Isolation of Eastern Equine Encephalitis Virus in A549 and MRC-5 Cell Cultures

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Eastern equine encephalitis (EEE) has been diagnosed either serologically or by virus isolation. Until now, the recovery of EEE virus has been delegated to reference laboratories with the expertise and resources needed to amplify the virus in a susceptible vertebrate host and/or to isolate and identify the virus in cell culture. We report a case in which EEE virus was recovered directly from a patient's cerebrospinal fluid in A549 and MRC-5 cell cultures. Many clinical virology laboratories routinely use these cells to recover adenovirus, herpes simplex virus, and enterovirus. To the best of our knowledge, this is the first report of isolation of EEE virus in A549 cell culture. This report demonstrates the possibility of recovery of EEE virus in cell culture without the necessity of bioamplification or maintaining unusual cell lines.

Although eastern equine encephalitis (EEE) is a relatively rare disease, it constitutes a public health threat because of its clinical severity and because multiple cases cluster cyclically. Severe and fatal human cases have been reported along the Atlantic and Gulf coasts of North America, and a few inland foci have been found in Ontario, New York, Michigan, and South Dakota [1]. Numerous clinical reports of EEE have been published [2–6]. However, there have been relatively few reports of virus isolation.

In this report, we present a case of an adolescent with encephalitis and a focal temporal lesion on a CT scan. The diagnosis of EEE was based on virus isolation in A549 and MRC-5 cell cultures directly from a CSF specimen obtained during hospitalization. Although it has been reported that EEE virus can be isolated from serum during the febrile prodrome very early in the course of disease, recovery of the virus is most common after death from brain tissue and occasionally CSF [1, 7].

Case Report

On 5 September 1993, a 14-year-old male was admitted to a local hospital with a 3-day history of severe headache and fever (temperature, 101.7°F). The patient had a history of recent travel to Florida and then to Block Island off the coast of Rhode Island where he camped on 27–28 August 1993. During this period, he sustained numerous mosquito bites in a swampy area near the camp. A lumbar puncture was performed, and subsequent analysis of CSF revealed a WBC count of 1,087/mm³ (85% polymorphonuclear leukocytes, 6% lymphocytes, and 9% monocytes), RBC count of 40/mm³, protein level of 63 mg/dL, and glucose level of 80 mg/dL but no organisms. A bacterial culture of CSF was negative. A CT scan showed hypodensity and edema of the right temporal lobe.

Although therapy with antibiotics was started, his condition worsened with seizures and fatigued respiration. The patient was transferred to Rhode Island Hospital (Providence) on 7 September 1993, where his condition progressively worsened despite ventriculostomy. On 8 and 9 September 1993, blood, CSF, urine, respiratory, and rectal specimens were collected and submitted to the laboratory for serological testing and culture. The single CSF and serum samples submitted were negative for IgM antibodies to herpes simplex virus (HSV) types 1 and 2. The serum titer of IgG antibody to HSV type 1 was 1:10. The serum and CSF samples were not tested at this time for antibodies to arbovirus. The bacterial cultures were all negative.

Adenovirus, however, was recovered from the rectal specimen, and a second isolate, ultimately identified as EEE virus, was recovered from the CSF. On 10 September 1993, a CT scan revealed significant worsening of the brain swelling with a right to left shift suggesting impending herniation. Two days later, the patient had areflexia with high intracranial pressure and died the following day.

At autopsy, which began 5.5 hours after death, analysis of the brain revealed the following: focal necrosis and softening of the right temporal lobe, focal petechial hemorrhages (0.3 cm) in the right frontal and parietal lobes, diffuse congestion and swelling, prominence of parahippocampal gyrus and tonsils, and moderate ventricular dilatation. All of these findings are consistent with EEE. Postmortem cultures of CSF and brain (right temporal lobe) tissue specimens were negative for bacteria and viruses. Electron microscopy of the brain tissue specimen was suggestive of virus, but the results were incon-
clusive. However, a postmortem serum sample was positive for EEE virus, with an IgM antibody titer of 1:512. Following the patient’s death, the serum sample obtained on 8 September 1993 and the CSF sample obtained on 9 September 1993 were tested for antibody to arbovirus. The serum was positive for IgM antibody to EEE virus at a titer of 1:16, while the CSF was negative for antibody to EEE virus.

