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Review

The key mechanisms of multi-system responses triggered by central nervous system damage in hand, foot, and mouth disease severity

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ABSTRACT

Hand, foot, and mouth disease (HFMD) is a prevalent infectious affliction primarily affecting children, with a small portion of cases progressing to neurological complications. Notably, in a subset of severe HFMD cases, neurological manifestations may result in significant sequelae and pose a risk of mortality. We systematically conducted literature retrieval from the databases PubMed (1957–2023), Embase (1957–2023), and Web of Science (1957–2023), in addition to consulting authoritative guidelines. Subsequently, we rigorously selected the most relevant articles within the scope of this review for comprehensive analysis. It is widely recognized that the severity of HFMD is attributed to a multifaceted array of pathophysiological mechanisms. The implication of multi-system dysfunction appears to be perturbances of the human defense system; therefore, it contributes to the severity of HFMD. In this review, we provide an overview and analysis of recent insights into the molecular mechanisms contributing to the severity of HFMD, with a particular focus on cytokine release syndrome, the involvement of the renin-angiotensin system, regional immunity, endothelial dysfunction, catecholamine storm, viral invasion, and the molecular mechanisms of neurological damage. We speculate that the domino effect of diverse physiological systems, initiated by damage to the central nervous system, serve as the primary mechanisms governing the severity of HFMD. Simultaneously, we emphasize the knowledge gaps and research urgently required to delineate a quick roadmap for ongoing and essential studies on HFMD.

1. Introduction

Hand, foot, and mouth disease (HFMD) is an infectious illness that predominantly affects infants and young children under the age of 5 and exhibits a higher incidence in the Asia–Pacific regions and Europe [1]. Typically, HFMD presents with primary manifestations such as fever, vesicular rashes, and oral mucosa ulcers (Fig 1). However, certain cases may progress to more severe complications, including aseptic meningitis, encephalitis, acute flaccid paralysis, neonatal sepsis, pulmonary edema (PE), and even sudden death [1]. Enterovirus A71 (EVA71) is the most well-known pathogen within the group of human enteroviruses (HEVs) associated with HFMD and causes the most widespread morbidity and mortality [1]. EVA71 selectively targets neurons across various regions, including the spinal cord, cerebellum, medulla, pons, brainstem, and cervical spinal cord. This targeting is closely associated with the development of neurogenic PE [2], a major feature contributing to mortality in severe HFMD cases.

Annually, numerous children worldwide are hospitalized due to HFMD, with a subset experiencing severe neurological complications. In certain Asian–Pacific regions, the overall mortality rate among severe EVA71-

Abbreviations: HFMD, Hand, foot, and mouth disease; EVA71, Enterovirus A71; HEVs, Human enteroviruses; PE, Pulmonary edema; CNS, Central nervous system; IL, Interleukin; TNF, Tumor necrosis factor; IFN, Interferon; Th, T helper; NA, Noradrenaline; Epi, Epinephrine; RAS, Renin-angiotensin system; Ang, Angiotensin; NK, Natural killer; ROS, Reactive oxygen species; NO, Nitric oxide; CV, Coxsackie virus; dpi, day(s) post infection; BBB, Blood-brain barrier; TLRs, Toll-like receptors..

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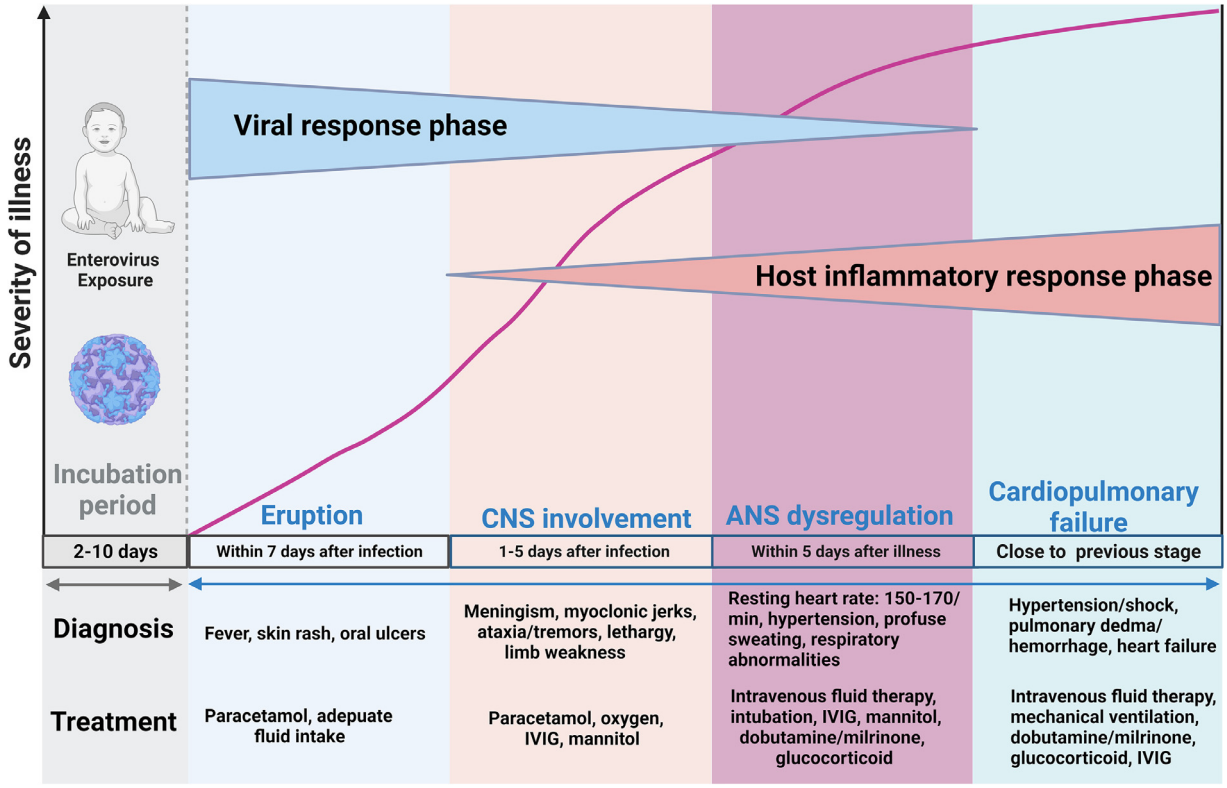

