Chronic Meningitis and Lyme Disease in Sweden

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INTRODUCTION

Chronic meningitis has been defined as a disease which fails to improve, or progresses both in its clinical and cerebrospinal fluid (CSF) abnormalities during a four-week period [1,2]. It can be associated with various infectious and non-infectious disorders. We have previously reported 21 patients with chronic meningitis, all of whom had a similar onset and course of disease [3]. Except for Streptococcus milleri antigen in the CSF in one patient, they had no demonstrable association with any infectious disease. The patients improved or recovered after therapy with intravenous penicillin G. In addition, all had indications of an intrathecal immune response with localized CSF production of oligoclonal immunoglobulin G (IgG). Since the previous report [3] we have treated 14 additional patients with similar characteristics. We report here clinical data on the 35 patients, serological examination for antibodies to the Lyme spirochete and the Swedish Ixodes ricinus spirochete, and results of imprint of immunofixation of electrofocused CSF and serum specimens.
PATIENTS AND METHODS

Patients

Between 1975 and 1983, we studied the 35 patients in the Department of Infectious Diseases, Danderyd Hospital (24 patients), in the Department of Neurology, South Hospital, Stockholm (eight patients), and, only during 1983, in the Roslagstull Hospital of Infectious Diseases (three patients). Chronic meningitis was defined as a disease, which failed to improve or worsened in its clinical and CSF abnormalities during a four-week period.

Laboratory Investigations

Blood tests done on all patients included an erythrocyte sedimentation rate, hemoglobin, leucocytes, alanine and aspartate aminotransferases, and creatinine. CSF samples were analyzed for cell counts, total protein, and CSF/blood glucose ratios. Samples of CSF, blood, nasopharynx, and urine were also cultured in many patients. Counter-current immunoelectrophoresis was used in an attempt to detect *Streptococcus milleri* antigen in CSF samples from 20 patients [4]. CSF specimens were also investigated for *Cryptococcus neoformans* and its antigen in 28 patients; for *Mycobacterium tuberculosis* in nine patients.

Sera from all patients were examined for antibodies against *Treponema pallidum*, *Listeria monocytogenes*, *Toxoplasma gondii*, and *Actinomyces israelii*. Paired sera from all patients and CSF samples from 22 patients were examined for complement-fixing antibodies to herpes simplex virus, varicella zoster virus, cytomegalovirus, coxsackie virus, echovirus, mumps virus, and tick-borne encephalitis virus [5]. Finally, sera from 34 patients were examined for antibodies to the Lyme spirochete and to the Swedish *I. ricinus* spirochete [8].

Agarose electrophoresis in combination with immunofixation was used to examine for oligoclonal IgG bands in 122 paired CSF and serum samples from the 35 patients [6]. Before the electrophoretic separation, the CSF was concentrated about 100 times in Minicon B 15 cells (Amicon Netherlands). Albumin and IgG levels were measured in CSF and serum samples using a nephelometric method. The IgG index was calculated as (CSF-IgG/serum-IgG):(CSF-albumin/serum-albumin) [7]. Imprint immunofixation (IIF) of electrofocused sera and CSF samples was done as described previously [9]. Briefly, paired serum and CSF, adjusted to equivalent concentration of IgG (2.5 g/l), were separated by an electrofocusing polyacrylamide gel with a pH 6.5–9.5 gradient. Agarose gel plates containing Lyme spirochete (120 μg/ml), *Treponema reiter*, measles virus, or cytomegalovirus antigen (25 to 80 μg/ml) were incubated sequentially in direct (gel-to-gel) contact with the separating gel for five minutes. Agarose gel plates without antigen were also included. After incubation, the separating gel was fixed and stained. The agarose gel plates containing antigen were washed and pressed to remove unbound antibodies, incubated in 125I-labeled rabbit antihuman IgG overnight, washed, pressed, dried, and autoradiographed for 17 hours.

RESULTS

Of the 35 patients with chronic meningitis, 15 were male and 20 female. Their ages ranged from 10 to 81 years (mean, 45). Except for one patient who was in remission from chronic myelogenous leukemia, all had previously been healthy and had not been treated with immunosuppressive drugs. The patients had their first symptoms
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during summer or early fall (June to October). Thirteen of the patients had been bitten by insects within a few weeks before onset of illness, eight of them reported bites by ticks, and one by a horsefly. Nine patients developed local skin reactions, which healed spontaneously, and five patients described erythema chronicum migrans (ECM). One of the latter patients had had ECM and meningitis 20 years earlier after a tick bite on the upper right thigh.

The time between onset of symptoms and the first medical consultation ranged from four days to 38 weeks (mean, four to five weeks). Treatment with intravenous penicillin G (3 g every six hours for two weeks) was given 12 weeks after onset of symptoms (range, 3–48 weeks). Most patients had clear clinical improvement within two to five days after starting therapy.

