Recurrent Lymphocytic Meningitis

The Role of Herpesviruses

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Background: Herpes simplex virus 2 (HSV-2) and HSV-1 have been recognized as causes of recurrent aseptic lymphocytic meningitis (RALM). However, the role of other herpesviruses has not been systematically assessed.

Objectives: To evaluate the cause of RALM by using polymerase chain reaction (PCR) tests detecting varicella-zoster virus (VZV), cytomegalovirus (CMV), or human herpesvirus 6 (HHV-6), in addition to HSV, on cerebrospinal fluid (CSF) samples; and to assess the utility of PCR and antibody analyses in consecutive episodes of RALM.

Design: The PCR and antibody results for herpesviruses were analyzed from 14 patients having 48 episodes of RALM.

Results: The CSF PCR results for VZV, CMV, and HHV-6 were negative in 12, 10, and 11 patients investigated, respectively, and antibodies against VZV, CMV, and HHV-6 showed only old immunity. Herpes simplex virus 2 was detected from the CSF in 10 patients, and HSV-1 in 1 patient. In 6 of these 11 patients, the HSV PCR result was positive in more than one disease episode. A significant increase of serum antibodies for HSV was seen in only 1 of 15 episodes examined. An intrathecal antibody response to HSV was not recognized in 9 episodes investigated in these 11 patients.

Conclusions: We could not find evidence of VZV, CMV, or HHV-6 in the pathogenesis of RALM, although most patients were previously infected by those viruses. Herpes simplex virus 2 was detected from the CSF in most patients, and often repeatedly, which further confirms the role of this virus in RALM. The causative diagnosis was obtained only by PCR, whereas antibody analysis was not clinically useful.

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HERPES SIMPLEX VIRUS 2 (HSV-2) and, less frequently, HSV-1 have been identified by polymerase chain reaction (PCR) assay from the cerebrospinal fluid (CSF) in patients with recurrent aseptic lymphocytic meningitis (RALM).1-6 Most articles presenting HSV-1 or HSV-2 as the causative agent of RALM are case reports.4,5,7,8 However, one report introduced 3 patients and another described 9 patients who had HSV-2 DNA in their CSF during RALM.2,3 A review of the literature revealed only one study in which CSF from the patients with RALM was systematically analyzed by the HSV PCR method; in each patient, only the last episode was examined.1 In that study, Tedder et al examined 13 patients with RALM, of whom HSV-2 DNA was detected by the PCR test from the CSF of 10 patients; HSV-1 DNA was detected from the CSF of 1 patient. Despite the etiologic role of HSV in RALM, there are still cases in which the cause remains unknown.

It can be assumed that to be capable of causing recurrent meningitis, a microbe should be harbored by the host and should have a propensity to infect the central nervous system. In addition to HSV, other herpesviruses also own such properties. In the recent decade, PCR analysis of CSF has broadened our understanding of the spectrum of the infections caused by other herpesviruses.9-12 Varicella-zoster virus (VZV) is recognized among the 3 most common agents in patients with central nervous system infections.9,13,14 Reactivation of a VZV infection is known to cause meningitis or encephalitis even in the absence of skin manifestations.15-17 Cytomegalovirus (CMV) and human herpesvirus 6 (HHV-6) have also been associated with central nervous system infections in patients without a known immunodeficiency.18,19 By adulthood, most humans have become infected by VZV, CMV, and HHV-6, and subsequently harbor the viruses for their lifetime. This makes these herpesviruses credible candidates for being causative.
agents of RALM. However, we found only one report of 4 patients in whom PCR tests for VZV, CMV, and Epstein-Barr virus also were applied in the diagnostics of RALM.3

In the present study, we investigated the cause of RALM in patients treated in a Finnish university hospital. Because there is a paucity of reports in the use of PCR for herpesviruses other than HSV in the diagnosis of RALM, we also included the PCR tests for VZV, CMV, and HHV-6 in the causative analysis in our study. The clinical utility of PCR and antibody analyses was evaluated in consecutive episodes of RALM.

