J Neurol (1990) 237:113–116

Journal of
Neurology

Histopathological findings in the central and peripheral nervous systems in neuroborreliosis

A report of three cases

B. Meurers, W. Kohlhepp, R. Gold, E. Rohrbach, and H. G. Mertens

Neurologische Universitätsklinik und Poliklinik, Josef-Schneider-Strasse 11, D-8700 Würzburg, Federal Republic of Germany

Received May 16, 1989 / Received in revised form October 5, 1989 / Accepted October 12, 1989

Summary. Very little is known about tissue changes and pathophysiological mechanisms in Garin-Boujadoux-Bannwarth syndrome. We report histopathological findings in the central and peripheral nervous systems of three typical cases. In the acute stage of the disease mononuclear perivascular infiltrations with mainly T-helper cells were the prominent finding, whereas after treatment there was no vessel involvement. The fibre changes in the peripheral nervous system consisted of axonal degeneration. No *Borrelia burgdorferi*-specific antigen could be detected by immunohistochemical methods. Thus vasculitis might be one of the primary pathophysiological mechanisms for the involvement of the nervous system.

Key words: Neuroborreliosis – Vasculitis – Parenchymal infiltration – Axonal degeneration – Monoclonal antibodies

Introduction

In 1922 Garin and Boujadoux were the first to describe a paralysis caused by tick bite [11]. Since then the disease has been well described in numerous reports [1–3, 6, 12, 15, 20, 21]. However, in contrast to the well-known clinical conditions there have been very few reports on histopathological findings [8, 20, 26]. The reasons for this deficit include the benign character of the disease and the availability of effective treatment with antibiotics [25, 27]. Although the causative agent, a spirochaete named *Borrelia burgdorferi*, has been identified recently [7, 23], very little is known about the pathophysiological mechanisms of the disease, i. e. the differences between the acute and chronic forms and whether the effects are due to direct invasion of borrelia or to a pathological immune reaction. Because of its therapeutic implications this question is of special interest in chronic or chronic relapsing disease and in patients with inadequate treatment response [10, 13, 25, 28, 29]. Further histological studies might help to answer some of these questions. As the nervous system is one of the organ systems mainly affected in the European form of the disease, we report the results of histological examinations of the central and peripheral nervous systems in three typical cases.

Offprint requests to: B. Meurers

Case reports

Case 1. A 75-year-old female patient was admitted with a 10-day history of paraparesis, starting in the right leg and later extending to the left leg. Four weeks prior to admission she had noticed erythema chronicum migrans after a tick bite on her right thigh, followed by a painful neuropathy 10 days later. There was bladder dysfunction for about 1 week. She had no previous history of medical or neuromuscular diseases, other than an umbilical hernia with suppurative inflammation of the navel which had lasted for over 6 months. Examination on admission revealed a severe paraparesis with inability to move the legs against gravity. The deep tendon reflexes were abolished and the plantar responses flexor. Apart from painful paraesthesiae in the right L3–5 dermatomes there were no sensory deficits. Laboratory studies revealed normal peripheral red and white blood cell counts, erythrocyte sedimentation rate, and normal levels of urea and electrolytes, transaminases, creatine kinase, and aldolase. Tests for *Treponema pallidum*, lupus erythematosus, cryoglobulins and rheumatoid factor were negative. Cerebrospinal fluid (CSF) showed 62 leucocytes/μl, predominantly lymphocytes (86%). Total protein was elevated to 107 mg/dl (normal, less than 45 mg/dl). IgG and IgA percentages of total protein were raised to 23.8% and 1.6% respectively (normal, less than 9% and 1% respectively). The IgG index was 1.27 (normal, ranging from 0.3 to 0.7) and serum: CSF albumin ratio 56 (normal, greater than 130). Isoelectric focusing of immunoglobulins showed oligoclonal bands in blood and CSF. *B. burgdorferi* titres (IFT) were raised in serum (IgG > 1:1000) and CSF (IgG > 1:64). Evoked potentials and nerve conduction velocities were not obtained. Our treatment consisted of intravenously administered doxycycline, starting with 200 mg/day for 2 days, followed by 100 mg/day. This led to a gradual relief of pain and paraparesis. After 8 days the patient was able to walk a few steps unaided. On the 10th day she developed paralytic ileus and despite all therapy she died 13 days after admission.