**Signs and Symptoms**

The illness began slowly and was accompanied by profound fatigue, loss of appetite, weight loss, migrating muscular pain, and sometimes burning and smarting dysaesthesia and/or facial nerve paralysis (Table 1). Only two-thirds of the patients complained of headache. When present, it was usually not a dominant symptom and was often periodic. Only five patients had severe headache. Two-thirds of the patients had fever, which was usually minor; only five patients had high fever.

Cranial nerve paresis developed in 16 patients. Twelve of them had unilateral peripheral paralysis of the seventh cranial nerve; two, bilateral facial nerve paralysis; and three, paralysis of the sixth cranial nerve. Moderate deafness developed in two patients. Cranial nerve pareses often disappeared spontaneously within a few weeks.

One 61-year-old woman slowly developed spastic paraparesis. Another patient, a

| TABLE 1 |
| --- |
| Symptoms and Signs in 35 Patients with Chronic Meningitis |
| Symptoms and Signs | No. of Patients |
| Profound fatigue | 29 |
| Malaise | 20 |
| Vomiting | 11 |
| Weight loss* | 19 |
| Headache | 22 |
| Muscle ache | 22 |
| Fever: 37.5–38.5°C | 16 |
| >38.5°C | 5 |
| Parasthesia/hyperesthesia | 11 |
| Sensory disturbances | 7 |
| Cranial-nerve paralysis | 16 |
| Facial nerve | 12 |
| Oculomotor and/or abducens nerve | 3 |
| Auditory nerve | 2 |
| Other paralysis* | 7 |
| Neck stiffness | 6 |
| Other* | 4 |

*Mean, 6.9 kg

*Paraparesis: spastic type (one), lower-motor-neuron type (one), partial paralysis of one leg (two) or of arms (one), hemiparesis (two)

*Ataxia (one), diplopia (two), somnolence (one)
57-year-old man, experienced the rapid onset of lower-motor-neuron paraparesis. Paresis of the proximal muscles of the left leg appeared in a 72-year-old man and a 64-year-old woman. Partial paralysis of the arms developed in a 75-year-old man. A left-sided hemiparesis developed slowly in a 70-year-old woman about six months after the acute onset of headache, malaise, and weight loss. Finally, a 56-year-old woman also developed hemiparesis. Other neurological signs included paresis and hyperesthesia with different localizations. Seven patients had impairment of sensation localized to one or more thoracic or lumbar segments. Six patients had transient neck stiffness. Only one patient had slight clinical evidence of encephalitis with somnolence, and no patient had papilledema.

After treatment, 21 patients were followed for seven months to eight years (mean, 3.2 years); and the 14 patients from 1983, two to five months. Most symptoms and neurological signs showed rapid regression. The spastic paraparesis in the 61-year-old woman showed some regression. The flaccid paraparesis in the 57-year-old man disappeared within four months. The unilateral paresis of leg muscles in the 64-year-old woman and the 72-year-old man also disappeared within a few months. The left-sided hemiparesis of the 70-year-old woman showed moderate regression, as did the hemiparesis of the 56-year-old woman. There were no other sequelae and no relapses.

**Laboratory Findings**

Seven patients had slight or moderate elevations of erythrocyte sedimentation rates (maximum 47 mm/hour); a few cases had slight elevations of liver transferases, and hemoglobin and creatine were normal.

**CSF Analyses** One hundred and eighty-seven lumbar punctures were done in the 35 patients, 97 before treatment with penicillin G and 90 after (Table 2). The highest measured CSF leucocyte count was 1,100 cells/mm³. The percentage of mononuclear cells was usually more than 90 percent and often 98 to 100 percent. Only three patients had values below 50 percent, the lowest being 44 percent. After treatment with penicillin G, the CSF leucocyte counts fell, in some cases to normal. Before treatment, the CSF protein was 0.6 to 13.0 g/l (mean, 2.35 g/l); after treatment, the levels were lower or normal. The lowest value for the CSF/blood glucose ratio was 0.2 (CSF glucose being 1.2 g/l). Before treatment, the glucose ratios were low (0.2–0.4) in half of the patients and normal (≥0.5) in the others. After treatment, the values were usually above 0.5.

Before treatment, almost all CSF samples showed several pronounced oligoclonal immunoglobulin bands, determined by agarose electrophoresis and immunofixation (Table 2). The striking changes in CSF samples contrasted with the modest findings in the corresponding serum samples. The CSF-IgG index values were also increased, indicating intrathecal synthesis of IgG. After treatment, the immune response in CSF regressed very slowly. At long-term follow-up (mean, 2.2 years after treatment), eight of 20 patients had no demonstrable oligoclonal IgG bands, and all had normal CSF-IgG index values. Most of the other patients had only weak indications of oligoclonal immunoglobulin bands.

