* ticks at the time of birth. This seasonal correlation implies – analogous to the transmission of chronic hepatitis B infection during delivery – that one form of MS is caused by neonatal exposure to maternal *B. burgdorferi s.l.* infection. Apart from acute infections, no other disease exhibits equally marked clusters by season and locality, nurturing the hope that multiple scler-

osis might ultimately be preventable (referenced in [20]).

### Heat shock protein dependent pathogenesis

Intriguingly, the geographical gradient of MS sharply declines at 37° latitudes, entirely sparing the tropical belt where human treponematoses are endemic (see Fig. 6). In more temperate climates, *B. burgdorferi s.l.* infection rates of seabird ticks match MS prevalence rates worldwide. This uneven geographical distribution of MS versus endemic treponematoses can be explained by the different expression of heat shock proteins (HSPs), which not only protect from fluctuations in temperature, but also activate host immune defences. Molecular evidence reveals that the pathogenic spirochaetes have circumvented this immunological impasse differently. Whilst in the course of evolution *Treponema* as an exclusively human pathogen could afford to delete the capacity of heat shock resist-

ance and thus vector-borne transmission, *B. garinii* adapted to a broad range of changes in temperature by expressing HSPs. For rapid adaptation to preferentially 38° is critical when transmitted from the tick vector to the human brain as well as warm-blooded seabirds with an average temperature of 38° (referenced in [20]).

*Treponema pallidum*, being exquisitely sensitive to temperature, can be eliminated by malaria-induced fever and this was indeed the standard treatment for cerebral syphilis until penicillin became the established therapeutic choice. Tens of thousands of psychiatric patients were thus healed, and Dr. Wagner-Jauregg rightly won the Nobel Prize for this daring achievement in 1927. The spirochaetal deletion of HSPs is interesting for two other reasons. First, a fundamental tenet of microbial pathogenesis holds that bacterial pathogens must overcome host iron limitation to establish a successful infection, and HSP90 is reportedly linked to membrane bound iron metabolism [21]. While maintaining the genetic expression of most heat shock proteins [22], *B. burgdorferi* has bypassed this host defence by eliminating the need for iron [23] and HSP90 [24]. Second, in order to become functional and virulent, intracellular pathogens (like *B. burgdorferi*) sequester cytosolic HSP90 and other HSPs in membrane bound complexes from their human host [25,26]. Not surprisingly, auto-antibodies can therefore be demonstrated against HSPs during such infections [27,28], as well as in patients suffering from MS [29].

## Pathological correlate

Although still a controversial issue, molecular mimicry of *Borrelia* epitopes are supposed to misdirect antibodies against host tissues [15–17,29,30]. The tick-borne pathogen must avoid being destroyed by the immune response while maintaining access to a new host, and protracted antigenic exposure destabilises the immune system. The mechanisms of tissue damage in autoimmune diseases are essentially the same as those operative in other inflammatory disorders including chronic infections. In MS the immunologic response simply persists because the immune system is not able to remove the offending agent from the body. Worse still, hitherto hidden auto-antigens are released from damaged tissues thus amplifying the auto-aggressive response.

The CNS consists of numerous candidate auto-antigens, among which are major myelin constitu-

ents including the myelin basic protein and several members of the HSP family, among which HSP70 is involved in myelin folding [31]. MS lesions also contain T-lymphocytes which have been suggested to attack oligodendrocytes expressing HSPs. In the complete collection of proteins extracted from MS-affected myelin, however, the dominant human antigen appears to be  $\alpha$ B-crystallin [32]. Within demyelinating MS lesions, this small HSP is present at enhanced levels in the cytosol of oligodendrocytes and astrocytes, where it is up-regulated at the earliest stages of pathologic formation and where its expression correlates with immunologic activity. Histologically located in close proximity to T cells, perivascular macrophages not only express MHC receptors. Inside its lysosomal vesicles macrophages also harbour  $\alpha$ B-crystallin HSP, a reason why these macrophages have been suggested to present myelin-derived HSP to infiltrating T cells [33]. The expression of  $\alpha$ B-crystallin is thus unlikely on its own to trigger demyelinating autoimmunity, but may, according to the authors, contribute to the amplification of local inflammatory responses in parallel with stress-producing events such as local immune responses to infectious antigens [34].

