

## Brief Report

LIFE-THREATENING CACHE VALLEY  
VIRUS INFECTION

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THE Bunyamwera serogroup (family Bunyaviridae, genus bunyavirus) contains more than 20 serologically cross-reactive viruses, 7 of which have been isolated in North America.<sup>1</sup> Cache Valley virus, first isolated in Utah in 1956,<sup>2</sup> has been recovered mainly from mosquitoes (genera *Culiseta*, *Aedes*, and *Anopheles*) and occasionally from vertebrates and has the widest apparent distribution among this serogroup of viruses. Antibodies against Cache Valley virus and other viruses of the Bunyamwera serogroup are prevalent in livestock, large wild mammals, and humans from Alaska to Argentina.<sup>3</sup> An association between Cache Valley virus infections and congenital malformations (various musculoskeletal and central nervous system defects) was described in sheep<sup>4</sup> and later experimentally reproduced in sheep<sup>5</sup> and cattle. Recent serologic studies suggest that the viruses of the Bunyamwera serogroup may also be etiologic agents of congenital defects of the central nervous system in humans.<sup>6</sup> Although antibodies against Cache Valley virus have been reported in humans, no acute infections or isolations of the virus have been reported, although a closely related virus was isolated from a febrile patient in Panama.<sup>7</sup> We describe the isolation of Cache Valley virus from a patient with severe encephalitis and multiorgan failure.

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## CASE REPORT

A 28-year-old, previously healthy man became ill on November 2, 1995, with myalgias, fever, chills, and headache. The following day, he began to vomit. On the third day of illness, he was admitted to a community hospital. His white-cell count was 5100 per cubic millimeter, with 76 percent segmented neutrophils and 13 percent band forms, and his platelet count was 91,000 per cubic millimeter. Studies of liver function were normal. A spinal tap disclosed clear cerebrospinal fluid containing 58 leukocytes per cubic millimeter (89 percent of which were segmented cells). Doxycycline and ceftriaxone were administered. Because of persistent fever, nausea and vomiting, and confusion, the patient was transferred to Duke University Medical Center on November 8, 1995. At the time of the transfer, a history of exposure to a dead mouse in his house was elicited. He also recalled having received numerous mosquito bites during a deer-hunting trip to southern North Carolina two weeks before the onset of his illness.

On the day of transfer, the patient appeared severely ill, with confusion, tachycardia, and fever and a maculopapular rash on his chest, face, and abdomen. The rash became vesiculopustular in some areas (Fig. 1). Other findings included bilateral conjunctivitis and meningismus. Laboratory studies showed thrombocytopenia (platelet count, 73,000 per cubic millimeter) and a normal white-cell count (6700 per cubic millimeter). The prothrombin time and partial-thromboplastin time were normal, but serum D-dimer concentrations were elevated (>1.25 mg per liter). Elevated serum concentrations of aspartate aminotransferase (269 U per liter) and alanine aminotransferase (189 U per liter) were found, but the serum alkaline phosphatase and bilirubin concentrations remained normal. Hypoxemia (partial pressure of arterial oxygen, 74 mm Hg) was present. The serum creatinine concentration was 1.2 mg per deciliter (106  $\mu$ mol per liter), and urinalysis showed proteinuria (3+), more than 2 white cells per high-power field, more than 2 red cells per high-power field, more than 4 granular casts per high-power field, and a specific gravity of 1.027. A chest radiograph revealed no abnormalities. Computed tomography of the head showed no focal parenchymal abnormalities or leptomeningeal enhancement with contrast medium.

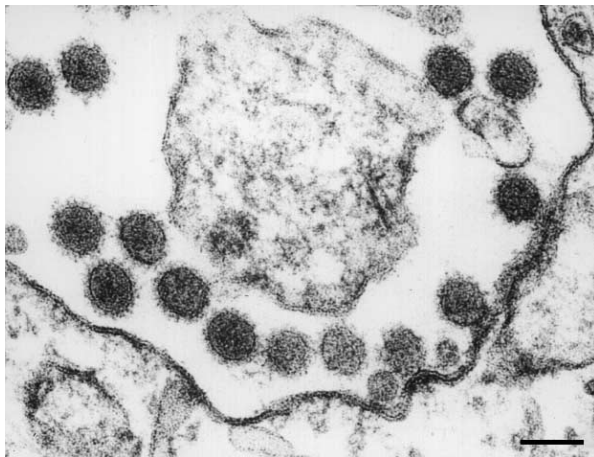
Ceftriaxone and doxycycline were continued. On November 9, the patient became hypotensive and delirious. Thereafter, his course was complicated by severe hypotension, requiring the use of multiple pressors; respiratory failure, requiring mechanical ventilation; seizures; and necrosis of the fingers and toes. Lumbar puncture disclosed clear cerebrospinal fluid with 40 leukocytes per cubic millimeter and a predominantly mononuclear-cell profile (92 percent mononuclear cells and 8 percent granulocytes; 8 erythrocytes per cubic millimeter). The protein concentration in cerebrospinal fluid was 154 mg per deciliter, and the glucose concentration was 49 mg per deciliter (2.7 mmol per liter); the serum glucose concentration was 129 mg per deciliter (7.2 mmol per liter). Aerosolized ribavirin and intravenous ampicillin were added to the treatment regimen, and continuous venovenous hemodialysis was required to manage acute renal failure.