**METHODS**

**PATIENTS**

We included in this study all adult patients treated for RALM between January 1, 1993, and June 31, 2003, at the Department of Neurology or the Department of Medicine, Turku University Central Hospital.

The patients were diagnosed as having RALM if they had at least 2 different episodes of meningitis with sudden onset, the CSF white blood cell (WBC) count was at least 5/µL with lymphocytic predominance, and the meningitis resolved without residual sequelae and with simultaneous clearing of CSF from leukocytes. In addition, the criteria included that all serum and CSF samples investigated were negative for microbes known to cause isolated acute meningitis (enteroviruses, respiratory viruses, arboviruses, or Mycoplasma pneumoniae).

The hospital records of the patients were retrospectively reviewed, and data were collected on age, sex, number of episodes, clinical symptoms, CSF findings, and the results of microbiological examinations. For each patient, the episodes of meningitis encountered before 1993 were also included in the analysis.

**MICROBIOLOGICAL METHODS**

Viral cultures on CSF specimens were performed at the Department of Virology, University of Turku, using standard methods. Because the HSV PCR test was applied in clinical use at the Department of Virology in 1993, the succeeding episodes were analyzed for the presence of HSV nucleic acids in CSF specimens by this assay. The HSV PCR assay was performed between January 1, 1993, and December 31, 1998 using a method previously described by Alanen and Hukkanen, and from January 1, 1999, onward, using a method described by Hukkanen et al.20,21

After the introduction of the PCR methods for CMV, VZV, and HHV-6 in our laboratory in the late 1990s, these assays were performed on CSF specimens. These PCR methods have been described previously by Hukkanen and Vuorinen.14 Antibodies against HSV, VZV, and CMV in patient sera and CSF were determined by indirect enzyme immunoassay.21 The HHV-6 antibodies in sera were measured by indirect immunofluorescence assay using HSB-2 cells (human T-cell acute lymphoblastic leukemia cell line) infected with the GS strain as an antigen.

**STATISTICAL METHODS**

An unpaired Mann-Whitney test was used to compare CSF samples, which were either positive or negative for HSV by PCR, in relation to the time from the onset of the symptoms and in relation to WBC counts and protein concentrations in CSF.

The disease history of 14 patients fulfilled the criteria of RALM (Table 1). The patients had 48 episodes of meningitis, verified between October 2, 1976, and June 17, 2003. Of these episodes, 31 occurred between 1993 and 2003, when the HSV PCR assay on CSF was in clinical use at the Department of Virology, University of Turku. The HSV DNA could be amplified by PCR at least once from the CSF of 11 patients, while the HSV DNA could not be detected from the CSF in the remaining 3 patients (Table 2). From the CSF samples, the VZV PCR result was negative in 12 patients, the CMV PCR result was negative in 10 patients, and the HHV-6 result was negative in 11 patients examined.
PATIENTS WITH HSV DNA IN THEIR CSF

Of the 11 patients with HSV DNA in their CSF, 10 were women and 1 was a man (patients 1-11; Tables 1 and 2). The patients had 39 episodes of meningitis, of which 28 occurred between 1993 and 2003. The onset of the symptoms was usually sudden, with headache, photophobia, nuchal rigidity, nausea, and low back pain. Often, the most profound symptoms of meningeal irritation resolved in a few days. The median temperature on admission was 37.3°C (range, 36.2°C-39.0°C). The median duration of headache was 6.5 days (range, 2-37 days), and the median duration of mildly elevated temperature was 3 days (range, 1-22 days). Five patients (patients 1, 2, and 4-6) had herpetic skin lesions associated with at least 1 disease episode: patients 1, 5, and 6 experienced these lesions during 1 episode, while patients 2 and 4, during 2 episodes. The vesicles were in the genital area in 4 patients (patients 1, 2, 5, and 6) and in the gluteal area in 1 patient (patient 4).