The cardinal features of pathology in MS are inflammatory demyelination with relative but not complete preservation of axons and astrocytic gliosis culminating in the multifocal sclerotic plaques. Inflammation increases the permeability in the blood–brain barrier (BBB) as the earliest detectable sign in MS followed by a focal infiltration of lymphocytes around small blood vessels in the brain and spinal cord [35,36]. As most infectious agents are assumed to enter the CNS from the bloodstream, it is tempting to speculate that these inflammatory cells are at the onset of MS reacting against foreign antigens located in the CNS.

There are several anatomical features of the nervous system that influence immune responses to CNS infection. These include the BBB, which stands as a physical barrier against the passage of immune elements from the periphery into the CNS, the Virchow-Robin spaces surrounding blood vessels that penetrate into the brain where important immunological reactions occur, and CSF recirculation pathways, which may disseminate microorganisms via particular routes throughout the neuraxis. Effective immunologic defence is in fact limited to the Virchow-Robin spaces which contain macrophage-like cells with phagocytic and antigen-presenting capacities that are normally not found within the brain parenchyma itself. Cerebrovascular endothelial cells maintain tight intercellular junctions and very low rates of vesicular transport across dense basement membrane

that differentiate them from the more permeable endothelium found in other tissues. Under these circumstances, the BBB generally prevents the entry of infectious pathogens, inflammatory cells, and circulating proteins such as antibodies and cytokines into the CNS. Infectious pathogens circulating in the bloodstream are thus effectively excluded from the brain. If the BBB is breached, however, the pathogens often take firm hold within the CNS, where the intrinsic immune capacity of neural tissues is limited and where effective host responses must surmount the BBB from the periphery. While this minimises the risk of CNS autoimmunity, it may also allow infectious pathogens to remain sequestered behind the BBB and hidden from the immune system [37].

The brain parenchyma itself has a surprisingly deficient capacity to initiate a primary immune response. The paucity of major histocompatibility (MHC) antigen expression within the CNS, particularly of neurons, limits direct T-cell mediated clearance of intracellular pathogens and may thus predispose to chronic or relapsing neurological infections. CSF is produced by cells of the choroid plexus within the ventricles of the brain. Its protein content, including antibodies and complement which are the primary immune mediators at baseline, are present at less than 1% of circulating blood levels. Worse still, CSF contains abundant nutrients such as glucose and amino acids on which most infectious pathogens depend. This fluid normally circulates within the subarachnoid space, thus disseminating invading microorganisms throughout the neuraxis [37]. Not surprisingly after all, the majority of plaques tend to be periventricular in distribution, although any part of the CNS can be affected in MS and despite the fact that the initial lesion is frequently obscured by large confluent lesions [5].

## Therapeutic implications

One of the greatest triumphs of epidemiology stems from the control of cholera before the responsible organism, let alone its pathogenesis had been identified. Major progress in prevention is thus possible by focusing on those variables that are known and can be influenced. To reduce the risk of chronic spirochaetal infection and to prevent future generations from being afflicted by MS, public awareness is warranted. Since both tinidazole [38] and hydroxychloroquine (HCQ) are effective against L-forms of *B. burgdorferi* [39], there also emerges new hope that a curative treat-

ment might ultimately be attainable. So why not prescribe tinidazole or HCQ so as to eradicate *B. burgdorferi* in at least one form of infectious MS?

## Antibiotic effects of tinidazole on *Borrelia* L-forms

Protozoa such as *Toxoplasma gondii* may establish latent CNS infection in the form of inert cysts that evade host immune clearance. It is also known that *B. burgdorferi* is capable of adopting cystic L-forms both in vivo and in vitro when exposed to antibiotics [8]. This phenomenon, combined with the ability of the cysts to reconvert to mobile spirochaetes [11], may explain the frequent reactivations of the disease after an illusionary 'cure'. Recently, *B. burgdorferi* L-forms were thus exposed to tinidazole [38], a 5-nitroimidazole antibiotic which exhibits selective activity against anaerobic bacteria and an excellent capacity to pass the BBB [40]. Having a low molecular weight it also penetrates cystic cell membranes. The accumulation in susceptible microorganisms is mediated by a reduction of the tinidazole molecule to reactive intermediates, and when the intracellular concentration decreases more tinidazole can enter the cell [40]. The production of blebs subsequently decreases and the cystic structures of *B. burgdorferi* dissolve [38]. This observation suggests that tinidazole in combination with a spirochaetocidal antibiotic could eradicate both cystic and mobile forms of *B. burgdorferi* in the treatment of chronic Lyme disease and MS.

