Isolation and Characterization of Adenovirus 5 from the Brain of an Infant with Fatal Cerebral Edema

This report describes a fatal case of cerebral edema caused by adenovirus in a previously healthy 18-month-old infant who developed skin rash, pulmonary congestion, and fever and who died 6 days later. Adenovirus hexon gene sequences were detected in brain tissue and brain tissue cultures. The virus was typed as adenovirus 5.

Adenoviruses are commonly associated with respiratory and eye infections in normal and immunocompromised adults and children, but encephalitis caused by viral replication in the brain is relatively rare [1–3]. Several serotypes (e.g., serotypes 2, 7, 11, 19, 31, 32, and 49) have been isolated from brain in a few reported fatal cases [2–6]. Viral infection has been diagnosed primarily by serological tests (e.g., immunofluorescent antibody [IFA] staining, complement fixation, and neutralization) and sometimes by electron microscopy (EM), restriction endonuclease analysis, or hybridization [4, 6–9]. However, isolation of adenovirus from brain tissues or CSF is rare. Furthermore, comprehensive characterization of an adenovirus isolated from brain is also lacking.

We report a fatal case of cerebral edema caused by adenovirus in an infant with fever and skin rash. For the first time, adenovirus 5 was isolated from the brain of an infant by means of cell culture and was characterized by IFA, neutralization, and EM. In addition, the genotype of the virus was compared with that of the wild-type adenovirus by means of PCR and nucleotide sequencing of the viral hexon antigen gene.

An 18-month-old white male infant with a history of skin rash, pulmonary congestion, and fever was found dead on the morning of 4 May 1998. He had been delivered vaginally and without complications to a gravida IV para IV mother after 38 weeks’ gestation. He had received vaccinations against diphtheria-tetanus-pertussis and polio but not chickenpox, and he had no known allergies. Other children in the family had no significant medical problems, and there was no history of immunodeficiency in the siblings or in other relatives from either the maternal or the paternal side of the family.

Approximately 6 days before his death, the infant developed upper respiratory symptoms with mild fever. Three days later, a maculopapular rash appeared on his face and abdomen, and he was seen by his pediatrician, who thought the centripetal rash was due to chickenpox. The child began receiving acyclovir and acetaminophen. On day 5 in the evening, the infant’s fever increased to 39.4°C, but he had no nausea, vomiting, or drowsiness. The pediatrician advised that the infant be seen in the emergency room. The infant was given acetaminophen at home, and his fever subsided. He was later given an over-the-counter mixture of doxylamine succinate, dextromethorphan hydrobromide, acetaminophen, and pseudoephedrine hydrochloride, and he was put to bed at ~10:00 p.m., after a normal feeding. The infant had no history of diarrhea, vomiting, opisthotonos, neck rigidity, seizures, or irritability. On day 6, at ~6:00 a.m., his mother was unable to awaken him; paramedics failed to resuscitate the infant, and he was pronounced dead in the emergency room of a local hospital (Ellis Hospital, Schenectady, NY), where an autopsy was done. No areas of laceration, abrasion, or contusion were present on the surface of the body. Skin lesions that were thought to be compatible with varicella were present on the surface of the body.

The brain (weight, ~1150 g) appeared congested and swollen. It contained some mononuclear cells within the meninges; the sulci were partially obliterated, and the gyri were shallow. The blood vessels at the base of the brain were identified, and both the vessels and the cranial nerves were found to be distributed in a normal manner. The lungs (weight: right lung, 75 g; left lung, 85 g) appeared to be somewhat congested, but no areas of consolidation, abscess formation, or infarction could be visually identified in either lung. The spleen, liver, pancreas, thyroid, parathyroid, gastrointestinal tract, kidneys, neck, and nasopharynx appeared unremarkable. The peritoneal cavity, pleural cavities, and pericardial cavity were devoid of free fluids, and the mediastinum and thymus were devoid of tumor masses. Electrolyte and liver enzyme levels had not been determined during antemortem examination. However, there was no histological evidence of steatosis in the liver, ruling out the possibility of Reye’s syndrome. No congenital anomalies or other abnormalities were detected in the heart after careful examination. Cerebral edema was the reported cause of death. Toxicological assays on blood, performed by the New York State Police laboratory, revealed no abnormal findings.