Fig. 1. The time course, diagnosis, and treatment of HFMD. The data were collected and summarized according to "A Guide to Clinical Management and Public Health Response for Hand, Foot and Mouth Disease (HFMD)" from the World Health Organization; "Management of Hand Foot Mouth Disease (HFMD) in Health Care Settings (May 2007)" formulated by the Centre for Health Protection, Hong Kong, China; as well as Chinese guidelines for the diagnosis and treatment of hand, foot, and mouth disease (2018 edition). Notably, severe patients with encephalomyelitis and persistent high fever and critical cases might be considered for IVIG treatment (1.0 g/kg/day for 2 days). Autonomic nervous system, ANS; intravenous immunoglobulin, IVIG. Created with BioRender.com.

associated HFMD cases has been reported to reach up to 19%. In 1998, Chinese Taiwan encountered the most significant epidemic of HFMD, with 405 patients suffering from severe illness. Unfortunately, among these cases, 78 patients (19.3%) succumbed to the disease [3]. Between 2008 and 2015, China reported approximately 13.7 million cases of HFMD, with a case fatality rate of 2.7% in severe cases [4]. While the survival rate of children with severe neurological complications has shown improvement, concerns persist regarding long-term sequelae, particularly in severe cases. A retrospective cohort study revealed that HFMD cases linked with neurological complications may manifest abnormalities across various domains, including neurological, motor, language, and cognition functions, as well as adaptive and ventilatory functions [5]. The fatal outcomes, severity of the disease, and long-term sequelae underscore the significant health challenges posed to children and adolescents by HFMD. The administration of EVA71 vaccines has demonstrated a positive protective effect against severe HFMD. Nonetheless, the emergence of new pathogens and alterations in the molecular epidemiology of viruses present challenges in effectively preventing severe disease cases. Despite ongoing efforts, the underlying mechanisms contributing to HFMD severity remain poorly understood. The severity of the disease is contingent upon various factors, including the nature of the virus, host factors, and environmental influences. All of these elements collectively contribute to viral infection and subsequent damage to organ-specific tissues. Of note, emerging evidence suggests that the severity of HFMD results from a complex interplay of several pathophysiological mechanisms involving both systemic and local inflammatory responses, alongside the substantial release of neurotransmitters, leading to central nervous system (CNS) damage. Recently, we provided a comprehensive summary of our current general knowledge of HFMD, encompassing aspects such as virology, epidemiology, sequelae, and vaccine development [6]. However, there remains a significant lack of comprehensive analyses focusing on the severity of HFMD. We conducted the comprehensive and meticulous retrieval of HFMD-related studies from multiple literature sources (including some authoritative guidelines [7-9]), excluding low-quality studies. Importantly, all studies included in the review were selected following extensive literature retrieval methods and multiple keyword searches. Different retrieval strategies were employed based on the mechanisms discussed in different chapters. In summary, this review is written to address the gaps in our understanding by delving into recent advances in the rapidly expanding field of HFMD research. It aims to not only enhance our understanding of the pathophysiology of HFMD but also

lay the groundwork for the development of both novel and repurposed treatment modalities.

2. Cytokine storm

2.1. Dynamic changes in cytokines and chemokines

The occurrence and progression of severe HFMD are intricately linked to a dynamic cytokine imbalance [10]. A previous case-control study demonstrated significantly elevated levels of interleukin (IL)-4, IL-6, IL-10, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ in patients with severe HFMD during the 2nd to 5th day post-disease onset compared with mild cases [11]. During the acute phase, mild cytokine storms (IL-10, IL-4) were observed in severe HFMD patients. Conversely, mild HFMD patients exhibited elevated cytokine levels (IL-10, IL-8) during the early onset phase of the illness [10]. The levels of granulocyte-macrophage colony stimulating factor, macrophage inflammatory protein 1β, IL-2, and IL-33 in the circulating blood each exhibited an increase, reaching their peaks 48–72 hours following hospitalization [12]. The levels of IL-8, CCL5, CXCL9, and IP-10 were notably elevated in HFMD children with encephalitis, but they exhibited a significant decrease during the convalescence phase [13]. The levels of granulocyte colony-stimulating factor, IL-8, MCP-1, IP-10, and IL-6 in the cerebrospinal fluid were found to be higher than the corresponding plasma levels in patients exhibiting neurological symptoms, suggesting that these cytokines may act as predominant mediators that induce neurological damage [12]. Notably, the level of IL-6 remained consistently elevated during the initial 2 days of CNS involvement, declining thereafter [14]. This trend may be associated with the initiation of a catecholamine storm [15].

Fluctuations in the cytokine cascade may underlie the inconsistencies observed in cytokine dysregulation across various publications focusing on HFMD. Case-control studies alone may not accurately reflect the dynamic immune response. However, the prospective and sequential collection of biological samples from patients could address these limitations and provide more reliable insights. In conclusion, cytokine profiles, encompassing T helper (Th) cell type 1 cytokines (e.g., IL-12, IFN-γ), Th2 cytokines (e.g. IL-4, IL-10), and proinflammatory cytokines (e.g., IL-1β, IL-6, TNF-α), vary at different stages of disease progression (Fig 2A), indicating that cytokine storms are associated with the severity of HFMD.

