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-
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 lumbosacular 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 ≥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-
Seven patients returned for follow-up and neurophysiological investigation 26–29 months after discharge from the hospital. Four had made a full recovery, but 3 had marked flaccid weakness and wasting in 1 limb. The 3 children who experienced wasting had electrophysiological changes characteristic of chronic denervation (i.e., reduced motor-unit recruitment and large-amplitude polyphasic units) and markedly reduced compound muscle action potentials, consistent with axonal or and chronic denervation noted on EMG.

**Patient 1.** A 12-month-old Chinese girl presented with a 5-day history of fever and watery diarrhea and a 2-day history of reduced movement of the right leg. During examination, she was febrile and lethargic, and there were no rashes. Her right leg was flaccid and had a Medical Research Council (MRC) power grade of 0/5 in all groups. The findings of the examination were otherwise normal. The findings of laboratory investigations are shown in table 2. Power gradually improved in the right leg to 3/5 at the time of discharge (day 8 of hospitalization), but the patient was still unable to walk. T2-weighted MRI on day 24 of illness revealed a high-signal intensity lesion extending from T10 to L1 on the anterior right side of the spinal cord (figure 5). At follow-up 2 years later, the patient had a wasted right thigh and lower leg, with loss of the medial arch of the foot. Her knee jerk was reduced on that side. She could walk independently but with a right-foot drop.

**Patient 2.** After 3 days of fever, this 8-month-old Iban boy stopped using his left hand and developed noisy breathing. He had a macular rash over his left shoulder and audible wheezing, but he was otherwise healthy. His left arm was flaccid and areflexic, with no movement, and his condition remained unchanged at discharge from the hospital 10 days later. He was treated with antibiotics for pneumonia, but the results of culture of a blood sample obtained at the time of admission were negative. At follow-up, the findings of an examination were entirely normal.

### Table 1. Clinical features of children with acute flaccid paralysis (AFP).

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex, age in months</th>
<th>Initial presentation</th>
<th>Follow-up finding(s)</th>
<th>Neurophysiological finding(s)</th>
<th>Anti-ganglioside antibody finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F, 12</td>
<td>Fever, diarrhea, left-leg AFP; increased signal on spinal MRI</td>
<td>Weak, wasted right leg</td>
<td>Reduced right posterior tibial motor amplitude (100 mV); healthy right common peroneal, left tibial, and right and left sural nerves; chronic denervation of right tibialis anterior (giant units up to 5 mV) with a reduced interference pattern noted on EMG</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>M, 8</td>
<td>Fever, rash, pneumonia, left-arm AFP</td>
<td>Full recovery</td>
<td>Reduced motor velocity (41 m/s; normal, &gt;45 m/s) and reduced sensory velocity (30.8 m/s; normal, &gt;50 m/s) in left median nerve; healthy left ulnar nerve; normal study findings for right arm and both legs; findings of EMG of left brachials were normal</td>
<td>Negative</td>
</tr>
<tr>
<td>3</td>
<td>M, 11</td>
<td>Fever, seizures, right-arm AFP</td>
<td>Improving at discharge</td>
<td>Did not return for follow-up</td>
<td>—</td>
</tr>
<tr>
<td>4</td>
<td>F, 9</td>
<td>Fever, macular rash, left-arm AFP</td>
<td>Full recovery</td>
<td>Reduced left median nerve motor velocity (37.5 m/s) with normal distal latency and amplitude</td>
<td>Positive for GD1A</td>
</tr>
<tr>
<td>5</td>
<td>M, 14</td>
<td>Fever, HFMD, left-leg weakness</td>
<td>Full recovery</td>
<td>Reduced velocity of left sural (sensory) nerve (42.9 m/s; normal, &gt;44 m/s); normal motor conduction in left posterior tibia; normal findings of sensory and motor studies of right leg and left arm</td>
<td>Weakly positive for GM1</td>
</tr>
<tr>
<td>6</td>
<td>M, 19</td>
<td>Fever, constipation, AFP in the right followed by left leg</td>
<td>Wasted right buttock, mild foot drop</td>
<td>Reduced right tibial motor amplitude compared with left tibia (8.4 vs. 17.4 mV) with velocity of 34.7 m/s; healthy right sural, right common peroneal, median (sensory and motor), and ulnar (sensory and motor) nerves; chronic denervation noted on EMG of right vastus lateralis</td>
<td>Negative</td>
</tr>
<tr>
<td>7</td>
<td>F, 14</td>
<td>Fever, HFMD, AFP in both legs, seizures</td>
<td>Full recovery</td>
<td>Slightly reduced left tibial motor velocity; normal right tibial motor velocity; healthy sural nerves</td>
<td>Negative</td>
</tr>
<tr>
<td>8</td>
<td>M, 4</td>
<td>Mouth ulcers, rash on arms and soles, left-then right-arm AFP followed by lower cranial nerve palsies</td>
<td>Wasting and flaccid weakness in left deltoid, biceps, and triceps</td>
<td>Reduced motor and sensory velocities in left median nerve (38 and 36 m/s; normal, &gt;49 m/s) and reduced amplitudes (200 and 41.4 mV); normal distal latencies; healthy left ulnar nerve; chronic partial denervation of left deltoid and brachialis noted on EMG</td>
<td>Weakly positive for GM1</td>
</tr>
</tbody>
</table>