Only postmortem brain and lung tissues were available for virological testing. In brief, the tissues were inoculated into A549, human embryonic lung, and Rhesus monkey kidney cell cultures, to grow and isolate the virus present in the tissues. The utility of A549 cells for the isolation of adenoviruses has been demonstrated [10]. Cultures showing cytopathic effects underwent further testing. IFA staining was done with fluorescent monoclonal antibody to adenovirus (Chemicon, Temecula, CA), and serum neutralization assay was done in A549 cell cultures with the use of adenovirus type-specific antisera (Centers for Disease Control and Prevention, Atlanta).

For PCR and nucleotide sequencing, total cellular DNA or RNA was isolated by use of the commercial kits IsoQuick...
Figure 1. Electron micrographs of brain tissue cultures. A, Thin section showing nuclear inclusion containing electron-dense virus crystals. Arrows, disrupted nuclear membrane with released virions. B, Virions in higher magnification. C, Negatively stained virions showing surface to be composed of capsomeres arranged in an icosahedron pattern.

(Orca Research, Bothell, WA) or Ultraspec-3 RNA (Biotecx Laboratories, Houston), respectively. Stratagene (La Jolla, CA) supplied primers and instructions for adenovirus hexon antigen gene PCR. PCR analyses for Coxsackie virus, echovirus, poliovirus, herpes simplex virus types 1 and 2, cytomegalovirus, and varicella-zoster virus were done with our laboratory’s routinely used assays.

Adenovirus PCR was done with total cellular DNA (~40 ng) in 100-μL reactions in a thermal cycler (model 480; Perkin Elmer Cetus, Norwalk, CT), with use of primers (catalog no. 728083) and instructions from Stratagene. The PCR amplification included denaturation at 94°C for 3 min and annealing at 53°C for 3 min, followed by 30 cycles of at 94°C for 1 min, at 53°C for 1 min, and at 72°C for 2 min, with final extension at 72°C of 10 min. The PCR products were separated by means of 3% agarose gel electrophoresis in Tris-acetate buffer, were stained with ethidium bromide, and were visualized with ultraviolet light to isolate an adenovirus-specific amplified cDNA product for automated nucleotide sequencing (ABI Prism 377; PE Biosystems, Foster City, CA).

For EM, brain tissues and their A549 cultures were fixed in 2% glutaraldehyde in 0.2 M sodium cacodylate buffer (pH 7.4) for 3 h and were postfixed for 1 h in 1% osmium
Figure 2. Alignment of nucleotides from PCR products of hexon gene from brain tissue (suspension) and its cell culture with adenovirus 5 hexon (locus, ADRA1) and adenovirus 2 genome (locus, ADRCG). Hyphens denote dissimilar nucleotides; dots were used to facilitate alignment. Alignment was done with University of Wisconsin Genetics Computer Group software programs [12].

| adeno genome | CTTCTTATT GTTTGGTTTG AAGCTTTTG AGGTTGTCGG TGGCCACCC AGGGCCACCC GGGCCATCT GGAGACTGTG |
| adeno hexon | CTTCTTATT GTTTGGTTTG AAGCTTTTG AGGTTGTCGG TGGCCACCC AGGGCCACCC GGGCCATCT GGAGACTGTG |
| brain suspension | CTTCTTATT GTTTGGTTTG AAGCTTTTG AGGTTGTCGG TGGCCACCC AGGGCCACCC GGGCCATCT GGAGACTGTG |
| brain cell culture | CTTCTTATT GTTTGGTTTG AAGCTTTTG AGGTTGTCGG TGGCCACCC AGGGCCACCC GGGCCATCT GGAGACTGTG |
| CONSENSUS | C-TTCTTATT GTT-GTTTG AAGCTTTTG AGGTTGTCGG TGGCCACCC AGGGCCACCC GGGCCATCT GGAGACTGTG |

According to the results of PCR, brain tissues were negative for Coxsackie virus, poliovirus, echovirus, herpes simplex virus types 1 and 2, cytomegalovirus, and varicella-zoster virus. However, positive PCR products of adenovirus antigen were detected in samples of the brain tissue and its A549 cell culture. Further analysis of the products, by means of nucleotide sequencing, revealed very similar hexon gene sequences in both cases (figure 2). To analyze the genetic diversity of the infant’s virus, we compared a 100-base region of the hexon gene with the sequences from wild-type adenovirus 2 genome [13] and adenovirus 5 hexon [14]. A remarkable similarity in the region was detected for the virus isolated from the infant and the reference wild-type strains (for comparison, see figure 2 for sequences with adenovirus genome and adenovirus hexon). However, the study did not establish that the virus replicated in the infant’s brain tissue. Therefore, the virologic, EM, and PCR data were compatible with a fatal case of edema of the brain caused by adenovirus 5 infection.