Case 2. In June 1988, a 56-year-old male patient presented with a 4-week history of low back pain radiating to the right thigh. Two weeks previously he had noticed a circular erythematous area on the right thigh, which had disappeared spontanously after several days. He had no recollection of having had a tick bite and there was no relevant past medical history. On admission, neurological examination revealed MRC grade 2–3 paresis and hypaesthesia in the S1 dermatome of the right leg. The right knee reflex was absent and plantar responses were flexor. Besides an elevation of the mean cell volume to 95 fl and of SGPT to 29 units/l all routine lab-

oratory investigations were normal. CSF contained 120 leucocytes/μl, predominantly lymphocytes (76%) and monocytes (8%). Total protein was raised to 163 mg/dl. Relative percentages of IgG, IgA, and IgM did not exceed normal ranges. IgG index was 1.0 and serum: CSF albumin ratio 82. Isoelectric focusing disclosed oligoclonal bands only in CSF. Anti-borrelia antibody titres (ELISA, IFT) were positive for IgG in serum (1:80) and CSF (1:10). IgM titres were negative. Neurophysiological investigations revealed prolonged latencies of visual evoked potentials, being 115 ms on the left and 113 ms on the right side (normal, ranging from 90 to 110 ms) and an isolated reduction of nerve conduction velocities (tibial nerve 36.1 m/s, absent stimulus response of the sural nerve) in the right leg. Treatment consisted of intravenously administered high-dose penicillin (20 million units/day) for 10 days. Because of an unsatisfying reduction of pain the patient also received corticosteroids (initial dosage 50 mg/day) for 6 weeks. On discharge, there remained only a slight paresis of the right plantar extensors. The sural nerve biopsy was performed on the 2nd hospital day before the onset of treatment.

Case 3. A 24-year-old male patient of previously good health was investigated for a left peroneal paresis of acute onset. During the following week he developed a mild fever, bilateral Bell's palsy and hearing loss. There was no history of tick bite. In addition to the signs already mentioned the initial neurological examination revealed slight anisocoria and hypaesthesia in the left S1 dermatome. Muscle tendon reflexes were normal and plantar responses flexor. Routine blood tests were normal. Initial CSF examination showed a raised leucocyte count (245 cells/μl), predominantly lymphocytes and monocytes (82% and 11%). Total protein was 219 mg/dl with 10.6% IgG, 1.1% IgA, and 0.2% IgM. IgG index was normal and serum: CSF albumin ratio decreased to 24.7, indicating a damaged blood-CSF barrier. Oligoclonal bands could not be detected. Anti-borrelia antibodies (ELISA) were positive in serum (IgG 1:100) and CSF (1:10). In a second CSF examination the leucocyte count had fallen to 90 cells/μl; total protein was 129 mg/dl and IgG index 0.47. Again there were no oligoclonal bands. Titres of anti-borrelia antibodies did not change. Other infections (syphilis, HIV, listeriosis, and toxoplasmosis) were excluded. There were no viral antibodies in CSF. Nerve conduction velocities of the right tibial nerve (39 m/s) and the left sural nerve (32 m/s) were reduced, while others (right sural nerve 48 m/s) were normal. In brain-stem auditory evoked potentials I-waves were absent on either side and V-waves showed a marked difference with latencies of 5.6 ms on the right and 6.1 ms on the left side (normal, 5.5–6.0 ms). Treatment consisted of intravenously administered high-dose penicillin (20 million units/day) for 12 days. Because of a further clinical deterioration with development of severe paraparesis, corticosteroids in an initial dosage of 1 g/day were added. On discharge 6 weeks later, the patient was able to walk unaided. The bilateral Bell's palsy and hypoacusis as well as the paresis of the left plantar extensor muscles were markedly improved. The sural nerve biopsy was performed in the healing phase of the disease, 4 weeks after the onset of symptoms and 2 weeks after the end of treatment. The sural nerve biopsies in cases 2 and 3 were mainly done for research purposes and informed consent was obtained from each patient.

Materials and methods

The brain, spinal cord, dorsal nerve roots, and abdominal autonomic nervous system from the autopsy (case 1) and two sural nerve biopsy specimens (cases 2, 3) were examined by light and electron microscopy. Routine stains (haematoxylin and eosin, luxol fast blue, methylene blue, and congo red) of all three cases were performed on formalin-fixed samples. The immunohistochemical preparations were performed on fresh frozen material, using the following monoclonal antibodies: Leu 4, Leu 2a, Leu 3a Il2 (Becton Dickinson, Heidelberg, FRG), and CD 19 (Dakopatts, Hamburg, FRG). For the demonstration of *B. burgdorferi* antigen we used the monoclonal antibody H 9724 (kind gift of Dr. Alan G. Barbour, University of Texas, San Antonio, USA). As there was no fresh frozen tissue available from case 1, immunohistochemistry for *B. burgdorferi* could not be performed.