**NOTE.** EMG, electromyography; HFMD, hand-foot-and-mouth disease.
Table 2. Findings of laboratory investigations for children with acute flaccid paralysis.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Blood sample</th>
<th>CSF sample</th>
<th>Bacterial microscopy and culture result</th>
<th>Virological test result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WBC count, cells × 10^9/L</td>
<td>Neutrophil differential, %</td>
<td>Lymphocyte differential, %</td>
<td>WBC count, cells/mL</td>
</tr>
<tr>
<td>1</td>
<td>16.1</td>
<td>62</td>
<td>35</td>
<td>280</td>
</tr>
<tr>
<td>2</td>
<td>19.6</td>
<td>29</td>
<td>71</td>
<td>17</td>
</tr>
<tr>
<td>3</td>
<td>14.5</td>
<td>75</td>
<td>21</td>
<td>205</td>
</tr>
<tr>
<td>4</td>
<td>14.4</td>
<td>31</td>
<td>65</td>
<td>152</td>
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<tr>
<td>5</td>
<td>17</td>
<td>70</td>
<td>30</td>
<td>188</td>
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<tr>
<td>6</td>
<td>19.7</td>
<td>30</td>
<td>61</td>
<td>10</td>
</tr>
<tr>
<td>7</td>
<td>9.9</td>
<td>64</td>
<td>34</td>
<td>72</td>
</tr>
<tr>
<td>8</td>
<td>19.8</td>
<td>72</td>
<td>28</td>
<td>161</td>
</tr>
</tbody>
</table>

**NOTE.** Ad21, adenovirus type 21; ^a, 5–10 RBCs per high-power field; ^b, 11–20 RBCs per high-power field; —, negative.

a Serum sample.

b CSF specimen.

**Patient 3.** One week after experiencing a mild upper respiratory tract infection, this 11-month-old boy had another febrile illness involving 2 brief, generalized tonic-clonic seizures. After the second seizure, the patient stopped using his right arm. Examination revealed tachypnea and a flaccid, hyporeflexic right arm with just a flicker of movement in the fingers. He was treated with antibiotics. The MRC power grade in the right arm improved and was 2/5 when he was discharged from the hospital 10 days later.

**Patient 4.** This 9-month-old girl had a brief history of high fever and rash, followed by reduced movement in her left arm. She had a diffuse macular rash over the trunk, but there were no oral ulcers or palm or sole lesions. She was treated with IVIG, and her condition remained unchanged. At follow-up, however, her arm was completely healthy.

**Patient 5.** After 2 days of fever, this 14-month-old Iban boy developed oral ulcers, vesicular lesions over his palms and soles, recurrent vomiting, and drooling of saliva. He looked lethargic and irritable, and he had cold extremities and slow capillary return, but the findings of a neurological examination were normal. His heart rate, blood pressure, and echocardiogram findings were normal. He was treated with IVIG and antibiotics. On the third day of hospitalization, his fever resolved, but he developed a flaccid, hyporeflexic left leg, with no movement except for a flickering movement of the toes. At discharge from the hospital on day 7, the MRC power grade