## Antibiotic and anti-inflammatory and effects of hydroxychloroquine

Since antimalarials are known to eradicate biologically active structures after penetrating cyst walls of protozoa, Brorson and Brorson [39] studied the susceptibility of cystic forms of *B. burgdorferi* to HCQ. In the presence of high concentrations of HCQ, they found that the amount of RNA decreased significantly and spirochaetal structures did not develop or they dissolved inside the cysts.

To my knowledge, only few human patients suffering from chronic Lyme borreliosis or MS have been treated with antimalarials. However, a sick horse with serologically confirmed borreliosis promptly improved upon treatment with HCQ (personal communication, Patric Luder, Pferdeklinik Cronau, Germany, 2003). The principle mechanism of HCQ relates to the intracellular alteration in pH, by which it interferes with the function of highly acidic compartments such as lysosomes. At a neutral serum pH, the uncharged, lipid soluble



form of HCQ readily permeates the cell membrane. With the subsequent acquisition of a second proton to produce a positively charged molecule, insoluble in lipid and incapable of passing back across the vesicle membrane, the protonated form remains trapped. As more hydrogen ions are pumped into the vesicles by ATP-dependent channels, more of HCQ will diffuse from serum into the cyst amounting to an over 100-fold excess concentration of the drug. These high concentrations of HCQ are known to inhibit RNA and DNA synthesis by complex formation and binding to DNA templates (referenced in [39,41,42]).

As the encysted forms are susceptible to HCQ at concentrations achievable in vivo and intracellularly at normal body temperature, HCQ alone may be sufficient in the treatment of intracellular cystic forms of *B. burgdorferi*. However, in order to eradicate also its mobile spirochaetal forms, which according to Steiner [5–7] and Simons [12] might play a specific pathogenic role during acute relapses of MS, a therapeutic combination of HCQ and minocycline is preferable to the application of HCQ alone. Conversely, the combination of minocycline plus HCQ or tinidazole is preferable to the application of minocycline alone, particularly in those forms of persistent Lyme borreliosis in which the dormant L-forms have developed antibiotic resistance. Examples for the effectiveness of therapeutic synergy are furthermore provided by the antiviral therapies against HIV infection or the combination of the immunosuppressive regimes routinely used in transplantation. As both pharmaceuticals, HCQ [42–44] and minocycline [45], are also well known for their immunomodulating anti-inflammatory effects, they might exert a beneficial effect during the inflammatory phase of MS. In addition, the potentially harmful Jarisch Herxheimer reactions which can be triggered by decaying *Borrelia* at treatment initiation may be reduced by these drugs.

### Antibiotic and anti-inflammatory effects of minocycline

Minocycline, which is known to effectively penetrate the BBB and to eliminate *B. burgdorferi* by its antibiotic effect [46–48], also impacts on several factors considered to be detrimental in MS [45]. Due to its chelating property, minocycline is a direct inhibitor of matrix metalloproteinase (MMP) activity by complex formation, and the reduction of MMP-9 decreases the transmigration of T cells across the matrix barrier of the

brain. In addition, minocycline has been shown to attenuate both mild and severe experimental autoimmune encephalomyelitis in mice, an animal model of MS. In further support of a pathogenic role for MMP-9, young mice genetically deficient of MMP-9 are relatively resistant to EAE (referenced in [45]).

MMP-9 elevation is apparently dependent on cell accumulation in the CSF and thus rather a consequence than a prerequisite for cellular invasion [49]. *B. burgdorferi*, being non-toxic to neurons and unable to express MMP activity directly, is able to induce the expression of MMP-9 by primary neural cultures [50]. However, since MMPs can digest myelin basic protein [51], *Borrelia* is thought to promote CNS injury by indirectly binding and inducing the expression of MMPs at glial [52] and other neuronal cells. Digestion of the brain extracellular matrix could thus facilitate the migration and dissemination of *B. burgdorferi* within the CNS [53]. Not surprisingly after all, elevated levels of cerebrospinal MMP-9 have been reported in the course of various pathological processes including Lyme borreliosis [53] and MS [54,55].