Results

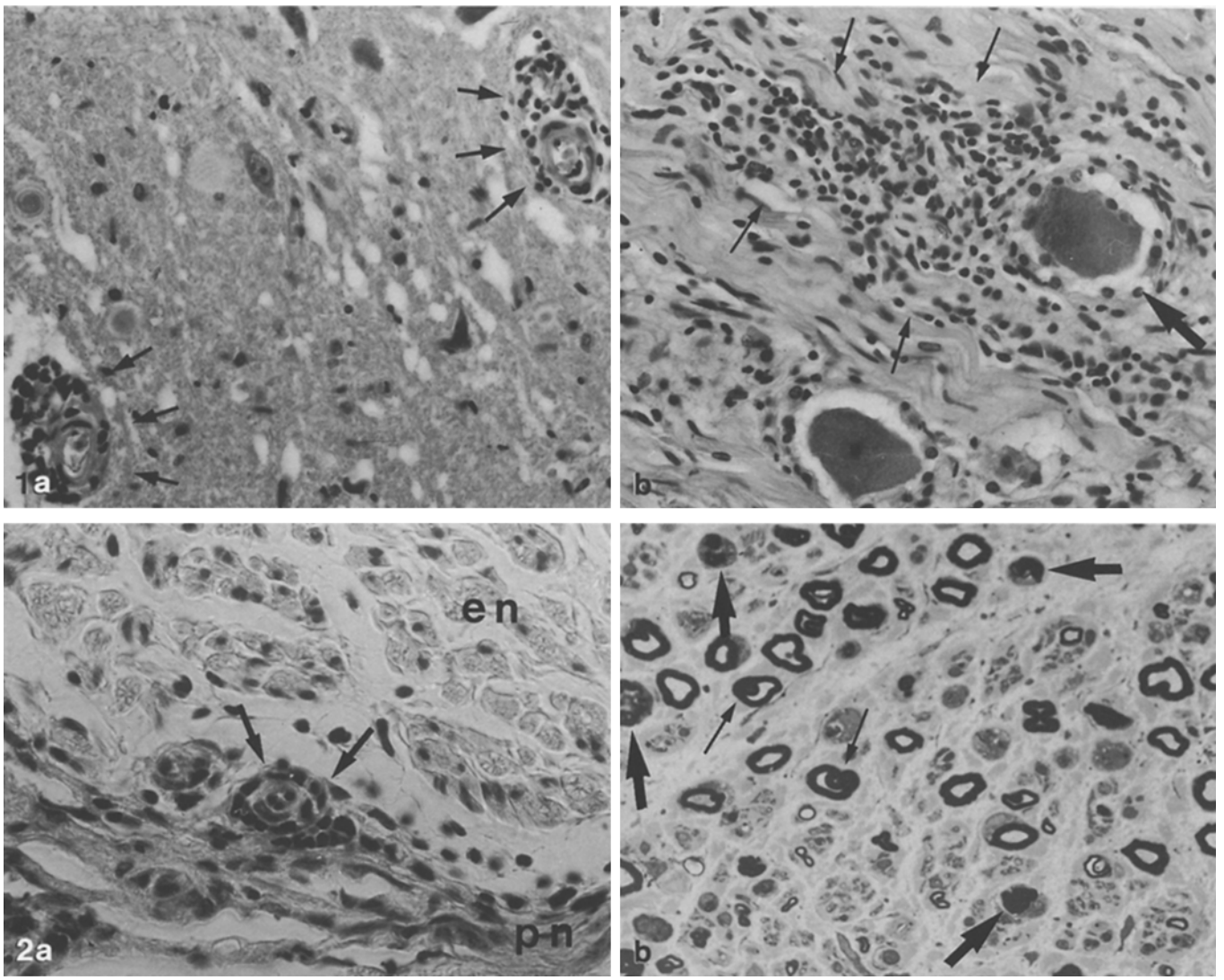
The spinal cord preparations showed mononuclear perivascular infiltrations in the meninges as well as in the white and grey matter (Fig. 1a). In contrast to this localization the infiltrations of the spinal ganglia and the dorsal nerve roots were parenchymal (Fig. 1b). Corresponding to the segmental nature of the disease, all these findings were limited to the segments L3–5. The brain, brain stem and abdominal autonomic nervous system had not been affected. Similar to the manifestations in the spinal cord, the sural nerve biopsy specimen of the acute case 2 showed mononuclear perivascular infiltrations around epi-, peri- and endoneural vessels (Fig. 2a). A slight diffuse infiltration of the endoneurium was detected by immunohistochemistry. The fibres were undergoing axonal degeneration (Fig. 2b). There was only slight myelin degeneration with few fibres showing a splitting of the myelin sheath. Hypomyelinated fibres and clustered sprouts were also rare. Electron microscopically the number of unmyelinated fibres was reduced. As a sign of regeneration after axonal damage occasional collagen pockets could be detected.

