Influenza B Virus Encephalitis

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Acute encephalitis and postinfectious encephalopathy have been reported infrequently in association with influenza A and B virus infections. We report herein a case of a 6-year-old girl with acute influenza B virus encephalitis resulting in neurological sequelae. The diagnosis was made by isolation of influenza B virus from the nasopharynx, seroconversion to influenza B, and reverse transcription polymerase chain reaction (RT-PCR) identification of the virus from the patient's cerebrospinal fluid. Direct sequencing of viral RNA from the patient's nasopharynx and cerebrospinal fluid revealed identical nucleotide sequences in the HA1 region of the hemagglutinin gene. This is the first report of influenza B virus encephalitis diagnosed by use of RT-PCR and illustrates the need for increased awareness of influenza virus as a cause of acute encephalitis. PCR may be a useful tool for diagnosing future cases.

According to Osler, ''Almost every form of disease of the CNS may follow influenza'' [1]. Two forms of neurological disease occur most commonly, an acute encephalitis and a postinfectious encephalopathy. Whether these are distinct entities or simply points along a continuum of disease remains to be proven. Although the association remains controversial, the estimated 500,000 neurological deaths following the 1918 influenza pandemic are thought to represent postinfectious influenzal encephalopathy [1], an association described further in numerous case reports (reviewed in [2]). Acute encephalitis during influenza has been reported since 1890 [3], mainly in association with the epidemics of 1890, 1918, and 1957. Identification and diagnosis of sporadic cases have been more difficult.

Attempts to demonstrate the presence of virus in the CSF or brains of patients believed to have influenza virus encephalitis have met with limited success until recently. Isolation of influenza virus at autopsy from the brain of a patient with fatal encephalitis has been reported [4], as has isolation from the CSF of three patients [5]. Evidence of infection with influenza virus has been observed by electron microscopy of brain biopsy material (R.J. Whitley, personal communication), and detection of the viral RNA of influenza A virus by reverse transcription (RT) and PCR from the CSF has been reported [6, 7].

We report herein a case of influenza B virus encephalitis diagnosed by isolation of the virus from the nasopharynx, seroconversion to influenza B virus, and detection of the virus in the CSF by RT-PCR. Nucleotide sequencing of the HA1 region of the hemagglutinin gene of the virus isolated from the nasopharynx and from the sequences amplified from the CSF showed no differences in amino acid composition. This is the first case of influenza B virus encephalitis diagnosed by use of PCR.

Case Report

A 6-year-old girl was admitted on 9 May 1997 to the Le Bonheur Children's Medical Center (Memphis, TN) because of lethargy and fever. She had a 3-day history of elevated temperature, rhinorrhea, and cough and a 1-day history of somnolence and inability to vocalize. Her initial physical examination was remarkable: oral temperature of 39.9°C, lethargy, poor cooperation, and ataxia. Her neurological examination was nonfocal. She progressed from her initial stage of excessive somnolence to a period of delirium characterized by hallucinations, bizarre behavior, and an agitated, emotionally labile state. This slowly resolved over several days, leaving her in a state of akinetic mutism with few purposeful movements and refusal or inability to answer questions or follow commands. She was discharged in this condition 12 days after admission.

Her initial evaluation included examination of CSF obtained by lumbar puncture, which revealed the following: WBC count of 22/mm^3, with 96% lymphocytes and 4% monocytes; glucose level of 63 mg/dL; and protein level of 28 mg/dL. CT and MRI studies of her head revealed normal findings. The following studies for a cause of her encephalitis had negative results: serologic studies for antibody to arboviruses, *Bartonella* species, varicella zoster virus, and Epstein-Barr virus and bacterial, viral, and fungal cultures of her CSF. Testing by PCR for the presence of herpes simplex virus DNA in her CSF was done and had negative results (courtesy of F. Lakeman, University of Alabama at Birmingham).