Tissue culture of brain and lung tissues in A549, human embryonic lung, and Rhesus monkey kidney cells showed an adenovirus-like cytopathic effect in A549 cells only. A negative result on hemadsorption testing of the lung culture monolayers ruled out the involvement of such viruses as influenza, parainfluenza, or mumps virus. Hemadsorption has been recommended for the detection of respiratory viruses [11]. IFA of the infected cultures revealed adenovirus antigens. Subsequent typing of the brain isolate, by means of neutralization with type-specific antisera, confirmed that the isolate was adenovirus 5. Cultures of lung tissue obtained from the dead infant also were typed as adenovirus 5, although postmortem examination did not reveal any noticeable and significant changes in the lungs. No serum was available at autopsy for further testing.

Adenovirus crystal arrays of hexagonal profile, which consisted of alternately arranged electron-dense particles and regions of low electron density, were seen in the nuclei of infected tissue culture cells (figure 1A). Virions measured 81–85 nm in diameter, with electron-dense cores measuring 55–60 nm. The center-to-center distance between virus particles was 75–85 nm (figure 1B). Disruption of the nuclear membrane released virions (shown as scattered particles in the cytoplasm in figure 1A [arrows]). When negatively stained (2% sodium phosphotungstate, pH 7.0) virions obtained from a clarified cell homogenate were viewed, the viral cores were shown to be enclosed within a series of capsomeres (diameter, 7–8 nm) arranged as icosahedrons (figure 1C). Despite repeated attempts, no such particles could be detected in the brain tissue, presumably because of their scarcity.

According to the results of PCR, brain tissues were negative for Coxsackie virus, poliovirus, echovirus, herpes simplex virus types 1 and 2, cytomegalovirus, and varicella-zoster virus. However, positive PCR products of adenovirus antigen were detected in samples of the brain tissue and its A549 cell culture. Further analysis of the products, by means of nucleotide sequencing, revealed very similar hexon gene sequences in both cases (figure 2). To analyze the genetic diversity of the infant’s virus, we compared a 100-base region of the hexon gene with the sequences from wild-type adenovirus 2 genome [13] and adenovirus 5 hexon [14]. A remarkable similarity in the region was detected for the virus isolated from the infant and the reference wild-type strains (for comparison, see figure 2 for sequences with adenovirus genome and adenovirus hexon). However, the study did not establish that the virus replicated in the infant’s brain tissue. Therefore, the virologic, EM, and PCR data were compatible with a fatal case of edema of the brain caused by adenovirus 5 infection.
cations [2, 3]. Because there was no evidence of either pre-existing immunodeficiency or another viral pathogen present in the infant, we conclude that the fatal brain edema could have been caused by a generalized infection with adenovirus 5 via hematogenous spread of the virus from the portal of entry to the CNS.

There are 3 unusual aspects of this study. First, adenovirus hexon gene sequences were detected for the first time in the brain, allowing for fine genotypic mapping of the infant’s virus and for comparison of this virus with wild-type strains. This region of the gene encodes adenovirus DNA-binding protein, which is needed for viral replication [13, 14]. In the case presented, it is of note that a high degree of sequence similarity in the region indicates little genotypic diversity in the infant’s virus. In contrast, new genotypes of adenoviruses 31 and 49 have been isolated from brain [6]. Second, the serotype of adenovirus most frequently isolated from CNS disease is type 7 [1–3, 7]. To our knowledge, this is the first case of fatal adenovirus 5 infection in an infant, in which the virus was isolated by tissue culture from the infant’s brain and lung tissues. However, our study did not establish that the virus replicated in the brain tissue. Death occurred ~6 days after the onset of symptoms. Third, recovery of adenovirus from the brain is rare, and its visualization in the tissue is even rarer. Although we were unable to visualize virus particles in brain cells by use of EM, we were able to do so in infected tissue culture samples. The inability to visualize the particles in brain cells could have been due to the scarcity of the particles in the tissue. Alternatively, the virus could have been in a masked (in smudgy intranuclear inclusions) [9] or latent state in the brain [15] and therefore could have escaped detection.

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References