The biopsy specimen obtained after treatment in case 3 showed no perivascular infiltrations. The nerve fibre changes were similar to those in case 2 with the exception of a mild proliferation of endoneural connective tissue (Fig. 2c). The immunohistochemical differentiation of the infiltration cells in case 2 disclosed 80% Leu 3a positive T-helper cells and only 20% Leu 2a positive T-suppressor cells. Activated T-cells and B-cells could not be found. In both sural nerve biopsy specimens no demonstration of *B. burgdorferi*-specific antigen was possible.

Discussion

From the few reports available on the pathological findings in neuroborreliosis, the major pathological change during the acute stage of the disease seems to be mononuclear perivascular infiltration. The involvement of meningeal and spinal cord vessels in our postmortem case is consistent with the only autopsy findings reported so far, by Schaltenbrand in 1949 [19]. As the aetiological agent was still unknown at that time, the disease was called "chronic aseptic meningitis". With regard to the peripheral nervous system, comparable reports have come from Camponovo and Meier [8] and Vallet et al. [26].

Having been demonstrated in the nervous system, vasculitis has also been found in the typical skin lesions of Bannwarth syndrome [5] and Lyme disease [17], suggesting that this is an important pathological feature. In the peripheral nervous system these inflammatory vessel changes may be the cause of the axonal fibre degeneration we saw in both sural nerve biopsy specimens.



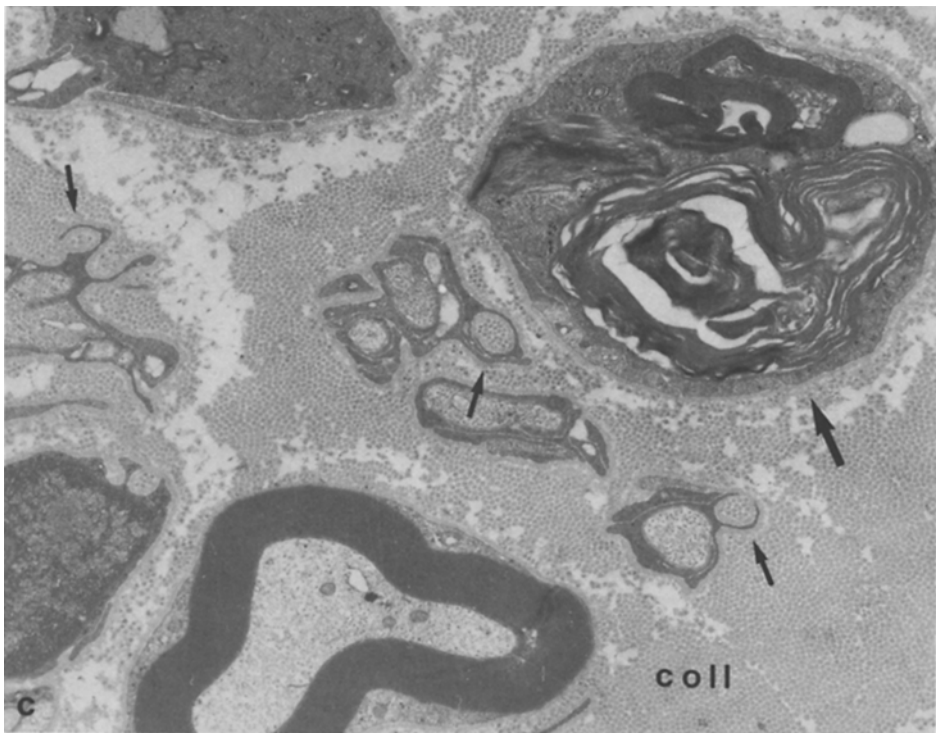


Fig. 1. a Anterior horn of spinal cord. Mononuclear perivascular infiltration around two small blood vessels *(arrows)* without necrotizing destruction of the vessel walls. No glial proliferation is visible. H & E, paraffin sections, × 250. **b** Spinal ganglion. Parenchymal infiltration *(small arrows)* gathering around a ganglia cell *(large arrow)*. H & E, paraffin section, × 250