The CSF samples were sent for HSV PCR analysis in 24 episodes, of which 18 were positive for disease (Table 2). The PCR test result was positive in 3 episodes in 1 patient, in 2 episodes in 5 patients, and in 1 episode in 5 patients.

The results of 4 CSF samples of the 15 episodes analyzed by the PCR method used from January 1, 1993, to December 31, 1998, were negative, as were 2 of the 9 CSF samples analyzed by the method used since January 1, 1999. Neither the duration of the symptoms before the lumbar puncture nor the WBC counts and the protein concentrations of the CSF differed significantly between the episodes being either positive or negative by HSV PCR analysis (Figure). Convalescent CSF samples were drawn for PCR analysis after acyclovir treatment in 7 episodes that were originally positive for HSV DNA. Six CSF samples were negative for HSV by PCR, but one was still positive after 17 days from the onset. A later convalescent CSF sample was not drawn from this patient. The CSF was analyzed by PCR for the presence of VZV DNA in 11 episodes, CMV DNA in 8 episodes, and HHV-6 DNA in 9 episodes from 9, 7, and 8 patients, respectively (Table 2). All CSF samples were obtained within 6 days from the onset of the symptoms. None of the test results were positive. Virus culture results from the 17 CSF samples obtained within 4 days after the onset of symptoms were also negative (Table 2).

All 11 patients who had HSV DNA in their CSF had serum IgG antibodies to HSV and VZV (Table 2). Convalescent sera were examined for HSV and VZV in 15 episodes, and obtained after a median of 22.5 days from the onset of the symptoms. More than a 3-fold increase of IgG titers to HSV was measured only during one episode of meningitis. No IgM antibodies against HSV and VZV in sera were detected.

Low amounts of HSV IgG antibodies in CSF were detected in 2 episodes, but paired CSF samples were not obtained or serum antibodies were not measured during these episodes. Convalescent CSF specimens were obtained in 9 episodes after a median of 27 days (range, 14-120 days) from the onset of symptoms. No appearance of intrathecal antibodies against HSV or VZV was detected.

Nine patients had serum IgG antibodies to CMV, and convalescent sera were examined for CMV antibodies in 8 episodes after a median of 27 days (range, 15-125 days) from the onset of the symptoms. Changes in IgG titers were not detected. Cerebrospinal fluid IgG antibodies for CMV were also investigated in 12 episodes, with convalescent CSF in 6 episodes, after a median of 24.5 days (range, 7-49 days). No positive titers were recognized. Five patients also had serum IgG antibodies to HHV-6 (Table 2).

PATIENTS WITH NO HSV DNA IN THEIR CSF

Of the 3 patients in whom HSV DNA was not detected in CSF, 2 were men and 1 was a woman (patients 12-14; Tables 1 and 2). The patients had 9 episodes of meningi-
The CSF samples were analyzed by HSV PCR in only one episode from each of these patients (Table 2). The PCR analyses were performed from September 3, 1995, to November 20, 1997. In 2 patients, a CSF sample was obtained for the HSV PCR assay within the first day, and in 1 patient, on the sixth day, from the onset of the symptoms. The WBC counts in the 3 CSF samples analyzed by HSV PCR were 100/µL, 304/µL, and 170/µL, and the protein concentrations of the samples were 0.0963 g/dL (96.3 mg/dL), 0.0441 g/dL (44.1 mg/dL), and 0.1260 g/dL (126 mg/dL). The CSF specimens of the last episodes were also analyzed. Herpes simplex virus DNA was identified from the CSF of several episodes from individual patients were analyzed. Herpes simplex virus DNA was identified from the CSF in 18 of 24 episodes in 11 patients. In 6 patients, HSV was detected from the CSF during more than one consecutive episode, further substantiating the causative role of this virus in RALM.