Extensive muscle and cutaneous necrosis ultimately required the amputation of one leg. Microscopic examination of muscle tissue revealed only necrosis; specific immunohistochemical analysis was not performed. The serum creatine kinase concentration rose to a peak of 69,500 U per liter on November 13. Magnetic resonance imaging of the head showed a small focal area of enhancement in the right inferior posterior parietal region involving both the leptomeninges and the cortex. Intensive support was required for the next month, and after three months of hospitalization the patient was transferred to a rehabilitation facility. Three months after the onset of his illness, the patient remained unable to speak, required assistance in eating, and could not walk. The patient died of pulmonary complications in June 1996; no post-mortem examination was done.

During the course of his illness, numerous blood and urine cultures were negative for bacterial pathogens. Serologic studies for the presence of antibodies against *Rickettsia rickettsii*, *Ehrlichia*



**Figure 1.** Vesiculopustular Rash, Which Appeared 8 to 10 Days after the Onset of Illness.



**Figure 2.** Electron Micrograph of a Vero E6 Cell Infected with Cache Valley Virus, Showing an Accumulation of Viral Particles within a Smooth-Membrane Vesicle.

The particles are filled with a moderately dense, finely granular matrix and are surrounded by fine 8-nm surface projections. The bar represents 100 nm.

*chaffeensis*, *Francisella tularensis*, hantavirus, and human immunodeficiency virus were all negative.

## RESULTS

### Pathological Analysis

No viral inclusions were seen in a skin-biopsy specimen taken from a vesiculopustular lesion. The epidermis showed focal dyskeratosis with a scattering of necrotic keratinocytes, a mixed inflammatory-cell infiltrate, and intercellular edema with vacuoli-

zation, bulla formation, and separation mainly at the dermal-epidermal junction. The dermis showed a mild, superficial, perivascular mononuclear-cell infiltration and extensive basophilic elastosis. No Cache Valley viral antigens were detected by immunohistochemical analysis.

### Isolation of Virus

Attempts to isolate the causative agent were made at the College of Veterinary Medicine, North Carolina State University, from blood collected on November 9. The blood was cultured with two cell lines (Vero cells and human endothelial cells, HEC-X). A cytopathic effect, consisting of rounding and detachment of virtually the entire cell monolayer of the Vero cells, was noticed within two to three days. The virus was again isolated at the Special Pathogens Branch laboratory of the Centers for Disease Control and Prevention from blood and serum specimens collected on November 9 and from cerebrospinal fluid, but not blood, collected on November 11. Thin-section electron microscopy of infected Vero E6 cells revealed numerous intracellular and extracellular viral particles. Consistent with the family Bunyviridae, the enveloped particles were observed individually and in groups within intracellular vacuoles of smooth cytoplasmic membranes and were mostly round, with an average diameter of 70 nm (range, 55 to 84) (Fig. 2).

Crude inactivated viral antigens were made by extracting infected cells and mock-infected cells with detergent and then irradiating them. The serogroup was identified by an enzyme-linked immunosorbent assay (ELISA) with the use of hyperimmune-mouse-group ascitic fluid reactive with various serogroups in the family Bunyviridae. Inactivated viral antigen and control antigens were adsorbed overnight at 4°C onto 96-well microplates. The various mouse ascitic fluids were allowed to react with the antigen, and bound antibodies were measured with the appropriate antimouse enzyme conjugate and substrate. The only positive reaction observed was with the Bunyamwera-serogroup ascitic fluid. Virus-specific ascitic fluids from this serogroup were then used in the same ELISA to narrow the identification. Positive results were observed with the immune ascitic fluids from all known North American Bunyamwera-serogroup viruses except Main Drain (Cache Valley, Lokern, Northway, Santa Rosa, Tensasaw, and Tlacotalpan).

After the isolate was recognized as a virus of the Bunyamwera serogroup, it was identified genetically as Cache Valley virus. The small genomic RNA segment of the virus was amplified by a reverse transcription-polymerase chain reaction as previously described,<sup>8</sup> and the amplified DNA product was cloned into the TA cloning vector pCRII (Invitrogen). Multiple clones were sequenced by the dye-

termination cycle-sequencing technique with an automated DNA sequencer (model 373A, Applied Biosystems). The derived sequence (920 nucleotides) was 99.3 percent homologous to the sequence previously reported for the small genomic RNA of Cache Valley virus and less than 87 percent homologous to the small genomic RNA sequences of other viruses of the Bunyamwera serogroup.<sup>8</sup>

Experimental IgG and IgM ELISAs were developed with standard techniques with antigens made from the viral isolate.<sup>9</sup> IgM was detectable in serum collected from November 29, 1995, to February 5, 1996 (the last sample), and was present in cerebrospinal fluid collected December 1, 1995. The serum IgG titer began to rise 20 days after the onset of illness and was strongly positive in the last sample, obtained on February 5, 1996. No IgG was detectable in the cerebrospinal fluid sample obtained on December 1, 1995.