Fig. 2 a–c. Sural nerve biopsies. **a** Case 2. Small vasculitic cuff *(arrows)* in the perineurium *(pn)*. *en*, endoneurium. **b** Case 2. Reduction and segmental loss of myelinated fibres. Wallerian degenerations *(large arrows)*. Myelin balls *(small arrows)*. There are no sprouts of regenerating fibres. Vinylcyclohexen-dioxide embedded semithin section, paraphenylenediamine, × 400. **c** Case 3. Wallerian degeneration *(large arrow)*. Reduction of unmyelinated fibres. Collagen pockets *(small arrows)*. Proliferation of endoneurial connective tissue. *coll*, Collagen fibrils. Electron microscopy. Vinylcyclohexen-dioxide embedding, × 10900

These findings consisted of segmental reduction in myelinated and unmyelinated fibres, scattered Wallerian degeneration and only mild involvement of the myelin sheath. In contrast to the sural nerve biopsied in the acute stage of the disease (case 2), the one biopsied after treatment (case 3) showed no vasculitis. As this patient received both antibiotics and immunosuppressive therapy with corticosteroids, no conclusions about the pathogenic origin of the vasculitis can be made. Two theories exist as to the pathophysiology of the vasculitis. The first assumes a direct inflammatory response to tissue invasion by spirochaetes with a persistence of the organism in chronic cases. The other assumes a pathological immune response to the spirochaete [8]. Evidence which supports the former theory is that antibiotics have been therapeutically effective in acute [24, 25, 29] and chronic [4, 13, 14] forms of the disease. The appearance of a new IgM response and the persistence or expansion of the IgG response in some late forms of the illness may also suggest that the spirochaetes remain alive [9, 16]. Further support for this theory has also been drawn from aetiological and clinical parallels between neuroborreliosis and Lues [3, 14]. Although *T. pallidum* has been shown histologically to persist throughout all stages of the disease [18], no such evidence exists as yet for neuroborreliosis. In our two sural nerve biopsy specimens no *B. burgdorferi*-specific antigen could be found.

In support of the latter immune response theory are the findings that not all cases of neuroborreliosis respond to antibiotics and that the administration of corticosteroids leads to a reduction of symptoms [15]. One might argue, however, that the failure to respond to antibiotic therapy might be due to insufficient dosage or duration of treatment and that the corticosteroid effect might not be one of immunosuppression but of non-specific reduction of oedema. More direct proof of the immune response theory lies in the fact that Sigal and Tatum [22] found *B. burgdorferi*-specific IgM antibodies cross-reacting with neuronal antigens. As there is evidence for both theories, further histological studies are needed to answer the important question about the pathophysiological mechanisms of nervous system involvement in neuroborreliosis.