However, the PCR result remained negative in 6 episodes examined in these same patients. The PCR-negative episodes did not differ from the PCR-positive episodes: the clinical symptoms were similar, as were the differential counts of the CSF leukocytes consisting almost completely of mononuclear cells. Therefore, and because no other agents were found, we suppose that HSV might also have been involved in the cause of these episodes. In that case, the reason for the PCR negativity remains obscure, because the sampling times of CSF samples were similar, as was the degree of inflammation when measured by WBC counts or protein concentrations in CSF.

The change of the PCR method in 1999 cannot explain the PCR negativity, considering that an equal proportion of the episodes investigated remained negative for HSV by both methods.

In this study, we assessed the role of different herpesviruses in the cause of RALM. To our knowledge, this is the first study in which herpesviruses other than HSV have been systematically investigated as potential causative agents of RALM. None of these viruses were, however, detected by PCR in any of the CSF samples tested, although most of our patients had a positive serologic result against VZV, CMV, and/or HHV-6, as an indication of a latent infection.

Herpes simplex virus 2 and HSV-1 were the only herpesviruses identified from CSF, being associated with the disease in 11 (79%) of the 14 patients. Herpes simplex virus 2 was the most common, in agreement with the previous report by Tedder et al.1 In that study, however, the CSF sample of only the last episode from each patient was examined, whereas in our study, the CSF samples of several episodes from individual patients were analyzed. Herpes simplex virus DNA was identified from the CSF in 18 of 24 episodes in 11 patients. In 6 patients, HSV was detected from the CSF during more than one consecutive episode, further substantiating the causative role of this virus in RALM.

COMMENT

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Only one of our patients had a significant increase in serum IgG titers to HSV during one episode of meningitis, which was her first. Genital herpes was associated with the episode, which possibly represented the primary infection of HSV-2. We did not find an increase of IgG titers to HSV between paired sera in her 2 consecutive episodes, or in any other disease episodes in our pa-
patients. We also did not find an intrathecal immune response to HSV in any of the paired CSF samples analyzed. Based on these findings, an antibody analysis of sera or CSF is of no diagnostic value in recurrent meningitis. Previous studies concerning the antibody response to HSV in RALM are limited.\(^3,12,23\) In one study describing 10 recurrences of probable herpes meningitis in 5 patients before the PCR era, no antibody response was detected in paired sera by immunoblot analysis.\(^23\) Instead, intrathecally produced IgG antibodies to HSV-2 type-specific glycoprotein were detected by the analysis in 6 of 9 CSF samples investigated. In another study describing 11 episodes of RALM, HSV antibodies were detected in CSF by immunoblot analysis, but paired CSF samples or sera were not analyzed.\(^1\)

In 3 of our patients, the cause of the disease remained undefined. The clinical symptoms, the length of the episodes, and the CSF features resembled those of the patients with HSV-associated disease; however, genital recurrences were not associated in any of the 9 episodes in these patients. One patient presented IgM positivity simultaneously against HSV, VZV, and CMV. However, IgG titers did not increase between paired sera; intrathecal IgG titers to HSV, VZV, and CMV were not detected; and PCR test results of the respective herpesviruses from CSF were negative during this episode. Therefore, IgM positivity was most probably due to an unspecific reaction caused by rheumatoid factor that was detected in this patient. Unfortunately, most of the episodes were encountered before the PCR era and, hence, nucleic acid–detecting methods of herpesviruses could be applied in the diagnostics only during the last episode of each patient.

In conclusion, the present study provides data on the role of different herpesviruses in RALM. Our study did not show evidence of the involvement of VZV, CMV, or HHV-6 in the cause of RALM. Herpes simplex virus 2 was detected from the CSF in most of the patients, and often repeatedly, which further confirms the role of this virus in RALM. The diagnosis was based only on PCR results; antibody analysis was not clinically useful.

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