Adenovirus in EV71-associated hand, foot, and mouth disease

Sir—Jane Cardosa and colleagues (Sept 18, p 987)[1] report the discovery of a new fastidious adenovirus among patients with suspected hand, foot, and mouth disease (HFMD) in Sarawak. Subsequent to that outbreak, at least five deaths among young children with almost similar clinical presentations were reported in the Malaysian Peninsula. Enterovirus 71 (EV71) was isolated and identified in all five cases,[2,3] which suggested that perhaps the two viral outbreaks were unrelated.

We detected enteroviruses including EV71 in about 51% (26 of 51) of samples from patients with suspected HFMD.[4] We detected and confirmed adenovirus infection by cell culture and immunofluorescence staining from throat and rectal swab samples in only one patient from Sarawak. However, the adenovirus genome was detected by PCR amplification of the *hexon* gene in at least six other patients with suspected HFMD, including a patient who succumbed to brainstem encephalomyelitis caused by EV71. From this patient, the amplification product was detected only in Vero cells inoculated with pericardial and cerebrospinal fluid but not in cells inoculated with other tissue materials. Nonetheless, the presence of adenovirus in suspected HFMD patients was confirmed by amplification of Vero cells inoculated with blood from a 10-year-old boy. Amino acid sequence from nucleotide sequencing of amplification products showed that the patient's adenovirus shared at least 95% identity with the *hexon* gene of adenovirus 7 and the new subgenus B adenovirus.[1] This finding suggests that perhaps a similar adenovirus was circulating in Sarawak and the Malaysian Peninsula during the HFMD outbreak.

Our attempts to propagate the virus or clone the sequence from the initial amplification product of other patients were not successful. However, EV71 was isolated from two of the six suspected adenovirus-positive patients. This includes the fatal infection attributed to EV71 and the HFMD case involving the 10-year-old boy from which EV71 and adenovirus were isolated from rectal swab and blood, respectively. In the latter case, the patient had fever, oral ulcer, and rashes on palms consistent with HFMD. Except for tachycardia and slight pleural effusion, no other overt neurological or cardiopulmonary symptoms were noted and the patient was discharged.

Although there is no specific evidence to associate an adenovirus with the fatal HFMD-associated cases in the Malaysian Peninsula, EV71 was identified in almost all cases, not directly from patients' tissues but after inoculation of cell cultures mainly suitable for enterovirus isolations. Perhaps the presence of a fastidious adenovirus in the suspected HFMD cases was missed because of use of an unsuitable cell culture system.[5] However, Cardosa and colleagues' report[1] and our own findings suggest that the potential role of a fastidious adenovirus in EV71-associated HFMD needs to be examined further.

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4 AbuBakar S, Chee H-Y, Shafee N, Chua KB, Lam SK. Molecular detection of enteroviruses from an outbreak of hand, foot and mouth disease in Malaysia in 1977. *Scand J Infect Dis* 1999; **31:** 331–35.
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Sir—In 1997, soon after the Sarawak outbreak of enterovirus 71 (EV71)-associated hand, foot, and mouth disease that Jane Cardosa and colleagues[1] report, a similar outbreak in Kuala Lumpur, Malaysia. There is a striking similarity in the clinical picture of our patients in Kuala Lumpur,[2] and the children who died in the Sibu[1] and Taiwan[3] outbreaks.

We disagree with Cardosa and colleagues that our fatal cases of culture-proven EC71 encephalomyelitis had primary myocarditis because two of our patients had blood pressure of 120/90 mm Hg, which is incompatible with primary myocarditis. Furthermore, with a combination of inotropes and vasodilators, the heart can generate adequate cardiac output to prevent sudden death, as had occurred in our patients. Their refractoriness to inotropes therefore suggests secondary myocardial dysfunction. More importantly, histopathological evidence of myocarditis was lacking in all four cases. Echocardiographic findings of global hypokinesis, as seen in the Sibu patients, have been shown in patients with subarachnoid haemorrhage complicated by neurogenic pulmonary oedema.[4]

The sudden and rapid deterioration of our patients could be explained by neurogenic pulmonary oedema in association with destruction of the respiratory and vasomotor centres in the medulla, as shown by necropsy findings in all our patients. Magnetic resonance imaging and necropsy findings in the Taiwan outbreak also showed the brain stem to be severely affected.[3] Furthermore, immunohistochemical evidence of EV71 antigens in the damaged neurones of the brainstem and anterior horn cell[5] provides a strong causal link between EV71 and destruction of the brain stem.

Several pathogens were isolated in the Sibu cases. In the absence of a full postmortem, histopathological examination, immunohistochemical staining, and viral cultures from the brain stem and spinal cord, Cardosa and colleagues were unable to determine with certainty the role of each pathogen. Viral cultures of biopsy samples from selected organs would have misrepresented the true picture. However, EV71 by itself is a well-known cause of central nervous system disease during a hand, foot, and mouth disease outbreak, as reported in Bulgaria and Taiwan,[3] and to consider EV71 as the common cause is epidemiologically more acceptable.

We suggest that children with non-fatal acute flaccid paralysis had EV71 infection of the spinal cord, and children who died of apparent myocardial failure had additional involvement of the respiratory and vasomotor centres in the brainstem that resulted in neurogenic pulmonary oedema with secondary myocardial dysfunction. Detection of the virus in tissue samples by immunohistochemistry or by in-situ hybridisation is important to confirm the role of adenovirus in the disease.

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