**Figure 1.** Cytopathic effect in A549 cells. The figure shows A549 cells 7 days after mock infection (A), 7 days after inoculation with early-passage virus isolate MY7/1 (B), and 7 days after inoculation with late-passage virus isolate MY7/1 (C). When late-passage material was diluted to provide an inoculum at a very low multiplicity of infection, the characteristic early-passage cytopathic effect was reproduced.
Table 3. Results of neutralization tests that used type-specific antisera.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Ad3</th>
<th>Ad7</th>
<th>Ad11</th>
<th>Ad14</th>
<th>Ad16</th>
<th>Ad21</th>
<th>Ad34</th>
<th>Ad35</th>
<th>Ad50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ad3</td>
<td>2560</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
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<td>—</td>
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</tr>
<tr>
<td>Ad7</td>
<td>—</td>
<td>2560</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ad11</td>
<td>—</td>
<td>—</td>
<td>2560</td>
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<td>—</td>
</tr>
<tr>
<td>Ad21</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>1280</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>&gt;2560</td>
<td>&lt;20</td>
<td>320</td>
<td>160</td>
</tr>
<tr>
<td>Ad34</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>160</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ad35</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>160</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ad50</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1280</td>
<td>—</td>
<td>320</td>
</tr>
<tr>
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<td>&gt;2560</td>
<td>&lt;20</td>
<td>320</td>
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<tr>
<td>MY7/1</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>1280</td>
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<td>&lt;20</td>
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<td>160</td>
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<tr>
<td>MY8/1</td>
<td>&lt;20</td>
<td>&lt;20</td>
<td>1280</td>
<td>40</td>
<td>&lt;20</td>
<td>&gt;2560</td>
<td>&lt;20</td>
<td>160</td>
<td>160</td>
</tr>
</tbody>
</table>

NOTE. The Sarawak (Malaysia) isolates were neutralized by specific antisera against adenovirus types (Ad) 21, 50, and 11 at titers close to the homologous titer. Two monoclonal antibodies raised against purified hexon protein from one of our isolates were found to be specific against Ad21 prototype and our isolates SIBU97, MY7/1, and MY8/1, but they did not react with any other species B human adenovirus, including Ad50.

in the leg was 2/5, but the findings of a follow-up examination 2 years later were entirely normal.

**Patient 6.** This 19-month-old Iban boy had a brief febrile illness with abdominal distension and constipation that had resolved spontaneously after 3 days. During the next 3 weeks, he had progressive weakness of the right leg followed by the left leg. At the time of admission to the hospital, he had flaccid weakness of both legs (MRC grades of 2/5 and 3–4/5 on the right and left legs, respectively) and an absent right-knee jerk. His anal tone was normal, and there was no evidence of bladder involvement. The findings of a CT myelogram and head scan were normal, and he received IVIG. By day 4 of hospitalization, power had improved in his legs so that he could walk with support. At follow-up, he had a wasted right buttock and thigh (figure 6, left), and he walked with an abnormal gait and a mild right foot drop.

**Patient 7.** A 14-month-old Iban girl had a 3-day febrile illness with rhinorrhea followed by a period of fluctuating consciousness and inability to stand. Examination revealed a scanty macular rash on the soles of her feet and a few painful oral ulcers on her hard palate and buccal mucosa. She had cold, mottled extremities and a capillary refill time of >2 s. Her heart rate was 145 beats/min, and her blood pressure was 130/70 mm Hg. Her legs were flaccid and areflexic, with no movement. Shortly after admission to the hospital, the patient was noted to have a labile heart rate with runs of 180–220 beats/min. An electrocardiogram showed sinus tachycardia, but the troponin T level and the findings of an echocardiogram were normal. She developed progressively severe stridor and continuous generalized seizures. Therefore, she underwent intubation and ventilation and was treated with phenytoin. She successfully underwent extubation after 2 days, and, at the time of discharge, she could stand with support. At follow-up 2 years later, the findings of an examination were completely normal.

