Diagnosis of Jamestown Canyon Encephalitis by Polymerase Chain Reaction

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In recent years, polymerase chain reaction (PCR) has been under study as a potential technique to improve the accuracy of diagnosis of suspected central nervous system viral infections. We describe a case of severe encephalitis in a previously healthy 20-year-old woman from New York who presented with headache, fever, and photophobia. Her illness was characterized by progressive worsening of her neurological status, leading to confusion, delirium, and status epilepticus. The diagnosis of Jamestown Canyon encephalitis was established by positive reverse transcriptase (RT)–PCR and nucleic acid sequencing of the band from both cerebrospinal fluid and brain tissue. The nucleotide sequence and the deduced amino acid sequence of the Jamestown Canyon virus from this patient were very similar to Jamestown Canyon virus isolates from mosquito pools in New York. This report suggests that RT-PCR assays could be important tools in the diagnostic workup of cases of encephalitis.

Nucleic acid amplification technology, specifically PCR, has been shown to improve the diagnosis of CNS infections due to enteroviruses and herpesviruses, and is rapidly becoming the approach of choice for such purposes [1, 2]. We used this technique to study a patient with encephalitis, in the hopes that it would hold similar promise with respect to infections caused by California serogroup viruses.

California encephalitis virus, the first of the California serogroup viruses to be discovered, was isolated from mosquitoes in 1943 [3]. Subsequently, in 1945, it was linked by serology to three human CNS infections that occurred in Kern County, California [4]. Fifteen years later, in 1960, La Crosse virus, the next member of this group to be associated with human CNS infections, was isolated postmortem from the brain tissue of a child who had died of encephalitis in La Crosse, Wisconsin [5]. Since then, >1,000 cases of CNS infections with La Crosse virus have been documented serologically, but there have been only five instances in which the virus was actually recovered from a patient; four of these were from brain tissue (three postmortem) and one was from CSF [5–9]. Other members of this group of viruses that have been linked to human CNS infections are Jamestown Canyon, snowshoe hare, and trivittatus viruses [10–13]. There is no report that any of the latter have ever been recovered from a patient, and until this account, all previous evidence for their role in human infection has been based solely on serological grounds. In the summer of 1997, a case of encephalitis occurred in Monroe County, New York, from which PCR was used to detect Jamestown Canyon virus genomic sequences in both brain biopsy tissue and CSF.

Case Report

A 20-year-old woman without significant medical history was hospitalized on 8 June 1997, after a 3-week illness characterized by headache, fever, neck stiffness, photophobia, nausea, and vomiting. On admission, she also complained of recent mild confusion and forgetfulness. She lived in Monroe County, New York, and denied any recent travel, contacts with ill persons, or insect bites, although she spent some time outside in a wooded area. On admission, she was alert but had difficulty concentrating. Her temperature was 38.6°C, and Kernig and Brudzinski signs were positive. No focal neurological deficit was found. Examination of CSF showed 608 cells/mm³ (normal, 0–5 cells/mm³) with 89% mononuclear cells; 148 mg/dL protein (normal, 15–45 mg/dL); and 51 mg/dL glucose (normal, 40–70 mg/dL) (table 1). Initial CT of the brain and electroencephalography were normal, but MRI showed an enhancement in the sulci and gyri over the cerebellum, with signs suggestive of leptomeningeal inflammation.

Over the next 4 weeks, the patient’s neurological condition deteriorated dramatically. She became increasingly febrile, confused, and delirious, with intermittent seizures. Repeated lumbar punctures consistently showed CSF pleocytosis with a predominance of mononuclear cells, with a mildly elevated protein level and a normal glucose level (table 1). Cultures of CSF for bacteria, viruses (herpes simplex virus [HSV] and enteroviruses), fungi, and acid-fast bacilli yielded negative results. PCR testing of CSF for HSV and enteroviruses showed negative results. Histological examination of the CSF showed no malignant cells. Repeated cultures of blood yielded negative results. Immunofluorescence assays for cytomegalovirus and Epstein-Barr virus (EBV) were negative, as was an EIA for Leptospira species. Serological tests for Lyme disease were
Table 1. Sequential CSF findings in a patient with Jamestown Canyon encephalitis.