### DISCUSSION

We describe disease caused by Cache Valley virus in a human. Viremia was detected seven days after the onset of fever, an unusually long period of viremia in the case of most Bunyaviridae infections, and the virus had clearly entered the central nervous system, as evidenced by isolation from cerebrospinal fluid on day 9 and the presence of IgM. This patient had severe disease with substantial functional sequelae. The pathogenesis of Bunyaviridae infections in rodents usually suggests direct viral damage as the chief mechanism, and the strain we isolated induced an obvious cytopathic effect in cell culture. In the absence of any direct evidence *in vivo*, however, a lytic effect of the virus cannot be assumed. We have no data that would allow us to attribute the neurologic lesions in our patient and his subsequent neurologic sequelae to direct lysis of neuronal cells. Lysis of virus-infected neurons by cytotoxic T lymphocytes is unlikely, since these cells have insufficient expression of class I and II major-histocompatibility-complex antigens.<sup>10</sup> But there are other possibilities, such as the release of mediators after interactions between T cells and infected dendritic cells. Furthermore, activation of the fibrinolytic system and the consequent marked circulatory failure can readily account for the patient's multiorgan failure.

*In utero* infection with Cache Valley virus caused congenital defects (ovine arthrogryposis with hydro-

cephalus or hydranencephaly) in sheep consisting of areas of cell necrosis and loss of the paraventricular neuropil and motor neurons in the central nervous system, along with myositis, with poorly developed myotubular myocytes in skeletal muscles.<sup>4</sup> Retrospective serologic studies in humans suggest that Cache Valley virus may be the cause of some cases of congenital macrocephaly.<sup>6</sup> No pathological or immunopathological data are available on the actual cell tropisms of Cache Valley virus and the types of lesions observed during acute human infection.

The previously known distribution of Cache Valley virus did not include North Carolina, but isolates of the virus have been recovered from mosquitoes trapped in Tennessee and Virginia.<sup>3</sup> Because of the widespread distribution of Cache Valley virus and other viruses of the Bunyamwera serogroup in the United States, more extensive studies should be undertaken to determine the role of these viruses in cases of otherwise unexplained human viral encephalitis, severe multiorgan failure, and congenital anomalies.

*We are indebted to Barbara C. Hegerty, who first recognized the cytopathic effect in tissue cultures of the patient's clinical specimens.*

### REFERENCES

1. Gonzalez-Scarano F, Nathanson N. Bunyaviridae. In: Fields BN, Knipe DM, Howley PM, eds. *Fields virology*. 3rd ed. Vol. 1. Philadelphia: Lippincott-Raven, 1996:1473-504.
2. Holden P, Hess AD. Cache Valley virus, a previously undescribed mosquito-borne agent. *Science* 1959;130:1187-8.
3. Calisher CH, Franci DB, Smith GC, et al. Distribution of Bunyamwera serogroup viruses in North America, 1956-1984. *Am J Trop Med Hyg* 1986;35:429-43.
4. Edwards JF, Livingston CW, Chung SI, Collisson EC. Ovine arthrogryposis and central nervous system malformations associated with *in utero* Cache Valley virus infection: spontaneous disease. *Vet Pathol* 1989;26:33-9.
5. Chung SI, Livingston CW Jr, Edwards JF, Gauer BB, Collisson EW. Congenital malformations in sheep resulting from *in utero* inoculation of Cache Valley virus. *Am J Vet Res* 1990;51:1645-8.
6. Calisher CH, Sever JL. Are North American Bunyamwera serogroup viruses etiologic agents of human congenital defects of the central nervous system? *Emerg Infect Dis* 1995;1:147-51.
7. Mangiafico JA, Sanchez JL, Figueiredo LT, LeDuc JW, Peters CJ. Isolation of a newly recognized Bunyamwera serogroup virus from a febrile human in Panama. *Am J Trop Med Hyg* 1988;39:593-6.
8. Dunn EF, Pritlove DC, Elliott RM. The S RNA genome segments of Batai, Cache Valley, Guaroa, Kairi, Lumbo, Main Drain and Northway bunyaviruses: sequence determination and analysis. *J Gen Virol* 1994;75: 597-608.
9. Ksiazek TG, Peters CJ, Rollin PE, et al. Identification of a new North American hantavirus that causes acute pulmonary insufficiency. *Am J Trop Med Hyg* 1995;52:117-23.
10. Griffin DE. Arboviruses and the central nervous system. *Springer Sem in Immunopathol* 1995;17:121-32.