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Identification of an Antigenic Subtype of Eastern Equine Encephalitis Virus Isolated from a Human

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Eastern equine encephalitis (EEE) virus was isolated from the cerebrospinal fluid of a 6-year-old male who had clinically diagnosed aseptic meningitis and subsequently died. Several standard serologic tests that use polyclonal antibody and indirect immunofluorescence and hemagglutination inhibition tests that use monoclonal antibody provided evidence that the isolate was an antigenic subtype of prototype North American EEE virus. We believe that this is the first evidence of an antigenic subtype of EEE virus.

Viruses in the family *Togaviridae*, genus *Alphavirus*, have been classified by comparison of antigenic characteristics (2). Membership in the serogroup is determined by any antigenic reactions between two viruses; if the viruses are closely related, they are considered members of the same antigenic complex. Classification of members of a given antigenic complex into categories that denote further relationships (species, subtype, and variety) is done by use of increasingly more discriminative tests (complement fixation, neutralization, and kinetic hemagglutination inhibition [HI]) or reagents (monoclonal antibody, infection-immune serum, and others).

Eastern equine encephalitis (EEE) virus is the sole representative of the EEE antigenic complex in the family *Togaviridae* (2). Although two antigenic varieties (North American and South American) have been distinguished (3), antigenic subtypes have not been identified. We report the identification of a subtype of EEE virus isolated from the cerebrospinal fluid of a human with central nervous system manifestations.

Virus isolate. On 20 September 1983, a 6-year-old male from Magee, Miss., 60 km southeast of Jackson, Miss., was admitted to a general hospital in Jackson with a temperature of 39.7°C for 3 days. On admission he was described as having seizures, headache, rhinitis, meningismus, and interstitial pneumonia. Although he was treated symptomatically and with antibiotics, his condition worsened and he died 2 days after admission. At autopsy, brain tissue and cerebrospinal fluid specimens were collected and shipped on dry ice to the Centers for Disease Control, Atlanta, Ga., where they were given the accession number 4789. The specimens were tested for the presence of virus by inoculation of primary monkey kidney cells and three cell lines: RD (human embryonal rhabdomyosarcoma), diploid human lung fibroblast, and Buffalo green monkey kidney. Sensitivity to chloroform was tested by a standard method (7).

Serologic tests. The average survival time of suckling (2- to 4-day-old) mice inoculated with virus passed in RD cells was 2 days. Fifth-passage (RD2 SM3) virus was used in three ways: to prepare antigens (6) for hemagglutination, HI, and complement fixation (4) tests; to infect cells for indirect immunofluorescence tests (10); and as stock virus (aliquots of clarified 10% suspensions in a medium containing 1% bovine albumin fraction V; stored at −70°C until used) for serum dilution-plaque reduction neutralization tests (9), which included labile serum factor (5). Hyperimmune mouse ascitic fluid (1) and a four-dose (weekly injections of virus, exsanguination 8 days after administration of the last dose) serum were prepared and used for certain tests; monoclonal antibody was used for others, as indicated below.

Monoclonal antibody (8) was prepared against the North American (strain NJ/60) subtype of EEE virus. This antibody (1B5C-3) had been certified to react in enzyme-linked immunosorbent assays, indirect immunofluorescence tests, and HI tests with only the North American subtype of EEE virus (A. R. Hunt and J. T. Roehrig, unpublished data).

Complete cytopathic effects were observed in RD cells within 2 days after inoculation with cerebrospinal fluid but not brain tissue; cytopathic effects were seen in RD cells but not in other cells. When initial efforts with antibodies to various enteroviruses failed to identify the isolate, it was passed to suckling mice by intracranial inoculation; third-passage (RD3) virus was found to be sensitive to chloroform. Vero cells subsequently were found to support the replication of the isolate, with cytopathic effects and plaques apparent within 2 days after inoculation.

Strain 4789 reacted in both complement fixation and HI tests with polyclonal antibody to EEE virus but not with antibodies to other alphatogaviruses (western equine encephalitis and Highlands J) or to other arboviruses from North America. In both immunofluorescence and HI tests, strain 4789 reacted with monoclonal antibody 1B5C-3 (North American EEE virus specific). When strain 4789 was tested by neutralization with viruses and with antibodies to EEE virus strain NJ/60, western equine encephalitis virus, and Highlands J virus, it was found to react only with antibody to EEE virus (Table 1). Antibody to strain 4789 (homologous titer, 1,280) had a titer of 10,240 with EEE virus strain NJ/60; antibody to EEE virus strain NJ/60 (homologous titer, 40,960) had a titer of 1,280 with strain 4789. Because both homologous and heterologous antibodies had lower titers with strain 4789 than with strain NJ/60, the test was repeated without labile serum factor and with a second anti-strain 4789 antibody preparation. However, the same one-direction difference (i.e., titer of antibody to EEE virus at least

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TABLE 1. Serum dilution-plaque reduction neutralization tests comparing strain 4789 with prototype EEE, western equine encephalitis (WEE), and Highlands J (HJ) viruses

Virus	Strain	Titer of antibody to[a]:			
		4789	EEE	WEE	HJ
EEE	4789	**1,280**	1,280	—	—
EEE	NJ/60	10,240	**40,960**		
WEE	Fleming	—		**2,560**	10
HJ	B-230	—		320	**320**

[a] Boldface type indicates homologous titers. —, Titer of <10. Blank, Not tested.

fourfold lower with strain 4789 than with strain NJ/60) was seen in both tests.

Until now, all isolates of EEE virus from North America had been shown to be essentially identical, distinguishable only from South American strains by kinetic HI tests (3). However, the repeatable fourfold, one-direction difference between EEE virus strains 4789 and NJ/60 indicates that strain 4789 is a subtype of the prototype EEE virus, an alphavirus subtype, as previously defined (2). These results provide the first evidence for the existence of a subtype of EEE virus. Whether such a subtype has epidemiologic significance or simply is an antigenic oddity remains to be determined. However, it is interesting that this isolate came from the brain of a dead person; few isolates of EEE virus have been tested as completely as this one was. Comparative sequencing of strain 4789 and of prototype North and South American strains of EEE virus might indicate the mechanism by which EEE viral RNA expresses both pathogenicity and glycoproteins.

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