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At presentation, she was negative for CF antibodies to influenza B virus (titer, <1:8). A convalescent serum sample, obtained 6 weeks after presentation, was positive at a titer of 1:64. A nasopharyngeal swab for culture of respiratory viruses yielded influenza B virus (B/Memphis/12/97); serotyping of the virus demonstrated a titer of 1:320 to B/Beijing/184/93-like reference serum and undetectable titer to B/Guandong/8/93-like serum. RT of RNA obtained from her CSF and PCR amplification of the hemagglutinin gene from cDNA demonstrated the presence of influenza B virus RNA in her CSF. Sequencing of the HA1 region of the hemagglutinin gene revealed no sequence differences between the RNA sequences from the CSF and virus isolated from the nasopharynx.

The patient has been followed as an outpatient by one of us (S. F.) for 1 year. Her akinetic mutism resolved over an ~1-month period, but she continues to have ataxia characterized by a broad-based gait and tendency to fall. Initial dysmetria on finger-to-nose testing has resolved. Although she was reportedly an exemplary student before her illness, the patient has had great difficulty in school this past year resulting in her being held back in the second grade. No formal intelligence testing has been done. Follow-up MRI scans have had normal results.

Methods

Virus. Virus was obtained by primary isolation in Madin-Darby canine kidney (MDCK) cells from throat swab material. Virus was passaged once in MDCK cells to obtain stock for experiments. Serotyping was done with use of 1996–1997 reference serum from the Centers for Disease Control and Prevention (CDC) [8].

Antibody titers. CF tests for antibody to influenza A and B viruses were done by Specialty Labs (Santa Monica, CA) by use of serum collected at the time of the patient's illness (acute) and 6 weeks after the initial sample was obtained (convalescent).

RT-PCR and sequence analysis. RNA extraction from virus-containing material was done by means of the RNAeasy Kit (Qiagen, Chatsworth, CA). RT and PCR amplification of genes of interest were done by standard methodologies. The PCR reaction used primers for HA1 of influenza B virus at nucleotide position 2 (5′-GCAGAAGCAGAGCATTTTCT-3′) and, on the complementary strand, at position 1025 (5′-TTTGCAGGAGGTCTATATTT-3′), resulting in a 1,023-bp product. After purification by use of a QIAquick PCR purification kit (Qiagen), PCR products were electrophoresed in a 2% agarose gel and stained with ethidium bromide. The RT-PCR products were sequenced by Taq Dye Terminator chemistry and analyzed on an ABI 373 DNA sequencer (Applied Biosystems, Foster City, CA).

Discussion

Encephalitis is a poorly recognized and infrequently reported clinical manifestation of influenza virus infection. Previously, it had been diagnosed only by seroconversion to the virus or by association of neurological symptoms with a compatible clinical illness during an influenza epidemic. In recent reports of several cases during outbreaks of influenza A in Hokkaido and Nagoya City (Japan), the investigators used RT-PCR on CSF samples to aid in the diagnosis [6, 7]. Specific diagnosis of influenza B virus encephalitis by culture or PCR of CSF has not been previously reported. Because a specific diagnosis in cases of viral encephalitis is difficult, it is uncertain how frequently influenza is manifested by neurological disease. In one review, 4 of 432 patients evaluated for suspected herpes simplex virus encephalitis had evidence of influenza A virus infection [9]. Influenza virus may be a more common cause of encephalitis than is suspected. Preliminary evidence from the Hokkaido reports on PCR diagnosis of influenza A virus encephalitis and this report of PCR diagnosis of influenza B virus encephalitis suggest that this modality may be useful in identifying influenza virus as the etiologic agent in cases of viral encephalitis.

The case reported herein has features typical of many reported cases of encephalitis during acute influenza. Influenza virus can cause a wide spectrum of neurological disease, including somnolence, coma, delirium, psychosis, behavioral disturbances, and oculogyric crisis. The patient's CSF is typically normal or shows mild pleocytosis. The brain at autopsy is often congested, with edema and nonspecific inflammatory changes, although cases with extensive necrosis have been reported [10]. Morbidity and mortality are high in reported cases, although this may reflect the virulence of the pandemic strains that have led to most of the reports. The patient reported herein had residual neurological and behavioral difficulties.

