

EV71, A Virus with Complicated Pathogenesis in the CNS

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Abstract

Fatal cases of hand, foot, and mouth disease (HFMD) caused by enterovirus 71 (EV71) infection are characterized by inflammatory damage to the central nervous system (CNS) with slight degeneration of nerve cells. Here, we review several studies that identify potential host factors and possible mechanisms of neuropathogenesis of EV71 infection.

Keywords: Enterovirus 71 (EV71); Neuropathogenesis; Central Nervous System (CNS); Astrocytes

Introduction

Enterovirus 71 (EV71) is the primary neurological threat responsible for fatalities in outbreaks of hand, foot, and mouth disease (HFMD) [1-5]. Patients with HFMD are defined as severe cases if they develop any neurological or/and cardiopulmonary complications accompanied by fever, skin rashes or on the hands, feet, and mouth [5]. Aseptic meningitis, brainstem encephalitis, and encephalomyelitis are the cardinal signs of EV71 infection of the CNS [6,7]. With the increased severity of disease, the damage to the CNS leads to neurogenic pulmonary edema, functional cardiopulmonary failure, and, consequently, the high rate of mortality [8,9]. Therefore, it is critical to understand the pathological mechanisms behind the severe clinical manifestations, which has recently drawn increasing attention from researchers.

The faeco-oral route is the predominant mode of EV71 transmission. EV71 invades the CNS through either the disruption of the blood-brain barrier or retrograde spinal neural transport. Generally, animal studies [10] and assessment of the distribution of the virus in fatal human cases [11] indicate that the latter may be the major route of transmission of EV71.

Central Nervous System (CNS) by EV71

With infection of the CNS by EV71, viral antigen can be detected by staining in CNS tissues in a variety of cell types, including neurons, microglia, and astrocytes [12]. Viral entry into the CNS usually leads to a strong inflammatory response. The severity of inflammation is high in the spinal cord and the medulla oblongata and lower in the hypothalamus, thalamus and part of the cerebrum [11,13,14]. The concomitant histopathological manifestations in the CNS are typically detected as confined vascular inflammatory cell infiltration, variable edema, glial cell proliferation and glial nodule formation [15,16]. Correspondingly, the levels of inflammatory cytokines in the cerebral spinal fluid (CSF) are elevated [17]. Interestingly, unlike other neurotropic viruses (such as poliovirus) with distinctive necrosis and destruction of the majority of neural cells [18], EV71 only induces slight neurodegeneration, rather than the widespread necrosis of

neural cells in the CNS that is frequently associated with these pathological changes [19,20]. This suggests that the observed CNS damage by EV71 might be induced by unidentified inflammatory responses distinct from other neurotropic viruses.

Here, we try to raise some questions regarding the peculiar inflammatory response observed during the EV71 infection in the CNS. What are the possible mechanisms of neuropathogenesis of EV71 infection? As demonstrated by our previous studies of the pathogenic mechanisms of EV71 infection in the CNS, the viral load in the CNS peaks during the infectious process in neonatal rhesus monkey models [21]. Moreover, the peak of viral replication in the CNS has been shown to be closely related to the substantial inflammatory reaction in the CNS tissues [21,22]. Additionally, K. T. Wong et al also found that the regions in which EV71 associated inflammation occurred partially correlated to the regions where viral RNA and antigens were detected [11]. These findings indicate an association between EV71 replications in the CNS tissue and the induction of an inflammatory response.

Multiple studies have found that several types of immune cells are involved in EV71 infection. For example, the interaction between neutrophils (or leukocytes) and vascular smooth muscle cells may contribute to inflammation and immune response during EV71 infection [23]. Human dendritic cells might be infected by the EV71 virus, triggering the release of proinflammatory factors by the dendritic cells, such as IL-6, IL-12 and TNF- α [24]. Additionally, EV71 infection activates NF- κ B and leads to the production of many proinflammatory factors. This study also showed that up-regulation of COX-2 is associated with the release of PGE2 from EV71-infected SK-N-SH cells *in vitro* [25]. These findings suggest that these signaling pathways may also contribute to EV71 infection.

Because immune cells are a minority cell type in the CNS, we moved our focus to other cell populations—neurons and glia in the CNS. As previously described, neuronal degeneration and necrosis were slight and observed limitedly in the spinal cord as well as in a small section of upper motor neurons [10,12,26,27]. Based on these results, researchers also aimed to study the other cell populations in the CNS, such as glia. Previous studies have shown that the microglia and astrocytes, the most abundant cells in the CNS, act as important functional cells [28]. Current interest in these cells was based on not only their important neurobiological function of selectively regulating

neural cell activities and synaptic transmission, [29,30] but also on their phagocytic capability and their ability to express multiple immune factors similar to other immune cells [31-33]. To date, the data concerning the role of microglia and astrocytes in EV71 infection are limited and primarily from autopsy studies, in which immunohistochemical analysis demonstrated that EV71 antigen was observed in microglia and astrocytes in the CNS of fatal human cases [34,35]. In addition, two *in vitro* study using the established human astrocytoma cell lines, to the extent demonstrated that astrocytes were permissive to infection by EV71 [36,37]. However, astrocytes are a focus of our study of EV71 infection and pathogenesis in the CNS. Recent work (unpublished data) in our lab identified astrocytes as the primary cell type involved in EV71 infection in the CNS. This work was done using *in vivo* experiments performed in neonatal rhesus monkey and suckling mouse models and *in vitro* experiments using a cellular model. Strikingly, astrocytes have the capacity to substantially modulate neuronal function via the canonical expression of proinflammatory cytokines during EV71 infection. This subsequently stimulates the neuronal secretion of adrenaline, an excitatory neurotransmitter that is considered a likely cause of the pathophysiological events in fatal HFMD cases, which are characterized by the development of neurogenic pulmonary edema and cardiopulmonary dysfunction.

In addition, the interaction between virus and receptors is considered to be important in the early steps of viral infection. It is usually a complex multistep process that sequentially involves the recognition of one receptor or the simultaneous recognition of multiple cell-surface receptors during virus attachment followed by entry into host cells [38]. To date, SCARB-2 [39], PSGL-1 [40] and DC-SIGN [24] have been identified as the receptors for EV71 infection, but they may be involved in different steps during the EV71 infection process. A recent autopsy study revealed that the expression of SCARB-2 and EV71 antigen colocalize in the human CNS, whereas SCARB-2 is poorly expressed in the mouse CNS [12]. Another recent study using transgenic mice expressing the human EV71 receptor, SCARB-2, found that the mice are more susceptible to EV71 infection than the wild type mice, confirming that SCARB-2 is important for EV71 infection *in vivo* [41]. However, our current study showed that specific antibodies against the EV71 receptor molecules, such as SCARB-2, PSGL-1 and DC-SIGN, frequently failed to inhibit viral replication in monkey astrocytes *in vitro*. These results suggested that EV71 infection of astrocytes may be directly attributed to the phagocytosis of the viruses based on the basic phagocytic function of astrocytes.

Conclusion

In summary, although studies have determined several potential factors involved in EV71 infection in the CNS, many questions remain unanswered. Which inflammatory mediators and cell populations protect adults from damage to the CNS during EV71 infection? Do astrocytes in the CNS phagocytose and kill destroy the EV71 virus? EV71 infection usually causes fatal cases in infants or young children. We should pay attention to patients in this specific age range in future work. These important questions merit further investigation, and the answers may provide novel therapeutic or prophylactic targets to cure this infectious disease.

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Conflict of Interest

Min Feng declares no conflict of interest. Qihan Li declares no conflict of interest.

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