It is well known that in patients with rheumatoid arthritis, minocycline improves laboratory parameters of disease activity, especially the acute-phase reactants and rheumatoid factor levels in the serum. Minocycline also suppresses the activation of microglia and this has been thought to be the mechanism by which it is neuroprotective in focal or global ischaemic models of stroke. Minocycline has also been reported to inhibit the expression of caspase delaying mortality in a transgenic mouse model of Huntington's disease, and in a model of Parkinson's disease the antibiotic reportedly protects against pharmacologically induced neurotoxicity. The most promising properties of minocycline, however, are the ones that favour its use in MS (referenced in [45]).

### Conclusion

In cases of latent or overt multiple sclerosis, there exists a problem. Its course presents over such remarkable variability over time or between events that uncontrolled clinical studies appear unacceptable. It should also be made clear whether we intend to address symptoms or cure the underlying disease, and whether the possible benefits would outweigh the side effects of a novel therapeutic intervention. Although these criteria seem simple enough, the importance of a scientifically sound approach cannot be overemphasised. A random-

ised, prospective, double-blinded trial with tinidazole or HCQ in addition to a spirochaetocidal antibiotic such as minocycline would be indispensable in patients from *B. burgdorferi* endemic areas with established MS and/or *Borrelia* L-forms in their cerebrospinal fluid. However, to yield reasonable significance within due time, the groups must be large enough and preferably taken together in a multi-centre study – while there appears an ethical problem for the physician in charge of the individual MS patient. Should we wait for the results of such a study, or does a rational, potentially curative and cost-effective treatment outweigh these caveats?

## Acknowledgements

Many thanks to Øystein Brorson and Sverre-Henning Brorson for all the information and the fantastic photographs taken of the *Borrelia burgdorferi* L-forms.