**Patient 8.** Seven days after this 4.5-month-old boy received his second dose of oral polio vaccine, he developed a febrile illness with a rash over his forearms and soles, ulcers on his buccal mucosa and tongue, recurrent vomiting, lethargy, and intermittent episodes of startled movements. He was pale, drowsy, and wheezy, and he cried with a hoarse voice. His heart rate was 124 beats/min, his blood pressure was 130/70 mm Hg, and his respiratory rate was 42 breaths/min. His chest was hyperinflated, with bilateral rhonchi. He had a full anterior fontanelle. His left arm was flaccid and hyporeflexic and could not be lifted against gravity. An echocardiogram revealed good left ventricular function. He was treated with IVIG, but his condition continued to deteriorate. Movements in his left arm were reduced to just a flicker, and his right arm also became flaccid and weak. His consciousness level fluctuated; he became tachypneic, with labored breathing; and he developed a bulbar palsy. He was treated again with IVIG, and his condition began to improve gradually. At the time of discharge from the hospital, the MRC power grade was 4/5 and 3/5 in the right and left arms, respectively. At follow-up, the patient was found to have marked wasting of the left deltoid, biceps, and triceps, which were flaccid (power, 0/5; figure 6, right). Wrist flexion and extension and intrinsic hand muscle functions were normal.
Figure 2. Restriction digests of strains SIBU97, MY7/1, and MY8/1 with use of enzymes BamHI (top), Smal (middle), and KpnI (bottom), compared with prototype adenovirus type 21 (Ad21) and other species B adenoviruses. The restriction digest shows that the 3 adenovirus isolates belong to Ad21 or adenovirus type 50 (Ad50) but differ from the prototype strains concerned. Comparison with previously published Ad21 restriction digest profiles [11, 12] shows that the Sarawak (Malaysia) isolates have an additional fragment between 500 and 1000 bp in the BamHI and the KpnI profiles and an additional 1000-bp fragment in the Smal profile. The first lane has molecular weight markers. Gel images were digitally scanned using the Typhoon 8600 Variable Mode Imager (Amersham Pharmacia Biotech). These images were edited and diagrammatic representations of the digest profiles were prepared with Adobe Photoshop, version 4.0.1 (Adobe Systems). Ad, adenovirus type.
Figure 3. Neighbor-joining phylogenetic tree of the hexon gene (301 nucleotides; bases 21–321 of the hexon gene) for SIBU97, MY7/1, and MY8/1 (boxed), showing their proximity to adenovirus types 21 (Ad21) and 50 (Ad50) and relationship to other species B adenoviruses. Two other Ad21 strains, which were isolated from patients in Singapore in 1997, are also included: SGH2289 (from a CSF specimen obtained from a child with acute flaccid paralysis) and SGH5253 (from a throat swab specimen obtained from an HIV-infected adult). The alignment was made using the Vector NTI suite software package, version 7.0 (Informax), and the phylogenetic tree was constructed using the neighbor-joining method on PAUP software, version 4.04a (Sinauer Associates). Horizontal branch lengths are proportional to genetic distance, and the scale indicates the proportion of nucleotide substitutions per site. The numbers above the branches are bootstrap support values, which are given as a percentage of 1000 replicates. Differences between the 3 isolates confirm that these were not contaminants. GenBank accession numbers are given in brackets.

DISCUSSION

Because the prevalence of poliomyelitis has decreased, it has become clear that a wide variety of other infectious and para-infectious processes can cause AFP. Para- or postinfectious causes include acute inflammatory demyelinating polyneuropathy (“classic” Guillain-Barré syndrome) and acute motor axonal neuropathy [13]. These causes are sometimes associated with characteristic patterns of antibody (including anti-ganglioside antibodies) responses against components of nerves [14]. Rarer causes of AFP include neuropathies of the lumbosacral and brachial plexus (neuralgic amyotrophy); these neuropathies are also thought to occur after infection [15–17]. Viral causes of AFP include infections with flaviviruses (Japanese encephalitis virus, tickborne encephalitis virus, and West Nile virus) [8, 18, 19] and nonpolio enteroviruses (coxsackieviruses, echoviruses, and EV71) [20, 21].

Epidemics of EV71 infection are associated with a wide variety of clinical features, including HFMD, herpangina, aseptic meningitis, encephalitis, and myelitis. The 1997 outbreak of HFMD in Sarawak was unusual because of the large number of severe and fatal cases. The following year, there was an outbreak in Taiwan with a similarly high mortality rate [22]. The factors that determine whether an outbreak of EV71 will be associated with HFMD, neurological disease, or both are not known. Given that our cases of AFP occurred during an EV71-associated outbreak of HFMD, the initial assumption was that the cases of flaccid paralysis were also due to EV71 infection. Indeed, although no EV71 isolates were recovered from patients with AFP, paralysis would have been attributed to EV71 infection had we not identified a fastidious adenovirus. An unusual cytopathic effect in A549 cells was seen initially (figure 1), and only after several passages did it display a more classic cytopathic effect; the results of immunofluorescence tests were only weakly positive, and the results of PCR using primers described by Allard et al. [23] were initially negative. We have since sequenced through the primer-binding sites of prototype species B adenoviruses and found up to 4 (16%) of 25 nucleotide mismatches in the forward primer and 6 (26%) of 23 nucleotide mismatches in the reverse primer, which explains the primer’s reduced sensitivity. New primers have now been developed [24]. Our neutralization tests identified the adenovirus as Ad21 and demonstrated a close relationship to Ad50 and Ad11. Restriction analysis showed that the isolates were different from the prototype Ad21, and the sequencing data indicated that our Ad21 isolates were very similar to Ad50. Taken together, these results suggest that the isolates recovered during this outbreak may be “intermediate” strains, as has been described elsewhere [25].

