Encephalitis without Meningitis
Due to Sandfly Fever Virus Serotype Toscana

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The role of Toscana (TOS) virus in producing encephalitis without meningitis is uncertain. We studied 2 cases of TOS virus encephalitis without meningitis by means of nested polymerase chain reaction assay and DNA sequencing. Findings confirm that TOS virus may directly cause encephalitis and suggest the usefulness of DNA sequencing in investigating relationships between TOS virus molecular patterns and the spectrum of neurological involvement.

Sandfly fever virus serotype Toscana (family Bunyaviridae, genus Phlebovirus) is the only sandfly-transmitted virus responsible for acute neurological disease [1–3]. Toscana (TOS) virus is an enveloped virion with a segmented negative-strand RNA genome consisting of 3 noncovalent closed, circular RNA species [4–6]. Antigenic, biological, and immunologic characterization of TOS virus, as well as humoral response elicited in acute disease, have been studied extensively [2, 7, 8]. TOS virus first was isolated from Phlebotomus perniciosus in Italy and is endemic in Mediterranean countries where the phlebotomus vectors (P. perniciosus and P. perfilievi) are present [1, 9]. This infection has been reported in residents of and in visitors returning from endemic areas, mainly central Italy, Portugal, Spain, and Cyprus [1, 10–13]. In central Italy, the infection occurs during the summer, reaching a peak in August, along with the maximum activity of the sandfly vectors [14–16]. Several methods have been developed for the diagnosis, including nested PCR (nPCR) assay and EIA, along with recombinant viral nucleoprotein EIA (rEIA) [17–19]. Symptoms such as fever, myalgia, severe frontal headache, vomiting, ocular pain, and neck rigidity have been reported in association with aseptic meningitis or, less frequently, meningoencephalitis related to TOS virus infection [3, 14, 15]. In addition, asymptomatic infections and infections without CNS involvement have been described elsewhere [20].

To our knowledge, there are only 5 cases of TOS virus encephalitis without meningitis mentioned in the available literature [14, 15]. Studies are required to clarify the role of TOS virus in this setting. Recently, 4 variants of TOS virus have been demonstrated by DNA sequencing in cases of acute meningitis [21]. These findings raise many questions about the role played by molecular variability in the protean pathomorphosis of TOS virus infection. We report 2 cases of TOS virus encephalitis without meningitis that were studied by use of nPCR assay and DNA sequencing.

CASE REPORTS

Patient 1. In September 1999, a 32-year-old Italian man was referred to the Pistoia Hospital because of a 1-day history of fever (38°C) with headache, vomiting, and photophobia. He was a resident of an area (Quarrata, near Pistoia, Italy) where sandflies are seasonally active. Examination indicated that the patient appeared to be alert, without any sign of focal neurological defects or meningeal irritation. Pupillar reflexes were normal. His pulse rate and blood pressure were 60 beats/min and 120/80 mm Hg, respectively.

Chest radiograph, electrocardiogram, blood cell count, and routine biochemical test results were normal. No brain lesions were evidenced by CT scan and MRI scan with gadolinium enhancement. Analysis of fluid taken via lumbar puncture revealed clear CSF with 160 lymphocytes/mm³, 98 mg protein/dL, 120 mEq chloride/L, and a normal glucose level (62 mg/dL, with a blood level of 102 mg/dL). An electroencephalogram (EEG) showed diffuse slow spikes with emphasis of the parietal and temporal right lobe. Treatment was begun with orally administered doxycycline and parenterally administered acyclovir. On day 3 after admission, the patient’s fever disappeared. After that, the patient rapidly improved, although he still complained of headache during the next 2 weeks. He was discharged on day 24 after clinical onset; he displayed no neurological symptoms and had an ameliorated EEG. At a

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follow-up visit 1 month later, he was asymptomatic, and his EEG showed further improvement.

**Patient 2.** In August 1999, a 45-year-old Italian man with a 3-day history of mild pharyngodynia and subcontinuous fever (range, 38.5–39°C) was hospitalized because of bitemporal and frontal headache that had developed abruptly, along with vomiting and somnolence. He was a horseman and lived in a region (Fucecchio, near Pistoia, Italy) where sandflies were present and where a West Nile epidemic in horses was discovered recently (authors’ unpublished data).

Physical examination revealed the patient to be slightly lethargic without focal neurological signs. Neither neck rigidity nor other signs of meningeal irritation were present. Pupillary reflexes were normal. His temperature was 38°C, and his heart rate and blood pressure were 60 beats/min and 135/85 mm Hg, respectively.

Analysis of chest radiograph and CT scan of the brain indicated no abnormalities. Analysis of fluid taken via lumbar puncture showed clear CSF with 34 lymphocytes/mm³, 98 mg protein/dL, and a normal glucose level (67 mg/dL, with a blood level of 119 mg/dL). Electrocardiogram, blood cultures, blood cell count, and routine biochemical test results were normal. EEG showed diffuse slow waves. Treatment was started with administration of doxycycline and acyclovir therapy. By day 3 after admission, both somnolence and headache improved. The patient was discharged, without neurological symptoms and with a normal EEG, on day 11 after the clinical onset of disease.