## References

- [1] Marie P. Sclerose en plaque et maladies infectueuses. *Prog Med* 1884;12:287–9.
- [2] Vasold M. Robert Koch. Heidelberg: Spektrum der Wissenschaft; 2002.
- [3] Adams DK, Blacklock WS, Cluskiie JAW. Spirochaetes in ventricular fluid of monkeys inoculated from cases of disseminated sclerosis. *J Pathol Bacteriol* 1925;28:117–8.
- [4] Coyle PK. Lyme disease. In: Asbury AK, McKhann GM, McDonald WI, Goadsby PJ, McArthur JC, editors. *Diseases of the nervous system*. Cambridge: Cambridge University Press; 2002. p. 1754–65.
- [5] Steiner G. Spirochäten im menschlichen Gehirn bei multipler Sklerose. *Der Nervenarzt* 1928;8:457–69.
- [6] Steiner G. Acute plaques in multiple sclerosis, their pathogenic significance and the role of spirochaetes as an etiological factor. *J Neuropathol Exp Neurol* 1952;11:343–72.
- [7] Steiner G. Morphology of *Spirochaeta myelophthora* in multiple sclerosis. *J Neuropathol Exp Neurol* 1954;13:221–9.
- [8] Mursic VP, Wanner G, Reinhardt S, Wilske B, Busch U, Marget W. Formation and cultivation of *Borrelia burgdorferi* spheroplast-L-form variants. *Infection* 1996;24:218–26.
- [9] Domingue Sr GJ, Woody HB. Bacterial persistence and expression of disease. *Clin Microbiol Rev* 1997;10:320–44.
- [10] Bergström S, Noppa L, Gylfe A, Ostberg Y. Molecular and cellular biology of *Borrelia burgdorferi sensu lato*. In: Gray O, Kahl O, Lane RS, Stanek G, editors. *Lyme borreliosis: biology, epidemiology and control*. Wallingford: CAB International; 2002. p. 175–200.
- [11] Brorson O, Brorson SH, Henriksen TH, Skogen PR, Schoyen R. Association between multiple sclerosis and cystic structures in cerebrospinal fluid. *Infection* 2001;29:315–9.
- [12] Simons HC. Spirochätenbefunde im Liquor bei multipler Sklerose. *Schweiz Med Wschr* 1957;18:544–5.
- [13] Satz N. Klinik der Lyme-Borreliose. Bern: Hans Huber; 2002.
- [14] Fumarola D. Multiple sclerosis and *Borrelia burgdorferi*. *Lancet* 1986;8506:575.
- [15] Martin R, Gran B, Zhao Y et al. Molecular mimicry and antigen-specific T cell responses in multiple sclerosis and chronic CNS Lyme disease. *J Autoimmunol* 2001;16:187–192.
- [16] Karussis D, Weiner HL, Abramsky O. Multiple sclerosis vs Lyme disease: a case presentation to a discussant and a review of the literature. *Mult Scler* 1999;5:395–402.
- [17] Baig S, Olsson T, Hojberg B, Link H. Cells secreting antibodies to myelin basic protein in cerebrospinal fluid of patients with Lyme neuroborreliosis. *Neurology* 1991;41:581–7.
- [18] Reik Jr L, Smith L, Khan A, Nelson W. Demyelinating encephalopathy in Lyme disease. *Neurology* 1985;35:267–9.
- [19] Wolfson C, Talbot P. Bacterial infection as a cause of multiple sclerosis. *Lancet* 2002;360:352–3.
- [20] Fritzsche M. Geographical and seasonal correlation of multiple sclerosis to sporadic schizophrenia. *Int J Health Geogr* 2002;1:5.
- [21] Kovar J, Stybrova H, Novak P et al. Heat shock protein 90 recognized as an iron-binding protein associated with the plasma membrane of HeLa cells. *Cell Physiol Biochem* 2004;14:41–6.
- [22] Fraser CM, Casjens S, Huang WM et al. Genomic sequence of a Lyme disease spirochaete, *Borrelia burgdorferi*. *Nature* 1997;390:580–6.
- [23] Posey JE, Gherardini FC. Lack of a role for iron in the Lyme disease pathogen. *Science* 2000;288:1651–3.
- [24] Ojaimi C, Brooks C, Casjens S et al. Profiling of temperature-induced changes in *Borrelia burgdorferi* gene expression by using whole genome arrays. *Infect Immunol* 2003;71:1689–705.
- [25] Hu J, Seeger C. Hsp90 is required for the activity of a hepatitis B virus reverse transcriptase. *Proc Natl Acad Sci USA* 1996;93:1060–4.
- [26] Banumathy G, Singh V, Tatu U. Host chaperones are recruited in membrane-bound complexes by *Plasmodium falciparum*. *J Biol Chem* 2002;277:3902–12.
- [27] Shanafelt MC, Hindersson P, Soderberg C et al. T cell and antibody reactivity with the *Borrelia burgdorferi* 60-kDa heat shock protein in Lyme arthritis. *J Immunol* 1991;146:3985–92.
- [28] Zhang M, Hisaeda H, Kano S et al. Antibodies specific for heat shock proteins in human and murine malaria. *Microbes Infect* 2001;3:363–7.
- [29] Cid C, Alvarez-Cermeno JC, Camafeita E, Salinas M, Alcazar A. Antibodies reactive to heat shock protein 90 induce oligodendrocyte precursor cell death in culture: implications for demyelination in multiple sclerosis. *FASEB J* 2004;18:409–11.
- [30] Aberer E, Brunner C, Suchanek G et al. Molecular mimicry and Lyme borreliosis: a shared antigenic determinant between *Borrelia burgdorferi* and human tissue. *Ann Neurol* 1989;26:732–7.
- [31] Aquino DA, Peng D, Lopez C, Farooq M. The constitutive heat shock protein-70 is required for optimal expression of myelin basic protein during differentiation of oligodendrocytes. *Neurochem Res* 1998;23:413–20.
- [32] Chabas D, Baranzini SE, Mitchell D et al. The influence of the proinflammatory cytokine, osteopontin, on autoimmune demyelinating disease. *Science* 2001;294:1731–5.