References

1. Ackermann R (1976) Durch Zecken übertragene Meningopolyneuritis. Münch Med Wochenschr 118:1621–1622
2. Ackermann R (1983) Erythema chronicum migrans und durch Zecken übertragene Meningopolyneuritis Garin-Boujadoux-Bannwarth: eine Borrelien-Infektion? Dtsch Med Wochenschr 108:577–580
3. Ackermann R (1986) Erythema-migrans-Borreliose und Frühsommer-Meningoenzephalitis. Dtsch Ärztebl 83:1765–1774
4. Ackermann R, Gollmer E, Rehse-Küpper B (1985) Progressive Borrelien-Encephalomyelitis. Chronische Manifestation der Erythema-chronicum-migrans-Krankheit am Nervensystem. Dtsch Med Wochenschr 110:1039–1042
5. Bammer H, Schenk K (1985) Meningo-Myelo-Radikulitis nach Zeckenbiß mit Erythem. Dtsch Z Nervenheilkd 187:25–34
6. Bannwarth A (1944) Zur Klinik und Pathogenese der chronischen lymphocytären Meningitis. Arch Psychiatr Nervenkr 117:161
7. Burgdorfer W, Barbour AG, Hayes SF, Benach JL, Grunwaldt E, Davis JP (1982) Lyme disease – a tick borne spirochetosis? Science 216:1317–1319
8. Camponovo F, Meier C (1986) Neuropathy of vasculitic origin in a case of Garin-Boujadoux-Bannwarth syndrome with positive borrelia antibody response. J Neurol 233:69–72
9. Craft JE, Fischer DK, Shimamoto GT, Steere AC (1986) Antigens of *Borrelia burgdorferi* recognized during Lyme disease. Appearance of a new immunoglobulin M response and expansion of the immunoglobulin G response late in the illness. J Clin Invest 78:934–939
10. Dattwyler RJ, Volkman DJ, Luft BJ, Halperin JJ, Thomas J, Golightly MG (1988) Seronegative Lyme disease. Dissociation of specific T- and B-lymphocyte response to *Borrelia burgdorferi*. N Engl J Med 319:1441–1446
11. Garin CH, Boujadoux C (1922) Paralysie par les tiques. J Med Lyon 71:765–767
12. Hörstrup P, Ackermann R (1973) Durch Zecken übertragene Meningopolyneuritis (Garin-Boujadoux-Bannwarth). Neurol Psychiatr 41:583–606
13. Klenk W, Heitmann R, Ackermann R (1985) Rezidivierende Erythema-chronicum-migrans-Krankheit des Nervensystems: Querschnittsmyelitis als Rückfall einer Meningopolyneuritis Garin-Boujadoux-Bannwarth. Aktuel Neurol 12:20–23
14. Kohlhepp W, Mertens HG (1985) Chronische Meningomyeloradikulitis durch Ixodes ricinus Spirochäten – eine der Metalues ähnliche Spirochätose? In: Gänshirt H, Berlit P, Haack G (eds) Verhandl Dtsch Ges Neurol, vol III. Springer, Berlin Heidelberg New York, pp 747–749
15. Kohlhepp W, Mertens HG, Oschmann P, Rohrbach E (1987) Akute und chronische Erkrankungen bei zeckenvermittelter Borreliose. Nervenarzt 58:557–563
16. Martin R, Martens U, Sticht-Groh V, Dörries R, Krüger H (1988) Persistent intrathecal secretion of oligoclonal, *Borrelia burgdorferi*-specific IgG in chronic meningoradiculomyelitis. J Neurol 235:229–233
17. Meyerhoff J (1983) Lyme disease. Am J Med 75:663–670
18. Peiffer J (1984) Neuropathologie. In: Remmele W (ed) Pathologie. Ein Lehr- und Nachschlagebuch, vol 4. Springer, Berlin Heidelberg New York, pp 161–164
19. Schaltenbrand G (1949) Chronische aseptische Meningitis. Nervenarzt 20:433–442
20. Schaltenbrand G (1966) Durch Arthropoden übertragene Erkrankungen der Haut und des Nervensystems. Verh Dtsch Ges Inn Med 72:1557–1562
21. Schmutzhard E, Stanek G, Pohl P (1985) Polyneuritis cranialis associated with *Borrelia burgdorferi*. J Neurol Neurosurg Psychiatry 48:1182–1184
22. Sigal LH, Tatum AH (1988) Lyme disease patients' serum contains IgM antibodies to *Borrelia burgdorferi* that cross react with neuronal antigens. Neurology 38:1439–1442
23. Steere AC, Grodzicki RL, Kornblatt AN, Craft JE, Barbour AG, Burgdorfer W, Schmid GP, Johnson E, Malawista SE (1983) The spirochetal etiology of Lyme disease. N Engl J Med 308:733
24. Steere AC, Hutchinson GJ, Rahn DW, Sigal LH, Craft JE, DeSanna ET, Malawista SE (1983) Treatment of the early manifestations of Lyme disease. Ann Intern Med 99:22–26
25. Steere AC, Pachner AR, Malawista SE (1983) Neurological abnormalities of Lyme disease: successful treatment with high dose intravenous penicillin. Ann Intern Med 99:767–772
26. Vallat JM, Hugon J, Lubeau M, Leboutet MJ, Dumas M, Desproges-Gotteron R (1987) Tick-bite meningoradiculoneuritis: clinical, electrophysiologic and histologic findings in 10 cases. Neurology 37:749–753
27. Weber K (1981) Treatment of Lyme disease (letter). Ann Intern Med 94:137
28. Wörth WD (1985) Diagnostik der Erythema-migrans-Krankheit (Lyme-Krankheit). Dtsch Med Wochenschr 110:1377–1379
29. Wörth WD (1985) Therapie der Erythema-migrans-Krankheit (Lyme Krankheit). Dtsch Med Wochenschr 110:1379–1380