MAJOR ARTICLE

Adenovirus Type 21–Associated Acute Flaccid Paralysis during an Outbreak of Hand-Foot-and-Mouth Disease in Sarawak, Malaysia

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We report the virological and clinical features of 8 children who presented with adenovirus-associated acute flaccid paralysis (AFP) during an epidemic of enterovirus type 71 (EV71)–associated hand-foot-and-mouth disease (HFMD) in Sarawak, Malaysia, in 1997. Neutralization tests and phylogenetic analysis revealed adenovirus type 21 (Ad21), although DNA restriction digests suggested that this virus was different from the prototype Ad21. Four children had upper-limb monoparesis, 2 had lower-limb monoparesis (one of whom had changes in the anterior spinal cord noted on magnetic resonance imaging), and 2 had flaccid paraparesis. At follow-up, 4 children were noted to have made full recoveries and 3 had residual flaccid weakness and wasting. Neurophysiological investigation revealed a mixture of axonal and demyelinating features in motor and sensory nerves, with denervation. These findings suggest that Ad21 might cause AFP by anterior horn cell damage or neuropathy of the brachial or lumbosacral plexus. The occurrence of these unusual adenovirus infections during an outbreak of EV71-associated HFMD suggests that an interaction between the 2 viruses may have occurred.

With the decrease in the number of cases of polio in the tropics, attention has moved to other causes of acute flaccid paralysis (AFP). In 1997, there was an unexpected increase in the number of children with AFP who presented to Sibu Hospital (Sarawak, Malaysia). The cases occurred during an outbreak of hand-foot-and mouth disease (HFMD) across Sarawak, and they coincided with an unexplained cluster of children with acute fatal myocardial dysfunction [1]. Although the outbreak of HFMD was clearly caused by enterovirus type 71 (EV71), obtaining a consensus on the cause of the cases involving paralysis and fatal cardiac cases has proven to be difficult [2, 3]. The clinical and pathological characteristics of the fatal cardiac cases have been described in detail elsewhere [4]. Here, we focus on the cases involving paralysis.

Although EV71 is known to cause polio-like flaccid paralysis, virological and epidemiological investigations conducted during this outbreak of disease showed that the virus that was most closely associated with the cases of AFP and myocarditis at Sibu Hospital was a species B human adenovirus [1]. As has been described else-

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where, this virus was found in CSF and/or serum samples obtained from 10 (63%) of 16 patients with fatal cases and from 5 (63%) of 8 patients with paralysis [1]. In contrast, EV71 was found in only 3 (19%) of the patients with fatal cases (from samples of nonsterile sites) and in none of the patients with paralysis. The adenovirus was isolated after multiple passages of clinical material in human pulmonary adenocarcinoma (A549) cells. The results of preliminary immunofluorescence tests were weakly positive for adenovirus hexon, and sequencing of the hexon gene showed similarity to species B human adenoviruses [1].

Adenoviruses are best known as causes of respiratory, diarrheal, and other simple febrile illnesses, but species B adenoviruses can be associated with more severe disease, including severe pneumonia, aseptic meningitis, encephalitis, and transverse myelitis [5]. However, adenoviruses have not been associated with AFP previously. Here, we report the further characterization of the species B adenovirus that we isolated as adenovirus type 21 (Ad21), and we describe the clinical presentations, the findings at follow-up, and the results of neurophysiological studies for patients with AFP. Our data suggest that Ad21 may have caused paralysis by directly attacking the anterior horn cells, or it may have done so indirectly by causing post- or parainfectious brachial or lumbosacral plexus neuropathy.

MATERIALS AND METHODS

During the HFMD outbreak in 1997, children with AFP were evaluated by viral culture of serum, throat swab, rectal swab, and CSF specimens, as described elsewhere [1]. PCR and RT-PCR were used to identify adenoviruses and enteroviruses, and direct immunofluorescence was used to identify adenoviruses and herpes simplex viruses. In addition, serological tests were performed for detection of Japanese encephalitis virus and dengue viruses [6]. Adenovirus isolates were further characterized by neutralization tests with use of reference antisera and prototype adenovirus strains [7], by sequencing part of the hexon gene and the complete fiber gene, and by restriction digest analysis with enzymes *Bam*HI, *Kpn*I, and *Sma*I, with use of standard methods.

In 1999, when electrophysiological facilities were made available, patients were observed with nerve conduction studies (on at least 1 nerve in the affected limb, and more, if possible) and electromyography with use of Medelec Synergy (Oxford Instruments), as described elsewhere [8]. Standard criteria were used to distinguish demyelinating from axonal/anterior horn cell disease [9]. Serum samples were obtained for assessment of IgG, IgA, and IgM anti-ganglioside antibodies to GM1, GA1, GD1a, GD1b, and GQ1b by ELISA with use of standard techniques [10].

The study was approved by the Director of Health for Sarawak (Malaysia) and the Ethics Committee of the Liverpool School of Tropical Medicine (Liverpool, England). Informed consent was obtained from each child's accompanying parent or guardian.

RESULTS

During the outbreak, 8 children (age range, 4–19 months) presented to Sibu Hospital with AFP (table 1). Adenoviruses were isolated from A549 cells obtained from 5 patients (table 2 and figure 1). There was no evidence of infection with enteroviruses, Japanese encephalitis virus, or any other viruses. In addition, 2 consecutive stool samples obtained from patients with AFP were submitted to the Malaysian World Health Organization Poliovirus Laboratory (Institute of Medical Research, Kuala Lumpur) and to $\geq$1 other independent laboratory, but none of the laboratories found evidence of any enterovirus infections. Adenovirus isolates (MY7/1 and MY8/1) recovered from the serum samples of the first 2 patients were serially passaged until grown to sufficient titer for further analysis, as was an isolate recovered from the CSF specimen of a patient who had a fatal cardiac case (SIBU97) [1].

Neutralization tests identified all 3 adenoviruses as Ad21 and showed they were closely related to adenovirus types 50 (Ad50) and 11 (Ad11; table 3). Restriction digests of genomic DNA extracted from the 3 isolates showed that they were identical to each other, but they differed from the prototype Ad21 profile and other published profiles for all 3 restriction enzymes (*Bam*HI, *Sma*I, and *Kpn*I; figure 2) [11, 12]. Sequencing of part of the hexon gene confirmed that the 3 isolates were adenoviruses and were closely related to each other and to the prototype Ad21 (figure 3). An alignment of the amino acid sequence of the fiber protein showed that our Ad21 strains differed from the prototype in the fiber knob structure that interacts with the cell-surface receptor, but they had the same 6–amino acid motif (AATSSK) as the recently identified Ad50 (figure 4) [7].

All children were up-to-date with their immunizations, including vaccination for polio, and all had normal developmental milestones. All developed neurological disease after a brief viral prodrome. Two children had lesions characteristic of HFMD; one other had suggestive lesions, and 2 others had a macular rash. Four children had upper-limb monoparesis; one of these children had lower cranial nerve involvement. Lower-limb monoparesis occurred in 2 children, one of whom had increased signal in the anterior horn of the spinal cord on T2-weighted MRI (figure 5). Two children had flaccid para-