- [33] van Noort JM, van Sechel AC et al. The small heat-shock protein alpha B-crystallin as candidate autoantigen in multiple sclerosis. *Nature* 1995;375:798–801.
- [34] Bajramovic JJ, Plomp AC, Goes A et al. Presentation of alpha B-crystallin to T cells in active multiple sclerosis lesions: an early event following inflammatory demyelination. *J Immunol* 2000;164:4359–66.
- [35] Compston A, Coles A. Multiple sclerosis. *Lancet* 2002;359:1221–31.
- [36] McDonald WI, Ron MA, Giovannoni G. Multiple sclerosis and its pathophysiology. In: Asbury AK, McKhann GM, McDonald PJ, Goadsby PJ, McArthur JC, editors. *Diseases of the nervous system*. Cambridge: Cambridge University Press; 2002. p. 1606–19.
- [37] Irani DN, Griffin DE. Host responses in central nervous system infection. In: Asbury AK, McKhann GM, McDonald PJ, Goadsby PJ, McArthur JC, editors. *Diseases of the nervous system*. Cambridge: Cambridge University Press; 2002. p. 1651–9.
- [38] Brorson O, Brorson SH. An in vitro study of the susceptibility of mobile and cystic forms of *Borrelia burgdorferi* to tinidazole. *Int Microbiol* 2004;7:139–42.
- [39] Brorson O, Brorson SH. An in vitro study of the susceptibility of mobile and cystic forms of *Borrelia burgdorferi* to hydroxychloroquine. *Int Microbiol* 2002;5:25–31.
- [40] Nord CE, Kager L. Tinidazole-microbiology, pharmacology and efficacy in anaerobic infections. *Infection* 1983;11:54–60.
- [41] Krogstad DJ, Schlesinger PH. Acid-vesicle function, intracellular pathogens, and the action of chloroquine against *Plasmodium falciparum*. *N Engl J Med* 1987;317:542–9.
- [42] Wolf R, Wolf D, Ruocco V. Antimalarials: unapproved uses or indications. *Clin Dermatol* 2000;18:17–35.
- [43] van den Borne BE, Dijkmans BA, de Rooij HH, le Cessie S, Verweij CL. Chloroquine and hydroxychloroquine equally affect tumor necrosis factor-alpha, interleukin 6, and interferon-gamma production by peripheral blood mononuclear cells. *J Rheumatol* 1997;24:55–60.
- [44] Fox R, Kang H. Mechanism of action of hydroxychloroquine as an antirheumatic drug. *Semin Arthritis Rheum* 1993;23:82–91.
- [45] Brundula V, Rewcastle NB, Metz LM, Bernard CC, Yong VW. Targeting leukocyte MMPs and transmigration: minocycline as a potential therapy for multiple sclerosis. *Brain* 2002;125:1297–308.
- [46] Cunha BA. Minocycline versus doxycycline in the treatment of Lyme neuroborreliosis. *Clin Infect Dis* 2000;30:237–8.
- [47] Dotevall L, Hagberg L. Adverse effects of minocycline versus doxycycline in the treatment of Lyme neuroborreliosis. *Clin Infect Dis* 2000;30:410–1.
- [48] De Maria A, Primavera A. Possibility of the use of oral long-acting tetracyclines in the treatment of Lyme neuroborreliosis. *Clin Infect Dis* 2000;31:848–9.
- [49] Yushchenko M, Weber F, Mader M et al. Matrix metalloproteinase-9 (MMP-9) in human cerebrospinal fluid (CSF): elevated levels are primarily related to CSF cell count. *J Neuroimmunol* 2000;110:244–51.
- [50] Perides G, Tanner-Brown LM, Eskildsen MA, Klemperer MS. *Borrelia burgdorferi* induces matrix metalloproteinases by neural cultures. *J Neurosci Res* 1999;58:779–90.
- [51] Chandler S, Coates R, Gearing A, Lury J, Wells G, Bone E. Matrix metalloproteinases degrade myelin basic protein. *Neurosci Lett* 1995;201:223–6.
- [52] Garcia-Monco JC, Fernandez-Villar B, Benach JL. Adherence of the Lyme disease spirochete to glial cells and cells of glial origin. *J Infect Dis* 1989;160:497–506.
- [53] Perides G, Charness ME, Tanner LM et al. Matrix metalloproteinases in the cerebrospinal fluid of patients with Lyme neuroborreliosis. *J Infect Dis* 1998;177:401–8.
- [54] Gijbels K, Masure S, Carton H, Opdenakker G. Gelatinase in the cerebrospinal fluid of patients with multiple sclerosis and other inflammatory neurological disorders. *J Neuroimmunol* 1992;41:29–34.
- [55] Leppert D, Ford J, Stabler G et al. Matrix metalloproteinase-9 (gelatinase B) is selectively elevated in CSF during relapses and stable phases of multiple sclerosis. *Brain* 1998;121:2327–34.

Available online at [www.sciencedirect.com](http://www.sciencedirect.com)