Materials and Methods

As with any specimen submitted for viral culture, the CSF sample obtained on 9 September 1993 was inoculated into primary rhesus monkey kidney, A549 (human lung carcinoma), and MRC-5 (human fetal lung fibroblast) cell cultures (Biowhittaker, Walkersville, MD), which were examined daily for evidence of viral replication. Reagents for the detection of EEE virus antigen in cell culture are not commercially available. Therefore, to test for EEE virus antigen, we used the positive control serum (polyvalent antibodies to arboviruses) from a commercially available arbovirus serology test (MRL Diagnostics, Cypress, CA).

Results

The A549 and MRC-5 cell lines began to show a cytopathic effect (CPE) on the third day of culture with complete development of the CPE on the seventh and eighth days. The observed CPE was more pronounced in the A549 cells. We were unable to detect adenovirus, HSV types 1 and 2, and enterovirus antigens in the infected cells with direct fluorescent antibodies specific to virus. Further testing revealed that virus infectivity could be neutralized by weak acid (pH 3.0) and chloroform, indicating the presence of an enveloped virus. After narrowing the search to enveloped viruses other than HSV that are neurotropic, able to grow in cell culture, and present in the summertime in New England, EEE virus emerged as the primary candidate. The infected A549 cells tested positive for arbovirus antigen with use of polyvalent arbovirus serum. We were then able to neutralize virus infectivity with horse antiserum specific to EEE virus (lot 78-0033, Centers for Disease Control and Prevention) and thereby show that the arbovirus was, in fact, EEE virus. The infected MRC-5 cells were sent to the Centers for Disease Control and Prevention where the identification of EEE virus was confirmed after passing the virus in Vero cells and detecting EEE virus antigen.

Discussion

Most arboviruses are capable of replication in vitro in a variety of mammalian cells with the production of the CPE. Those cell lines with the widest susceptibility to arboviruses are the continuous cell lines: Vero, BHK-21, LLC-MK2, and CER cells and primary cells cultivated from hamster kidney, chick embryo, and duck embryo [7]. A review of the literature showed that most cases of EEE were diagnosed serologically [2, 8]. In most of the reported cases involving virus isolation, EEE virus was recovered either in mice or in cell culture following virus amplification in mice [4, 6, 9]. However, the virus has also been isolated directly in cell culture [9]. Unfortunately, few laboratories have sufficient resources available to amplify virus in a susceptible vertebrate host or to maintain unusual cell lines, whereas many more laboratories can recover virus in routine cell culture as well as perform serological testing.

With arboviruses, the CPE may be detectable within 1–2 days for certain viruses, while for other viruses, the cells may need to be observed for 7 to 10 days [7]. The CPE observed in our case was similar to the report by Lubiniecki [10], where the CPE of EEE virus in primary chick embryo fibroblasts and rabbit kidney cell cultures was characterized as cell rounding accompanied by cytoplasmic shrinkage and nuclear pyknosis beginning on the third day.

In the present case, failure to recover EEE virus in cell culture from the postmortem brain tissue and CSF specimens was unexpected and could be related to the 5.5-hour interval between the death of the patient and the start of the autopsy and to extensive necrosis in the CNS. The absence of identifiable EEE virus by electron microscopy in the postmortem brain tissue sample supports this possibility. In a somewhat analogous case, Calisher et al. [9], who used human embryonal rhabdomyosarcoma cells, were unable to recover virus from a postmortem brain tissue specimen from a child with EEE. However, virus was recovered from CSF. In the future, it is likely that PCR analysis will be used to detect residual viral genome when the virus cannot be recovered.

Finally, epidemiological follow-up revealed that one of 16 individuals who camped with the victim had a serum titer of IgM antibody to EEE virus (1:512) without significant symptoms [11]. In terms of geographic incidence, this case illustrates the unpredictability of EEE. The virus had not been detected in birds and horses on Block Island. Furthermore, the island had not been considered to be a suitable habitat to support EEE virus [11].

On the basis of our experience, the routine use of the A549 cell line by clinical virology laboratories would be advantageous especially in those areas where EEE virus is endemic. The use of these cells could obviate the need for reference testing and, thereby, decrease the time required for a diagnosis of EEE. The potential value of virus culture for establishing an accurate diagnosis of EEE as well as for providing virus for epidemiological follow-up should not be overlooked.

In summary, to the best of our knowledge, this is the first reported isolation and identification of EEE virus from the CSF.
of a living patient with encephalitis and the first report of isolation of EEE virus in A549 cell culture.

References