<table>
<thead>
<tr>
<th>Date</th>
<th>WBC count (/mm³)</th>
<th>RBC count (/mm³)</th>
<th>PMNLs (%)</th>
<th>Mononuclear cells (%)</th>
<th>Glucose level (mg/dL)</th>
<th>Protein level (mg/dL)</th>
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<tr>
<td>8 June</td>
<td>608</td>
<td>3</td>
<td>11</td>
<td>89</td>
<td>51</td>
<td>148</td>
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<td>580</td>
<td>2</td>
<td>3</td>
<td>97</td>
<td>47</td>
<td>126</td>
</tr>
<tr>
<td>25 June</td>
<td>279</td>
<td>1</td>
<td>2</td>
<td>98</td>
<td>65</td>
<td>81</td>
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<td>1 July</td>
<td>280</td>
<td>356</td>
<td>0</td>
<td>100</td>
<td>60</td>
<td>55</td>
</tr>
</tbody>
</table>

NOTE. PMNLs = polymorphonuclear leukocytes.

Laboratory Studies

Serology. Serological testing by immunofluorescence assay for total anti-virus immunoglobulin used virus-infected cells as antigen. In addition to Jamestown Canyon virus, the test panel included eastern equine encephalitis, western equine encephalitis, St. Louis encephalitis, Powassan, and La Crosse viruses. A rise in titer was observed only in antibody to Jamestown Canyon virus, and this occurred slowly over a 3-month period.

PCR. PCR analyses for enteroviruses, HSV, and HIV-1 were done on CSF at other laboratories, and results were negative. Brain tissue surgically collected on 5 July was sent to the Wadsworth Center, New York State Department of Health, to be examined for evidence of arbovirus infection. A positive result was obtained in a reverse transcriptase (RT)–PCR that used universal primers directed toward the small genomic RNA segment of the California serogroup viruses [14]. Briefly, RNA from brain tissue as well as a control RNA template (GeneAmp RNA PCR kit, Perkin-Elmer Cetus, Norwalk, CT) were extracted by using Trizol (Life Technologies GIBCO BRL, Gaithersburg, MD). The advantages of using this control RNA template are that it minimizes the chance of contamination and it

Figure 1. Cross-section of the cerebellum of a normal adult, showing the outer eosinophilic molecular layer (ml), Purkinje cell (indicated by the arrow), and densely granular cellular layer (gcl). Hematoxylin-eosin stain; magnification, ×100.

Figure 2. Cross-section of the cerebellum of the index patient, with inflammatory cell infiltrate in the leptomeninges with lymphocytes and macrophages, diffuse and focal microglial infiltrate in the molecular layer (ml), and Purkinje cell loss and severe cell loss in granular cellular layer (gcl). Hematoxylin-eosin stain; magnification, ×100.
ensures the presence of all of the required reagents. The RNA was reverse-transcribed to generate first-strand cDNA in a reaction mixture (50 µL) containing random hexamers as described previously [14]. An aliquot of the cDNA reaction mixture (5 µL) was added to a 50-µL PCR reaction mixture containing universal primers. The amplification reaction was carried out in a DNA thermal cycler (PE480; Perkin-Elmer Cetus). The thermal profile for PCR consisted of an initial 3 minutes at 94°C, followed by 35 repetitive cycles of 30 seconds at 94°C, 30 seconds at 52°C, and 30 seconds at 72°C, followed by a 5-minute extension at 72°C for one cycle. The reaction product was analyzed on a 2% agarose gel containing Tris-borate-EDTA buffer and ethidium bromide. The PCR band with the expected size was observed on the gel from brain tissue. The band was recovered from the gel and directly sequenced by the dye-termination cycle-sequencing technique with an automated DNA sequencer (model 373A; Applied Biosystems, Foster City, CA). When the nucleotide sequence (718 nucleotides) and the deduced amino acid sequence (190 amino acids) were compared with published data for the reference strain of Jamestown Canyon virus, similarities of 89.6% and 97.9%, respectively, were observed. The sequence data were also compared with that for South River virus, which is antigenically very closely related to Jamestown Canyon virus and was isolated from mosquitoes in New Jersey in 1960 [15]. The nucleotide and amino acid sequence similarity between the patient’s virus and South River virus were 87.1% and 97.4%, respectively. The RT-PCR analysis was repeated on a CSF specimen that had been collected on 8 June, about 4 weeks before the brain biopsy. A positive PCR band was also detected in the CSF, and the viral sequences were identical to those found in the brain. Although the sequences detected in the two patient specimens were identical, they differed from those of any other example of Jamestown Canyon virus that had ever been handled in this laboratory. The relationships between the nucleotide sequences of Jamestown Canyon viruses are displayed in the dendrogram shown in Figure 3. Comparison of a Jamestown Canyon virus isolate (9730210) currently circulating in mosquitoes in New York State with the sequence of the virus detected in the patient’s specimens showed 98.9% similarity at the nucleotide level and 98.4% similarity at the amino acid level.