2.2. Imbalance of the immune response

As depicted in Fig. 2B, host cells recognize viral invasion and initiate an innate immune response through pattern recognition receptors, resulting in dynamic changes in cytokine levels. Notably, upon invasion of the CNS by a virus, the activation of glial cells may directly modulate the systematic immune cell response, thereby inducing significant alterations in cytokine production [16]. Monocytes activated by EVA71 can stimulate the proliferation of T cells and enhance the release of specific functional cytokines [17]. Additionally, EVA71 induces the production and secretion of IL-1β in macrophages and peripheral monocytes by activating the NLRP3 inflammasome [18]. Distinct cytokine secretion profiles are evident across various subsets of T cells, with alterations in the frequency of T cell subsets during infection leading to dynamic cytokine patterns. A previous study revealed that both mild and severe HFMD patients manifest significantly higher percentages of Th1 and cytotoxic T cells, alongside an increased Th1/Th2 ratio [19]. In contrast, a recent study noted a reduction in the frequency of total Th cells, cytotoxic T cells, and regulatory T cells, coupled with increased percentages of B cells and Th2 cells, among children with EVA71 in their peripheral blood [20]. Furthermore, patients exhibiting elevated levels of IL-4 expression in their $CD4^+$ T cells tended to experience a longer duration of illness [21]. While the results of various studies may differ, potentially attributed to differences in the sample sizes or the diverse stages of the disease, alterations in the frequency of immune cell subsets and a dynamic imbalance of cytokines are consistently reported to contribute to the severity of HFMD.

However, it is essential to note that significant alterations in cytokine levels typically occur only in severe cases, warranting further investigation into the underlying causes of these changes. It has been observed that catecholamines inhibit the cytokine response of Th1 cells while enhancing the cytokine response of Th2 cells [22], a phenomenon consistent with the clinical data mentioned above [20]. By targeting distinct adrenergic receptors and modulating cytokine and antibody production, circulating NA and epinephrine (EPi) can elicit stimulatory and/or inhibitory effects. Notably, catecholamines trigger the systemic release of IL-10 from unstimulated monocytes within minutes via a β-adrenoreceptor-mediated pathway [23]. The catecholamine storm triggered by CNS damage in HFMD patients could be responsible for the dysregulated cytokine expression due to a leukocyte circulation imbalance. An *in vitro* experiment revealed a significant increase in IL-6 levels in EVA71-infected monocytes following treatment with NA [24]. In a mouse model, the administration of the neurotoxin 6-hydroxydopamine, which selectively eliminates sympathetic nerves and reduces NA levels in innervated organs, resulted in improved clinical symptoms and a decrease in excessive inflammatory responses [25]. Plasma IFN-γ levels exhibited a significant decrease on days 4, 6, and 8 following 6-hydroxydopamine treatment [25]. Catecholamines exert differential effects on the pathogen-induced immune response depending on the concentra-

