

# Pathological findings in a mouse model for Coxsackievirus A16 infection

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## Abstract

Coxsackievirus A16 (CV-A16) is the leading cause of hand-foot-mouth disease (HFMD), which usually presents as mild and self-limiting symptoms in young children. Rarely, CV-A16 has been reported to cause severe and fatal neurological complications but little is known about these complications. In the present study, 1-day and 7-day old mouse models of CV-A16 were developed using a clinical strain via subcutaneous inoculation. All infected mice exhibited clinical signs of infection, including reduced mobility, limb weakness and paralysis between 3 to 6 days post-infection. Pathologically, the main organs involved were the central nervous system (CNS), skeletal muscles and brown fat. In the CNS, viral antigens as demonstrated by immunohistochemistry, were localized mainly to neurons in the brain stem and spinal cord, suggesting that CV-A16 is neurotropic although inflammation is very mild. The skeletal muscles showed necrosis and myositis due to viral infection as evidenced by the dense viral antigens. Focal viral antigens were also detected in the brown fat. These preliminary pathological findings indicate that our mouse models can be further developed to be useful models for pathogenesis studies, and vaccine and anti-viral drug evaluation.

## INTRODUCTION

Coxsackievirus A16 (CV-A16) is a non-enveloped, single-stranded, positive-sense RNA virus from the family of *Picornaviridae*. First isolated in South Africa in 1951<sup>1</sup>, it infects humans via faecal-oral or oral-oral routes.<sup>2,3</sup> Together with enterovirus 71 (EV-A71), CV-A16 is one of the most important pathogens that causes hand-foot-mouth disease (HFMD). The usual mild clinical manifestations of HFMD are fever, vesicles/rashes on the palmar and plantar skin, and ulcers on the buccal mucosa and tongue.<sup>4</sup> Very rarely, severe and fatal neurological complications e.g. rhombencephalitis and myocarditis have been associated with CV-A16.<sup>5-8</sup>

In contrast to EV-A71 encephalomyelitis in which human autopsies have been studied<sup>9-11</sup>, human pathology and neuropathogenesis of CV-A16 infection is unknown. Therefore, it is essential to develop animal models to better understand the infectious disease pathology of the virus, particularly its neurotropism. Previously, a few animal models of CV-A16 have been developed as tools for vaccine and anti-viral drug evaluation. In these models, the pathological findings suggest that the virus may have a predilection for the brainstem and cerebellum.<sup>12,13</sup> However,

we believe, this has not been convincingly demonstrated. In the present study, a mouse model was established to investigate the neurotropism and viral tropism for non-central nervous system (CNS) tissues of a clinically-isolated CV-A16 strain. The findings in this model extend our knowledge of CV-A16 pathogenesis. Moreover, this mouse model could also be potentially useful for testing new anti-viral drugs and vaccines.

## METHODS

### *Virus stock and cell culture*

A clinical isolate of CV-A16 (CV-A16-N132), obtained from a HFMD patient was used for our experiments. The virus was propagated in Vero cells maintained in Dulbecco's Modified Eagle Medium (DMEM, Sigma-Aldrich, USA) maintenance medium supplemented with 2% fetal bovine serum (FBS, JR Scientific, USA). The culture was kept until the cells showed >90% cytopathic effect. After 3 freeze-thaw cycles and centrifugation at 4000rpm for 10 min at 4°C, the supernatant was filtered through a 0.22µm pore-size filter (Minisart; Sartorius, Germany), aliquoted and stored at -80°C before use. The viral titers were determined by measuring 50%

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