The mechanism(s) by which influenza virus invades the brain and the potential virulence factors of these viruses necessary for neurotropism in humans are not known. The virus may enter the bloodstream and gain access to the CNS by penetration of the blood-brain barrier, or it may invade by infection of the olfactory nerve and direct extension into the brain. The relative contributions of direct cellular damage from infection and pathologic inflammatory changes in response to infection are poorly understood for neurotropic viruses [11]. Encephalitis and postinfectious encephalopathy seem to be more common clinical manifestations of infection with pandemic viruses than with nonpandemic viruses. This observation may be due to specific virulence factors of these viruses, or it may be an artifact of recognition of influenza virus as the etiologic agent during a period of heightened awareness.

An increased awareness of influenza A and B viruses as causes of viral encephalitis is necessary, and studies to determine the incidence of influenza virus encephalitis, especially during yearly epidemics, should be undertaken. Since antiviral agents effective against influenza virus exist, early diagnosis and treatment may help to improve outcome. No instances of administration of antiviral therapy to patients with influenza virus encephalitis have been reported. The recent epidemic of

avian influenza in Hong Kong has led to renewed concerns about the possibility of a novel pandemic strain emerging [12], and history seems to indicate that we should be prepared for an increased incidence of neurological complications should this occur. Further study of the mechanisms of neurovirulence of influenza virus is needed. This report of a case of influenza B virus encephalitis illustrates the need for increased awareness of influenza virus as a cause of acute encephalitis and suggests that PCR may be a useful tool for diagnosing future cases.

References

1. Ravenholt RT, Foege WH. 1918 influenza, encephalitis lethargica, parkinsonism. Lancet **1982**;2:860–4.
2. Hayase Y, Tobita K. Influenza virus and neurological diseases. Psychiatr Clin Neurosci **1997**;51:181–4.
3. Leichtenstern MH. Mittheilungen über die Influenzaepidemic in Köln. Dtsch Med Wochenschr **1890**;16:212–5, 338–9.
4. Flewett TH, Hoult JG. Influenzal encephalopathy and postinfluenzal encephalitis. Lancet **1958**;2:11–5.
5. Thraenhart O, Schley G, Kuwert E. Isolation of influenza virus ''A/Hong Kong/1/68 (H3N2)'' from liquor cerebrospinalis of patients with CNS involvement [in German]. Med Klin **1975**;70:1910–4.
6. Togashi T, Matsuzono Y, Anakura M, Nerome K. Acute encephalitis and encephalopathy at the height of influenza in childhood [in Japanese]. Nippon Rinsho **1997**;55:2699–705.
7. Fujimoto S, Kobayashi M, Uemura O, et al. PCR on cerebrospinal fluid to show influenza-associated acute encephalopathy or encephalitis. Lancet **1998**;352:873–5.
8. World Health Organization Collaborating Center for Reference and Research on Influenza. Concepts and procedures for laboratory-based influenza surveillance, B-19. Atlanta: Centers for Disease Control, **1982**.
9. Whitley RJ, Cobbs CG, Alford CAJ, et al. Diseases that mimic herpes simplex encephalitis. Diagnosis, presentation, and outcome. NIAID Collaborative Antiviral Study Group. JAMA **1989**;262:234–9.
10. Mitzuguchi M, Abe J, Mikkaichi K, et al. Acute necrotising encephalopathy of childhood: a new syndrome presenting with multifocal, symmetric brain lesions. J Neurol Neurosurg Psychiatry **1995**;58:555–61.
11. Johnson RT. The pathogenesis of acute viral encephalitis and postinfectious encephalomyelitis. J Infect Dis **1987**;155:359–64.
12. De Jong JC, Claas EC, Osterhaus AD, Webster RG, Lim WL. A pandemic warning? [letter]. Nature **1997**;389:554.