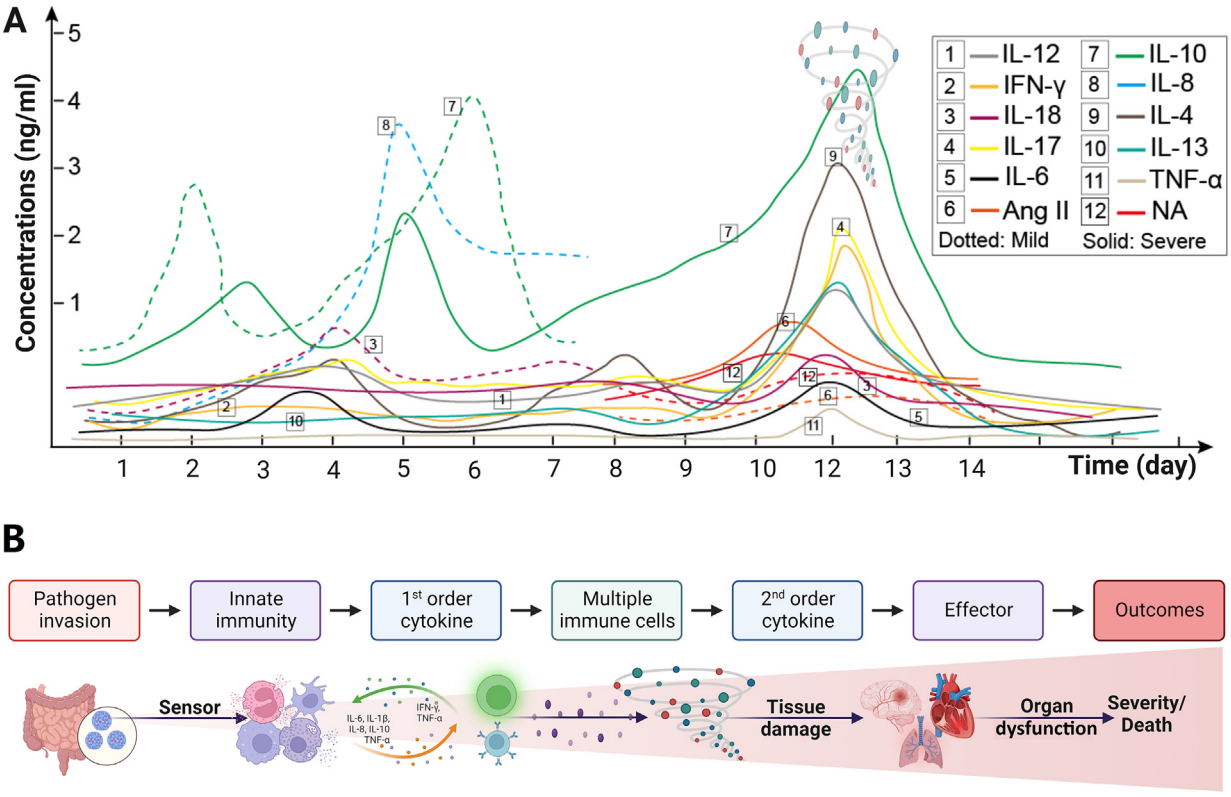

Fig. 2. Cytokine storm in HFMD pathogenesis. (A) The time-changing trend diagram illustrates the fluctuations in the serum cytokines, angiotensin (Ang) II, and noradrenaline (NA) as HFMD progresses. The dashed line denotes mild cases, and the solid line denotes severe cases. (B) If the initial immune response fails to prevent viral replication, the viruses spread throughout the body in a secondary viremia. The inflammatory response further prompts the activation of immune cells. Once the virus invades the CNS, activated glial cells, catecholamine storm, and excessive Ang II are subsequently involved with the dysregulation of cytokine expression. The extensive cytokine storm leads to incalculable damage to the body. Created with BioRender.com.

tion and duration of adrenergic receptor stimulation. However, it remains unclear how immune-cell activation leads to an increase in catecholamines and how catecholamines enhance cytokine production during the progression of severe HFMD.

3. Renin-angiotensin system

3.1. Involvement of the renin-angiotensin system in the progression of HFMD severity

The renin-angiotensin system (RAS) has significant importance in the human body, as it is tasked with regulating plasma sodium concentrations, arterial blood pressure, and extracellular volume. Additionally, RAS plays a pivotal role in modulating the inflammatory response and the sympathetic nervous system. Notably, certain components of RAS can interact with each other, further influencing its regulatory functions [26]. Clinical studies have demonstrated that severe HFMD patients exhibit significantly higher serum concentration of Ang II compared with mild cases. Furthermore, the elevation in Ang II concentration correlates positively with the development of HFMD severity [27,28]. Moreover, animal experiments have revealed elevated Ang II concentrations in the brain, skeletal muscle, and lung tissues of EVA71-infected mice [27]. Additionally, a retrospective observational study also suggested that continuous intravenous hemodialysis may be beneficial in treating severe HFMD patients complicated with cardiorespiratory failure, potentially by reducing their levels of renin-angiotensin-aldosterone system substances (e.g., renin, Ang II, and aldosterone) [29]. Evidently, the activation of RAS is implicated in the pathogenesis of HFMD severity.

3.2. The role of RAS in the development of HFMD severity

Viral infection can perturb the stability of RAS by eliciting a cascade of downstream responses [30,31]. The activation of the RAS entails a series of enzyme reactions involved in synthesizing and degrading angiotensin peptides. The initial phase involves the release of renin from renal juxtaglomerular cells, a process that can be stimulated by activating the β1 adrenoceptor through sympathetic activation. Notably, a certain correlation between Ang II and NA has been observed in severe HFMD patients [27]. Subsequently, liver-synthesized angiotensinogen is hydrolyzed by renin, resulting in the production of

Ang I. Upon circulation, this enzyme exerts local functions in various tissues. Angiotensin converting enzyme, which is abundantly present in the lung vascular endothelium, catalyzes the conversion of Ang I to Ang II. Upon binding to angiotensin type 1 receptors, Ang II triggers diverse physiological and pathophysiological effects, including sodium/water retention, vasoconstriction, pro-inflammatory and pro-thrombotic effects, and interactions with sympathetic nerves [32]. Excessive Ang II can synergistically interact with α1-adrenoceptors through catecholamines in the systemic vasculature, leading to a substantial increase in systemic vascular resistance [33]. In addition to sympathetic excitation, the inflammatory response can also activate the RAS system. Certain immune cells possess RAS elements and a complete repertoire of enzymes to synthesize their own Ang II. When inflammatory factors are recruited to the site of injury, the production of Ang II increases, thereby amplifying the inflammatory response. Ultimately, Ang II activates angiotensin type 1 receptors to promote the release of cytokines and chemokines, exacerbating aseptic lung injury. Although the involvement of the RAS system in the progression of severe HFMD is evident, the mechanism of its interaction with cytokines and the catecholamine storm remains unclear.