**Microbiological and Serologic Tests** All bacteriological cultures of samples taken from CSF, blood, and urine were negative, as were all tests for viruses, mycobacteria, and fungi, and all serological tests for *Treponema pallidum, Listeria monocytogenes, Toxoplasma gondii, Leptospira, and Actinomyces israelii*. Antigen
of *Streptococcus milleri* was detected in one of 20 CSF samples before treatment but not in a sample taken five weeks after treatment. Indirect immunofluorescence antibody titers against the Lyme spirochete and the Swedish *Ixodes ricinus* spirochete of $\geq 160$ were demonstrated in 26 of 34 chronic meningitis patients, but in only two of 26 control cases of acute meningoencephalitis. We found concordant results between the two spirochetes.

**Imprint Immunofixation** In one patient with chronic meningitis, local intrathecal synthesis of spirochetal antibodies was demonstrated in all four CSF samples collected during one year. Oligoclonal spirochete IgG antibodies appeared as bands in the autoradiographs (Fig. 1). Intrathecal synthesis of *Treponema reiter*, measles, or cytomegalovirus antibodies was not detected. In a patient with subacute sclerosing panencephalitis (SSPE), local intrathecal synthesis of oligoclonal measles virus antibodies was demonstrated (Fig. 1) but no local synthesis of antibodies to spirochete, *Treponema*, or varicella zoster virus was found. The other control patient with acute meningoencephalitis had no demonstrable intrathecal antibodies against any of these agents (Fig. 1).

### TABLE 2
Leucocytes, Glucose Ratios, and Proteins in CSF Specimens Sampled Initially, Before, and After Treatment with Penicillin G, and at Long-Term Follow-up

|                          | Initial | Before penicillin G | After penicillin G | Long-term follow-up |
|--------------------------|---------|---------------------|-------------------|---------------------|
| Time before treatment:  |         |                     |                   |                     |
| Mean                     | 6.0 wk  |                     |                   |                     |
| Range                    | 1-22 wk |                     | 1-2 wk            |                     |
| Time after treatment:    |         |                     |                   |                     |
| Mean                     |         |                     |                   | 2.2 yr              |
| Range                    |         |                     | 1-4 wk            | 1 mo-6 yr           |
| Spinal leucocytes        |         |                     |                   |                     |
| ($\times 10^9$)          |         |                     |                   |                     |
| Mean                     | 237     | 294                 | 45                | 3                   |
| Range                    | 36-412  | 66-840              | 2-180             | 0-28                |
| Mononuclear cells >90%   |         |                     |                   |                     |
| (n/n tested)             | 22/25   | 29/35               | 33/35             | 16/21               |
| CSF-glucose/blood-glucose ratio 0.2-0.4 |         |                     |                   |                     |
| (n/n tested)             | 8/24    | 16/35               | 3/35              | 0/21                |
| Spinal protein (g/l)     |         |                     |                   |                     |
| Mean                     | 2.20    | 2.35                | 1.02              | 0.49                |
| Range                    | 0.7-9.8 | 0.6-13.0            | 0.4-4.9           | 0.3-1.0             |
| Oligoclonal IgG bands    |         |                     |                   |                     |
| (agarose electrophoresis): |        |                     |                   |                     |
| Pronounced               | 24/25   | 34/35               | 31/33             | 0/20                |
| Weak                     | 1/25    | 1/35                | 1/33              | 12/20               |
| Absent                   | 0/25    | 0/35                | 1/33              | 8/20                |
| CSF-IgG index:*          |         |                     |                   |                     |
| Mean                     | 1.2     | 1.29                | 1.06              | 0.57                |
| Range                    | 1.0-1.3 | 0.6-3.7             | 0.5-2.46          | 0.34-0.97           |
| No. tested               | 4       | 30                  | 19                | 18                  |

*Normal range, 0.37-0.58*
FIG. 1. Characterization of antibodies by electofocusing (pH 6.5-9.5) and imprint immunofixation in (left to right) a patient with chronic meningitis (indirect immunofluorescence antibody titers: serum 1,280 and CSF 320), a patient with subacute sclerosing panencephalitis (SSPE), and a patient with chronic meningoencephalitis of unknown etiology. Each set of autoradiographs shows serum above and CSF below.

Note presence of oligoclonal CSF IgG in all three patients and of intrathecally synthesized antibodies to Lyme spirochete in the chronic meningitis patient and to measles virus in the SSPE patient.

DISCUSSION

The etiology of chronic meningitis is often difficult to determine [2]. The more common causes include mycobacterial, bacterial, fungal, and other infectious or non-infectious disorders [1]. Infections such as actinomycosis, nocardiosis, syphilis, leptospirosis, and brucellosis can be complicated by chronic meningitis. However, based on the antibody response against the Lyme spirochete and the Swedish *I. ricinus* spirochete, we have now demonstrated a spirochetal etiology for many cases of chronic meningitis observed in Sweden.