**RESULTS**

Antibody titers measured in CSF and serum samples by standard laboratory methods ruled out infections caused by *Herpes simplex* viruses types 1 and 2, cytomegalovirus, Epstein-Barr virus, mumps virus, adenovirus, Coxsackie and Echo viruses, *Listeria monocytogenes*, *Borrelia* species, *Leptospira* species, *Rickettsia* species, *Chlamydia* species, *Salmonella* species, *Brucella* species, *Treponema pallidum*, *Toxoplasma* species, and *Mycoplasma pneumoniae*. Direct examination and culture of the CSF were negative for bacteria and mycobacteria. Throat swabs for bacteria and herpesviruses were similarly negative. Separate laboratories carried out the subsequent diagnostic procedures for each patient.

**Patient 1.** A search for viral agents was done at the Department of Molecular Biology, Microbiology Section, University of Siena, in CSF and serum samples obtained at admission. RNA was extracted from 200 μL of the CSF sample by use of the procedure described by Chomczynsky and Sacchi [22]. DNA extraction was done on the CSF specimen by use of the QIamp kit (Qiagen). PCR assay was done according to the recommended guidelines [23]. The CSF sample tested negative for all the DNA neurotropic viruses considered (*H. simplex* virus types 1, 2, and 6; Epstein-Barr virus; cytomegalovirus; varicella-zoster virus; and parvovirus B19) and for *M. pneumoniae* and *Chlamydia pneumoniae* DNA. By reverse transcriptase (RT)–PCR assay, the RNA extracted was tested for enteroviruses, tickborne encephalitis virus, TOS virus, West Nile virus, measles virus, and mumps virus. It was possible to detect the TOS virus genome by the TOS-specific primers, as described elsewhere [17]. The patient’s serum was positive for TOS virus IgG and IgM by EIA (Enzywell TOS virus IgG/IgM, Diesse). The serological test was not done on the CSF sample.

To identify the TOS virus variant involved in the infection, the PCR product was purified by use of the QIA-quick spin PCR purification kit (Qiagen) and was sequenced by dideoxy chain termination with use of a Sequenase kit (USB Corporation) by using sense and antisense primers to sequence both strands [21]. The sequence was compared with the TOS reference sequence of the ISS Phleb.3 strain and with the 4 variants of TOS virus circulating in Tuscany [6, 21]. The variant SI 4 strain of the wild-type TOS virus was used as a positive control in all the RT-PCR and sequencing assays [21]. RNA extracted from mock-infected cells was used as a negative control. Comparison of sequences confirmed that the PCR product obtained referred to a strain of TOS virus that was very similar to the SI 2 variant, except for the change at position 473 (A→G). This point mutation did not result in amino acid changes.

**Patient 2.** CSF and serum samples taken at admission were sent to the Laboratory of Virology, Arbovirus Unit, Istituto Superiore di Sanità, Rome, for testing for TOS and West Nile viruses. Results were negative for West Nile infection both in serum (hemagglutination inhibition and EIA) and in CSF (virus isolation procedures and RT-PCR assay). Serum and CSF samples were positive, however, for TOS virus IgG and IgM by rEIA [19]. A 200-μL volume of the CSF sample was used for RNA extraction, and the presence of TOS virus genome was confirmed by an RT-nPCR assay [17, 22]. DNA sequencing was done as described for the first patient [21]. Sequences of PCR product obtained from CSF, compared with the TOS reference sequence of the ISS Phleb.3 strain, showed 2 nucleotide changes with C→T at position 359 and G→T at position 374 [6]. These point mutations did not result in amino acid changes.

**DISCUSSION**

TOS virus is the most common viral agent involved in acute CNS infections in both children and young adults in central Italy [14–16]. To our knowledge, no detailed reports of TOS virus encephalitis without meningitis are currently available. Two cases of TOS virus encephalitis without meningitis have been reported here, and the virus was found in CSF by use of
an nPCR assay. No other pathogens were detected, and no brain lesions were found by means of CT and MRI scans. Therefore, we believe that encephalitis was caused directly by TOS virus invasion of the CNS. Both patients recovered without neurological sequelae, although headache and EEG alterations lasted in 1 patient. In this patient, the DNA sequence of the ampiclon detected by RT-nPCR assay confirmed the presence of a wild-type strain of TOS virus in the sample. The nucleotide analysis revealed a high degree of similarity with the SI.2 variant of the ISS Phleb.3 strain, which has been in Tuscany since 1995, except for retromutation at the position 473 (A→G) of the studied sequence [21]. In the other patient, a close similarity of TOS virus strain in the sample with the ISS Phleb.3 strain itself was found by use of nucleotide analysis [6].

The evidence arising from this study may improve knowledge of the natural history of TOS virus infection. Indeed, it confirms that TOS virus must be considered among the direct causes of encephalitis without meningitis, although nothing is known to explain the sporadic occurrence of this entity during the course of the infection. To this end, DNA sequencing would be done to investigate relationships between the molecular patterns of TOS virus and the spectrum of CNS involvement [21]. This approach enabled us to establish a direct association between defined strains of TOS virus and 2 cases of benign encephalitis without meningitis. These results provide further information into this emerging manifestation of the infection.

Studies are in progress to characterize these TOS virus strains circulating in Tuscany, Italy, during summer 1999 and to identify potential epitopes involved in the development of encephalitis without meningitis.

References