4. Regional immunity imbalance

Innate immune cells, comprising both tissue-resident and recruited populations, serve as the frontline responders to pathogen encounters within tissues. Resident immune cells, including macrophages and natural killer (NK) cells, are intricately regulated by local signals and environmental factors governing tissue physiological activities and localized protective responses. Disruptions to regional immunity can precipitate organ-specific inflammatory injuries. Evidence for EVA71 replication has been provided by the detection of the virus in post-mortem human lung tissue [2,34]. Autopsy findings from fatal HFMD cases revealed severe edema accompanied by a significant infiltration of inflammatory cells, including macrophages, neutrophils, and NK cells, alongside evidence of necrotizing tracheitis [34]. Some T lymphocytes were identified in the alveolar septum and peri-bronchial regions, while the majority of mast cells were located around the bronchi [34]. Furthermore, M2 type macrophages in the alveoli were found to be positive for both EVA71-VP1 and CD163 in a robust oral infection mouse model [35]. Macrophages serve not only as crucial target cells but also as effectors, amplifying proinflammatory cytokine responses. Moreover, EVA71-VP1 promoted the production of neutrophil chemokines, thereby facilitating the recruitment of neutrophils to the lungs of infected mice [36]. Neutrophils play a pivotal role in amplifying the inflammatory response by releasing an array of cytokines and generating reactive oxygen species (ROS), which contribute to the development of PE. Rapid mast cell degranulation was observed in animal models challenged with SARS-CoV-2, which led to the induction of pro-inflammatory factors and subsequent disruption of tight junctions [37]. Similarly, degranulated mast cells were identified in the lungs of EVA71-infected mice and showed a positive correlation with the severity of symptoms induced by EVA71 infection [18]. Degranulated mast cells surrounding blood vessels release vasoactive mediators such as histamine and nitric oxide (NO), which can directly increase vascular permeability and alter local hemodynamics. Analysis of lung proteomics from EVA71-infected mice further revealed that local innate and adaptive immune responses play a significant role in the progression of severe illness [38]. Briefly, local immune dysregulation and the consequent inflammatory response contribute to the development of PE [39]. In addition, autopsy results have indicated the occurrence of myocardial interstitial congestion, myocardial edema, myocardial necrosis, and inflammatory infiltrates [40]. In mouse models, viral infection resulted in heart injury characterized by localized leukocyte infiltration and subsequent inflammatory responses [35,41]. Moreover, an analysis of miRNA expression profiles unveiled the involvement of inflammatory responses, T cell activation, antiviral immunity, and NK cell infiltration in the pathogenesis of heart injury caused by Coxsackievirus (CV) A2 [42]. Anti-CXCR3 neutralizing antibody therapy has shown promise in ameliorating clinical symptoms and mitigating pathological changes in multiple organs caused by CVA2 [43]. Through the modulation of the IFN response triggered by EVA71 in both the lungs and brains, IRF3 agonists have been shown to effectively alleviate EVA71-induced illness, whereas TBK1 inhibitors have been found to exacerbate disease progression [44]. Hence, regional immunity plays a pivotal role in maintaining immune homeostasis and protecting the host from harm.

5. Vascular endothelial barrier dysfunction

In addition to hemodynamic alterations (Fig. 3A), PE may be linked to the heightened pulmonary vascular permeability resulting from brainstem lesions and/or systemic inflammatory responses [45]. Evidently, numerous studies have underscored the substantial role of vascular endothelial injury in the progression of HFMD severity (Fig. 3B and C). We have elucidated that the proposed molecular mechanisms of endothelial activation and dysfunction are multitudinous [46]. HEVs may disrupt vascular homeostasis by directly infecting endothelial cells. EVA71 infection can induce endothelial apoptosis directly. Notably, EVA71-VP1 alone has been shown to enhance the permeability of brain endothelial cell monolayers, while claudin-5 levels are reduced in brain tis-