Discussion

This case is significant for several reasons: It represents the first instance in which a case of encephalitis attributable to a California serogroup virus was diagnosed by PCR and suggests that this method may offer improved opportunities for the diagnosis of such infections; it was possible to show that the viral genome detected in the patient was much more closely related to Jamestown Canyon virus variants currently circulating in mosquitoes in New York than to the prototype strain of the virus; and the course of the illness and its outcome were atypical compared with previous reports related to CNS infections with Jamestown Canyon virus.

There is an average of one reported case of Jamestown Canyon encephalitis in the United States each year (Centers for Disease Control and Prevention, personal communication). However, intensive surveillance and retrospective studies in the early 1980s in New York State uncovered 43 cases [11, 13], but after dissolution of surveillance programs in the mid-1980s, no further cases have been reported in that state. However, seroprevalence surveys as well as virus isolates in mosquitoes indicate that Jamestown Canyon virus is the prevalent
California serogroup virus in New York State [13]. Nationwide, after La Crosse virus, Jamestown Canyon virus is now generally accepted as the most important human pathogen among the California serogroup viruses [16]. To the best of our knowledge, all of the previously reported cases were identified by serology, and there was no opportunity for molecular characterization of the virus itself. Thus, the detection and amplification of viral genomic sequences in the present case also offered the first occasion in which the virus from a patient could be compared with other known isolates of Jamestown Canyon virus.

Jamestown Canyon virus has a wide geographic distribution in North America, and a serologically distinguishable variant, South River, has been recognized in the Northeast [15]. Consequently, the region of the viral genome amplified from the patient was compared with prototype Jamestown Canyon and South River viruses and with 10 isolates of Jamestown Canyon virus recovered in New York from mosquitoes during 1996–1997. The latter represent a broad geographic distribution ranging from Long Island in the southeast to the far western regions of the state. Interestingly, for the 718-nucleotide portion of the small genomic RNA used in the comparison, the patient’s virus was found to be much more similar to those from the mosquitoes than to either prototype Jamestown Canyon or South River virus.

The RT-PCR assay has been essential in establishing the diagnosis in this particular case for two reasons. First, the clinical course as well as the outcome for this patient were not typical: Jamestown Canyon virus infections have been largely described as mild febrile diseases or as cases of aseptic meningitis, cases of encephalitis have been described, and a full recovery is the general rule. A single fatal case has been reported, but coexistent HSV infection was also present [11]. Second, early serological studies were not conclusive. After >6 weeks of hospitalization, only a twofold rise in titer was observed, a fourfold rise being obtained only on the fourth specimen drawn 3 months after the onset of the disease. Hence, serology had limited diagnostic usefulness. On the other hand, RT-PCR findings rapidly established the diagnosis of Jamestown Canyon encephalitis in CSF and brain tissue, both showing identical nucleic acid sequences. We were unable to explain the slight elevation in HSV-1 and HSV-2 EIA titers. However, we felt that coinfection with HSV was highly unlikely because of the negative results on HSV-1 PCR, a test that is now considered the standard for diagnosis of encephalitis due to herpesvirus [1].

Accurate diagnosis of CNS infections is important to ensure appropriate treatment and to establish timely prognosis. Given the limitations associated with serological testing, this report suggests that RT-PCR assays for California serogroup viruses could be important tools in the diagnosis of cases of encephalitis occurring from May to October in states in which these viruses are endemic. However, since this is the first case to be diagnosed by this technique, further studies are needed to establish the sensitivity and the specificity of RT-PCR in human infections at different points in the disease.

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References