Ad21 has been associated with a variety of severe and sometimes fatal manifestations, including severe pneumonia, hemorrhagic keratoconjunctivitis, myocarditis, and disseminated infection [5]. Neurological disease has not been reported previously for Ad21; however, for other species B human adenoviruses, particularly adenovirus type 7, a variety of neurological complications have been reported, including aseptic meningitis, encephalopathy, encephalitis, and transverse myelitis [26–31].

The clinical features of Ad21-associated AFP in our patients bore similarities to AFP caused by polio viruses, nonpolio enteroviruses, and flaviviruses [19]. A brief febrile illness was followed by rapid onset of AFP in ≥1 limb. Enteroviruses and flaviviruses directly attack the anterior horn cells of the spinal
Figure 4. Alignment of the deduced amino acid sequence of the fiber protein of our isolates SIBU97, MY7/1, and MY8/1, compared with adenovirus type 21 (Ad21) and type 50 (Ad50) prototypes. The motif AATSSK is seen in all our isolates, as well as Ad50 (generously provided by Jan de Jong, Faculty of Medicine, Erasmus University Rotterdam, Rotterdam, The Netherlands), but not in Ad21.

cord to cause purely motor abnormalities; radiological changes consistent with this were seen in one of our patients. In cases of pure anterior horn cell damage, sensory changes and demyelination do not occur. However, in several of our patients, the findings of neurophysiological studies were consistent with previous demyelination of both sensory and motor nerves. Thus, these data suggest that 2 pathophysiological processes may occur in Ad21-associated flaccid paralysis: (1) direct invasion of the anterior horn cells, and (2) brachial or lumbo-sacral plexus neuropathy.

Figure 5. Axial (left) and sagittal (right) T2-weighted MRIs of the thoracic and lumbar spine of patient 1. The MRIs were obtained on day 24 of illness (day 20 after presentation) and show a high-signal intensity lesion extending from T2 to L1 on the anterior right side of the spinal cord.
Idiopathic neuropathy of the brachial plexus (also known as “neuralgic amyotrophy”) and its counterpart in the leg, lumbosacral plexus neuropathy, are rare disorders characterized by sudden onset of limb weakness and patchy sensory loss. In adults (but not children), limb pain often precedes weakness [15, 17, 32]. Typically, the upper part of the plexus is involved, causing proximal weakness in a single limb. However, bilateral cases occur [16], and any part of the plexus and even cranial nerves may be involved [33]. In cases of plexus neuropathy, electromyography typically reveals denervation in the affected muscles. Nerve conduction studies may show changes of axonal damage, changes of demyelination, and/or conduction block [34], as was seen in our patients. The findings of CSF examination may be normal or may reveal moderate pleocytosis. Most patients with plexus neuropathies have normal function by 2 years, although children appear to fare worse [32]. The pathogenesis of plexus neuropathy is not known, but it is thought to be immune mediated, because attacks typically occur after viral upper respiratory tract infections and immunizations [17, 32], oligoclonal bands are seen in CSF samples obtained from some patients, and multifocal mononuclear infiltrates are noted on nerve biopsy specimens [33, 35]. The anti-ganglioside antibodies seen in our patients may represent the tail end of an autoimmune response. Alternatively, the adenovirus penton protein may be implicated. The adenovirus penton protein, which is part of the capsid, is made in great excess, and it causes cellular abnormalities in vitro; levels of this protein were elevated in serum samples obtained from patients with fatal adenovirus infections [29].

There has been considerable debate about the cause of the unusual manifestations in cases of EV71-associated HFMD in Sarawak and peninsular Malaysia in 1997 and, subsequently, in Taiwan [1, 2, 4, 36–40]. Increased virulence of EV71 alone seems unlikely, given that several different EV71 genotypes were implicated in these outbreaks [40–42]. Noninflammatory cardiac dysfunction has now been recognized as a contributor to many of the fatal cases. Although some researchers have suggested that this may be the result of sympathetic overactivity [38], others suggest that another infectious agent might contribute [4]. Adenoviruses have not been described as a major cause of AFP previously. Whether the epidemic of EV71-associated HFMD was coincidental or whether the severe presentation was due to an interaction between the 2 viruses is not certain. Interestingly, plexus neuropathy and adenovirus encephalopathy have both been previously associated with dual viral infections [16, 43]. Clearly, more detailed prospective clinical, virological, and pathophysiological studies are needed to investigate the possible interaction between enteroviruses and adenoviruses and the patterns of disease that they cause.

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