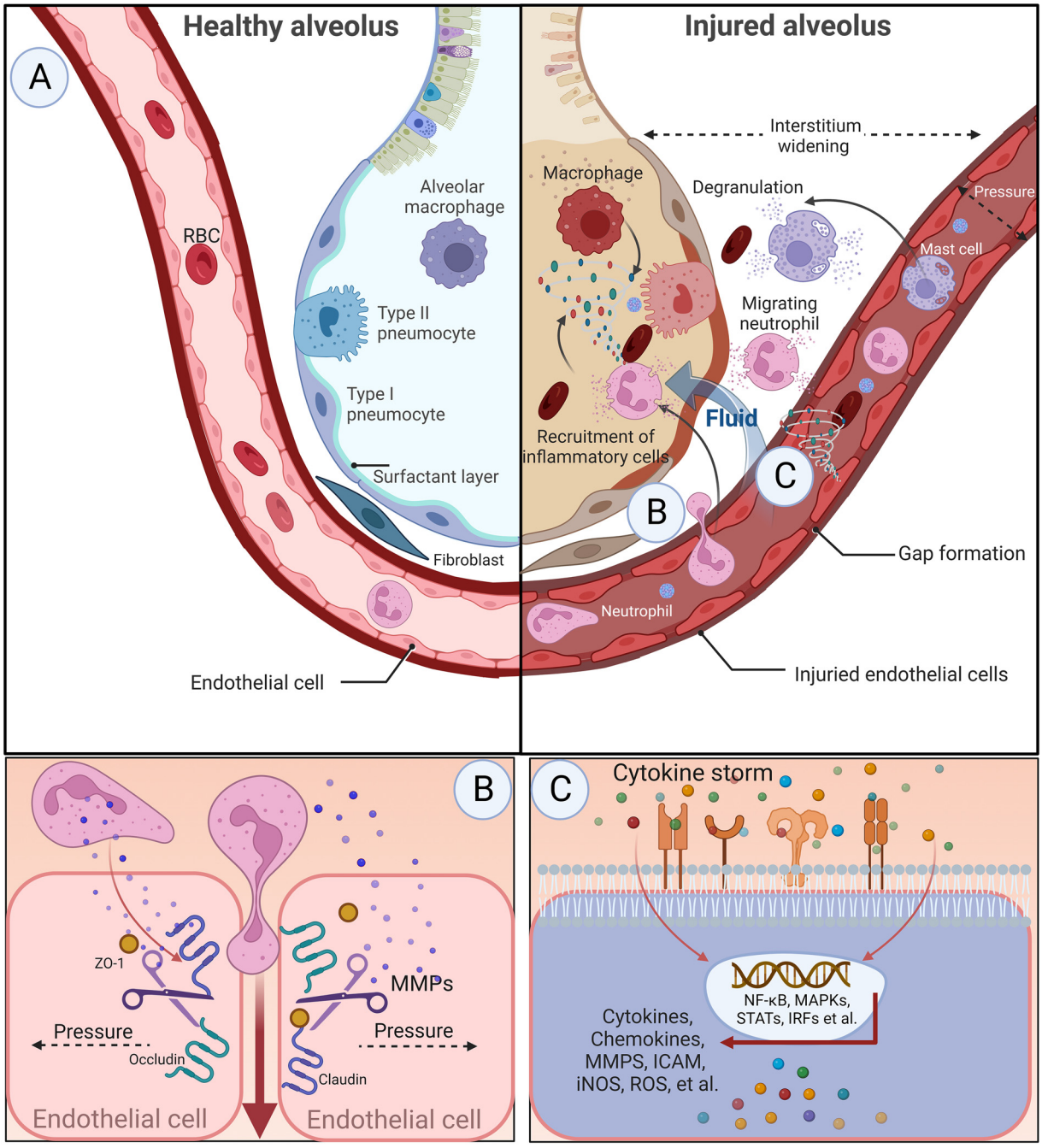

Fig. 3. Pathogenic mechanisms of PE in the progression of HFMD severity. (A) Hemodynamic changes and damage to the vascular endothelial barrier are the two primary factors contributing to pulmonary edema. Increased volume of pulmonary blood circulation; elevated pulmonary microvascular pressure resulting from pulmonary venous constriction; left heart insufficiency; and a combination of these factors cause a dramatic increase in intrathoracic blood volume with excessive hydrostatic pulmonary pressure. (B) Mechanical stress, overproduction of some hydrolases. (C) Circulating and local cytokines, ROS, and chemokine-mediated migratory infiltration of immune cells can damage the alveolar microvascular barrier, causing leakage. Inducible nitric-oxide synthase, iNOS; Intercellular cell adhesion molecule, ICAM. Created with BioRender.com.

sues or brain endothelial cells treated with VP1 [47]. VP1 also disrupts the integrity of the lung barrier by downregulating tight junction proteins [36]. Additionally, noncoding RNAs are implicated in the onset and progression of various human diseases associated with HEV infection [48]. The differential expression of miR-4516 may serve as the initial step in varying epithelial impairments by rupturing intercellular adhesion, ultimately leading to the distinct outcomes of EVA71 and CVA16 infections [49]. The elevated activity or expression of matrix metalloproteinase-9, which may target intercellular junction proteins or glycocalyx, was detected in both mouse brains and patient cerebrospinal fluid samples following EVA71 infection [50]. Infection with CVA16 results in the downregulation of miR-1303 expression, which in turn disrupts junctional complexes by directly regulating ma-

trix metalloproteinase-9, ultimately leading to pathological CNS changes [51]. In our mouse model, we also observed the disruption of tight junctions between endothelial cells, leading to the degradation of tight junction proteins such as ZO-1, claudin-5, and occludin [39,51,52]. The dysfunction of the endothelium has emerged as a pivotal contributor to the pathophysiology of severe HFMD, serving as an integrative and active platform for various mechanisms of injury. This dysfunction not only underlies the causative factors of cardiopulmonary failure but also facilitates neuronal invasion and injury.

6. Pivotal catecholamine storm

6.1. Fluctuating catecholamine levels

Catecholamines, including NA, EPi, and dopamine, mediate sympathetic activation, resulting in catecholamine disorders characterized by hypertension, tachycardia, and severe peripheral vasoconstriction. Tachycardia and systemic hypertension have been consistently observed in many clinical studies, with plasma levels of NA and EPi significantly elevated in 50% of severely ill patients [53]. A previous study demonstrated heightened sympathetic nerve activity, increased arterial pressure, and an elevated heart rate in patients with severe cases of EVA71 infections [54]. The concentrations of NA and Epi in the plasma are markedly elevated in HFMD patients with neurological injury and PE compared with those of mildly ill and/or healthy individuals, although sometimes no changes in Epi levels are observed [55]. The concentrations of NA in the sera of severe cases are notably higher than those in mild cases during the progression of HFMD from 1 day post infection (dpi) to 5 dpi. Furthermore, a peak in the concentration of NA in severe cases was observed at 3 dpi [27]. Moreover, levels of serum chromogranin A, co-released with catecholamines during exocytosis from sympathetic nerve terminals, are increased in severe HFMD patients [56]. The incidence rates of abnormal urinary catecholamine values (Epi and NA) are 100% in HFMD with hyper-sympathetic nervous activity and PE/hemorrhage, confirming the occurrence of a catecholamine storm [57]. In mouse models, the plasma levels of NA and EPi in EVA71-infected mice were increased at 2 dpi and peaked at 5 dpi [25]. The concentration of NA in EVA71-infected mouse brains was significantly elevated from 3 dpi to 7 dpi, in skeletal muscles at 5 dpi and 7 dpi, and in lungs at 7 dpi [27]. Elevated levels of pulmonary NA in the CVA6-infected mouse model were also detected from 3 dpi to 5 dpi [39]. After administering excessive catecholamine to a rodent model, most of the animals died around 5 h after NA infusion [58]. Additionally, intracerebral injection of NA exacerbated the severity of EVA71 infections in neonatal mice [16]. Therefore, the excessive production of catecholamines plays a role in disease deterioration. Future investigations should place emphasis on the mechanisms by which the infection activates sympathetic nerves and the subsequent biologic effects of excess catecholamines.