The patients in our series had intrathecal synthesis of considerable quantities of oligoclonal IgG. Oligoclonal IgG in CSF is demonstrable in multiple sclerosis and in various infectious diseases of the central nervous system such as neurosyphilis, measles encephalitis, herpes simplex encephalitis, and mumps meningoencephalitis [9,10,11]. Moreover, we demonstrated local synthesis in the CSF of oligoclonal spirochete-specific IgG. Local production of these specific antibodies strongly supports a direct relationship between spirochetal infection and chronic meningitis.

Many patients reported here had persistent or progressive symptoms and CSF abnormalities, which often lasted for months. They responded to intravenous penicillin. Neurological involvement has been described after ECM by Bannwarth in 1941 and 1944 and by Hörstrup and Ackermann in 1973, and named tick-borne meningopolyneuritis [12,13,14]. Some of our patients shared characteristics with those described for tick-borne meningopolyneuritis, but others in our series lacked radicular pain and had a more slowly progressive disease accompanied by profound fatigue, malaise, and considerable weight loss. Neurological abnormalities of Lyme
disease, first reported in the United States in 1979 [15], include aseptic meningitis, encephalitis, chorea, cerebellar ataxia, cranial neuropathy (including bilateral facial paralysis), motor and sensory peripheral radiculoneuritis, mononeuritis multiplex, and myelitis. In contrast, encephalitis is rare in European patients with ECM-associated neurological disorders and in our chronic meningitis patients.

We have cultivated spirochetes from about 20 percent of collected I. ricinus ticks [unpublished data]. These and other spirochete isolates will allow us to determine the natural history of this tick-borne spirochetosis in Sweden. For example, we know that chronic meningitis and ECM are parts of this illness, but cardiac and arthritic complications are not reported from Sweden. The future will show us if these complications do not occur there or have simply not yet been recognized.

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REFERENCES

1. Ellner JJ, Benett JE: Chronic meningitis. Medicine 55:341-369, 1976
2. Hopkins AP, Harvey PKP: Chronic benign lymphocytic meningitis. J Neurol Sci 18:443-453, 1973
3. Sköldenberg B, Stiernstedt G, Gärde A, et al: Chronic meningitis caused by a penicillin-sensitive microorganism? Lancet ii:75-78, 1983
4. Wadström T, Nord C-E, Lindberg A, Möllby R: Rapid grouping of streptococci by immunoelectrosmophoresis. Med Microbiol Immunol 159:191-200, 1974
5. Sköldenberg B: On the role of viruses in acute infectious diseases of the central nervous system. Scand J Infect Dis (Suppl 3):1-96, 1972
6. Johansson BG: Agarose gel electrophoresis. Scand J Clin Lab Invest 29 (Suppl 124):7-19, 1972
7. Tibbling G, Link H, Öhman S: Principles of albumin and IgG analyses in neurological disorders. I. Establishment of reference values. Scand J Clin Lab Invest 37:385-390, 1977
8. Steere AC, Grodzicki RL, Kornblatt AN, et al: The spirochetal etiology of Lyme disease. New Eng J Med 308:733-740, 1983
9. Vandvik B, Nordal HJ, Vartdal F, et al: Imprint immunofixation of antibodies separated by agarose electrophoresis or by electrofocusing. In Handbook of Immunoprecipitation-in-Gel Techniques. Edited by N Axelsen. Scand J Immunol (Suppl 10):33-37, 1983
10. Sköldenberg B, Kalimo K, Carlström A, Forsgren M, Halonen P: Herpes simplex encephalitis: A serological follow-up study. Acta Neurol Scand 63:273-285, 1981
11. Vandvik B, Nilsen RE, Vartdal F, Norrby E: Mumps meningitis: Specific and non-specific antibody response in the central nervous system. Acta Neurol Scand 65:468-487, 1982
12. Bannwarth A: Chronische lymphocytäre Meningitis, entzündliche Polyneuritis und "Rheumatismus." Arch Psychiat Nervenkr 113:284-376, 1941
13. Bannwarth A: Zur Klinik und Pathogenese der "chronischen lymphocytären Meningitis." Arch Psychiat Nervenkr 117:161-185, 1944
14. Hörsstrup P, Ackerman R: Durch Zecken übertragene Meningo-polyneuritis (Garin-Bujadoux, Bannwarth), Fortschr Neurol Psychiat 41:583-606, 1973
15. Reik L, Steere AC, Bartenhagen NH, Shope RE, Malawista SE: Neurologic abnormalities of Lyme disease. Medicine (Baltimore) 58:281-294, 1979