6.2. Catecholamine storm triggers and CNS injury

Circulating catecholamines primarily originate from both the peripheral (adrenal medulla) and central (sympathetic nerves) nervous systems. Stored within cytosolic granules, catecholamines are released via a Ca^{2+}-dependent mechanism prompted by the action potentials at adrenergic synapses and sympathetic discharges in the adrenal medulla. The sympathetic activation resulting from nervous system damage could be the primary instigator of the catecholamine storms observed in severe HFMD patients. Inflammation disrupts neurotransmission and neuroimmune communication, leading to sympathetic excitation. An animal model study demonstrated that proinflammatory cytokines, secreted by EVA71-infected astrocytes, stimulated neurons to release the monoamine neurotransmitter, Epi [15]. These cytokines can also activate adjacent uninfected cells and induce further neuroinflammation, thereby contributing to the dysregulation of the immune balance. Notably, the histopathological lesions caused by HEV infection are localized at some main sites (brainstem, pons, and medulla oblongata). They are pivotal sites of the CNS adrenergic system [59]. The destruction of the medial, ventral, and caudal medulla could lead to excessive sympathetic overactivation [54]. Overexcited sympathetic nerves release significant amounts of catecholamines, which function as neurotransmitters and neuromodulators. In areas of the brainstem and other regions with diffuse inflammation, a significant quantity of catecholamines influences neurons and immune cells, ultimately promoting brainstem encephalitis. Together, the evidence shows that the catecholamine storm is initiated by specific trigger zones in EVA71-related pathology. These excessive catecholamines directly damage multiple organs and exert various diverse biological effects on immune, metabolic, and other pathways, thereby exacerbating the severity of the illness.

6.3. Catecholamine storm contributes to HFMD severity

In fatal cases of EVA71 infection, markedly elevated levels of plasma catecholamines may exacerbate vascular permeability, peripheral vasoconstriction, and pulmonary venous constriction, ultimately culminating in cardiac dysfunction and neurogenic PE. Additionally, NA and EPi can potentially enhance the infectivity and replication of EVA71 [24]. Adrenergic receptors are primarily categorized into $\alpha1$, $\alpha2$, $\beta1$, $\beta2$, and $\beta3$ receptors, which mediate the transmission of external catecholamine stimuli

into intracellular signals. NA mainly stimulates α and β1 receptors, while EPi activates all subtypes of α- and β-adrenergic receptors. Stimulation of the α1-adrenergic receptor leads to heightened pulmonary microvascular permeability and capillary pressure. Catecholamine-related hemodynamic effects are major causative factors in the initial phase of pulmonary fluid displacement, whereas inflammation and increased capillary permeability serve as secondary factors [60]. Excessive NA induces cardiomyocyte apoptosis *in vitro* via the β-adrenergic pathway, a process associated with heart failure [61]. The high concentrations of catecholamines resulting from brainstem encephalitis are one of the causative factors of acute heart failure [62]. Subsequently, the left ventricular compliance decreases, leading to an increased intrathoracic blood volume with excessive hydrostatic pulmonary pressure, eventually resulting in PE. In a rodent model mimicking the fulminant course of heart failure induced by excessive catecholamine, the left ventricle volume at diastole significantly increased half an hour after NE infusion [58]. The high concentrations of catecholamines associated with brainstem encephalitis either precede or coincide with EVA71-related acute heart failure, ultimately resulting in increased mortality [53]. A pathological examination of cardiac ventricular tissue from fatal cases revealed the presence of coagulative myofibrillar lysis and cardiomyocyte apoptosis, which are characteristic features of catecholamine-related cardiotoxic effects [62]. Additionally, the vasoconstrictive effect of excessive catecholamines tends to diminish in the later stage of heart failure, contributing to vasodilatory shock, partially through the effect of NO via β2-mediated post-receptor signaling [45]. Therefore, it is proposed that the combined effects of NA cardiotoxicity and vasodilatory shock may be the dominant causes of heart failure and mortality among EVA71-infected patients.

Based on the evidence presented above, damage to the dorsal vagus nucleus and nucleus tractus solitarius in the brainstem may activate the sympathetic nerve, resulting in a sudden increase in catecholamine levels, which could lead to uncontrollable outcomes such as heart failure and PE.

7. Molecular mechanisms of neurological damage

7.1. Invasion of CNS by HEVs

After ingesting food and water contaminated by HEVs, the palatine tonsil and oral mucosa support active initial viral replication [63]. Upon shedding into the oral mucosa, HEVs can penetrate the intestinal tract and replicate within the intestinal mucosal epithelium. Following replication, the virus migrates to adjacent lymph nodes before entering the bloodstream, leading to a transient and mild viremia. Viremia usually manifests between day 1 to 7 from the onset of the disease (with a median on day 4), and it is more prevalent among infants. An extended duration of viremia is correlated with greater disease severity [64]. After 4 to 5 days, a secondary viremia occurs, allowing the virus to disseminate throughout the body, including the CNS [1]. It is plausible that multiple pathways may act synergistically to facilitate its subsequent invasion of the CNS (Fig. 4A and B).

Firstly, HEVs may breach the blood-brain barrier (BBB) by altering several BBB functions. The BBB typically acts as a barrier separating the CNS from the circulatory system, regulating the transport of molecules to and from the CNS. In a rhesus monkey model, researchers showed that, by exploiting the biological function of matrix metalloproteinase-9, CVA16 penetrates the BBB, gaining access to the CNS by disrupting junctional complexes [51]. The capsid protein VP1 of EVA71 can similarly disrupt the BBB and upregulate the expression of the viral receptor (vimentin), facilitating viral entry into the CNS [47]. Exosomes released from EVA71-infected cells possess the capability to establish a productive infection in human neuroblastoma cells and evade the immune system [65]. Indeed, EVA71 has been detected in exosomes isolated from the plasma of severe EVA71-infected children [66]. The binding between EVA71 3A and vacuolar protein sorting 25, which is associated with exosome production, promotes exosome biogenesis, thereby boosting viral replication [67]. Brain microvascular endothelial cells may endocytose small extracellular vesicles containing EVA71 viral components and release them on the abluminal side of the BBB [68]. During the early stage of infection, EVA71 contained within exosomes can infect astrocytes without disrupting the BBB, indicating a crucial role of exosomes in the pathogenesis of EVA71 in the CNS [68,69].

Secondly, EVA71 can penetrate the CNS by utilizing the retrograde axonal transport route. Recently, studies using microfluidic devices identified the neural pathway that facilitated the spread of EVA71 in adult human scavenger receptor class B, member 2 Tg mice [70]. Probable clinical evidence, such as the segmental distribution of lesions in the CNS, strongly supports the hypothesis of the retrograde axonal transport of EVA71 invasion [71]. Several studies have demonstrated that the virus may spread from skeletal muscles to the peripheral motor nerves and subsequently into the CNS in infected mice [72]. Nevertheless, the pronounced muscle tissue tropism observed in neonatal mice is not observed in human infections. Moreover, the virus can enter the CNS not only via the motor components of spinal nerves but also through cranial nerves [73]. Autopsy results have also provided direct pathological evidence supporting the potential for viral entry into the CNS through the peripheral nerves [74].

Thirdly, the virus may exploit infected immune cells in the peripheral circulation as vehicles for transporting

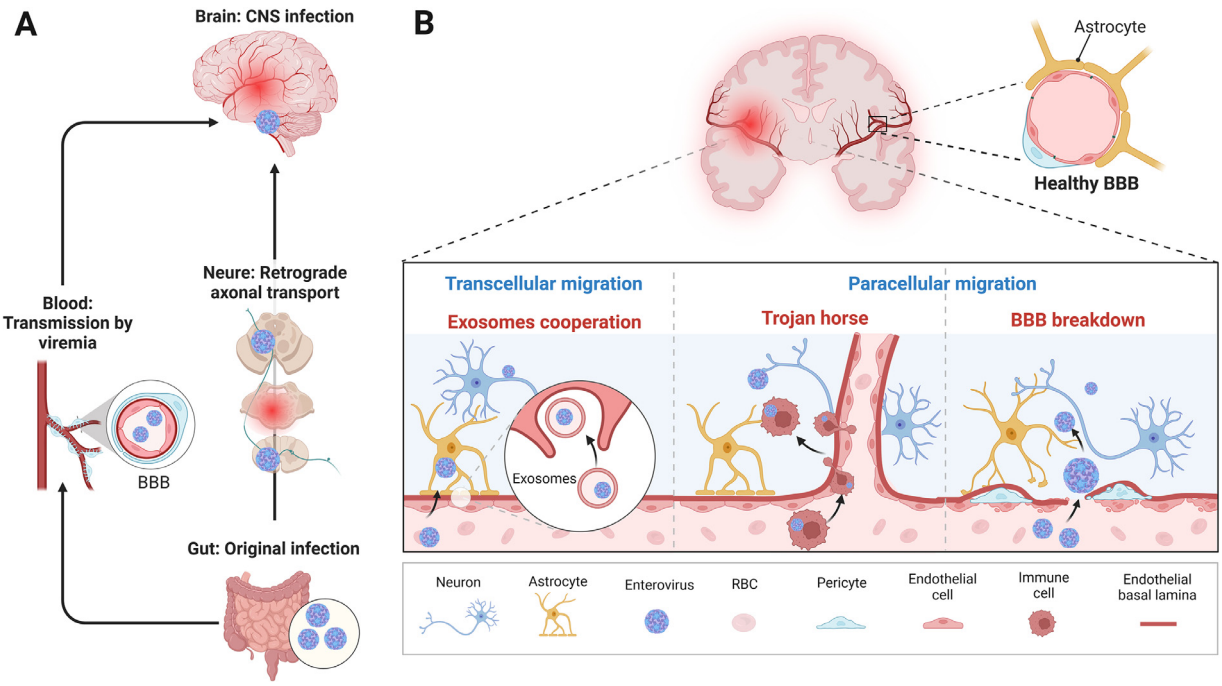

Fig. 4. Pathways by which HEVs invade the CNS. (A) The viruses replicate rapidly in intestinal epithelial cells and then invade the CNS through retrograde axonal transport along the nerve axis, or across the BBB. (B) The viruses could cross the BBB via a paracellular or transcellular pathway. Potentially, viruses can hitch-hike across the BBB in immune cells in a "Trojan horse" fashion. Created with BioRender.com.

intracellular viruses to the CNS, which is known as the "Trojan horse" route. Studies have indicated that EVA71 and several HEVs can infect various immune cells [17], suggesting the possibility that these viruses might hijack immune cells to infiltrate the CNS. Further studies are warranted to assess the extent of viral invasion of the CNS facilitated by immune cells as shuttles.

Taken together, evidence shows HEVs are transmitted to the CNS either through the BBB or via retrograde axonal transport. Investigating the virus' capacity for CNS invasion from primary infection sites is essential to understand the underlying mechanisms of HFMD severity. Apparently, blocking viral access to the CNS or preventing further neuropathology could potentially mitigate disease severity. Despite the extensive research conducted to comprehend CNS invasion, further studies are necessary to elucidate the invasive pathways.

7.2. The pathophysiology of CNS injury caused by HEVs

The CNS constitutes the primary component of the nervous system and is composed of the spinal cord and brain. The spinal cord, housed within the spinal canal, connects to both the brain and the peripheral nerves. The brain continues from the spinal cord and resides within the cranial cavity. The brain can be divided into four main parts: the brainstem, diencephalon, cerebrum, and cerebellum. Previous neuroimaging data have demonstrated that HFMD patients with neurological complications exhibit magnetic resonance imaging abnormalities in the posterior portions of the medulla oblongata and pons, along with the ventral horn and bilateral anterior horns of the spinal cord [71,75]. An autopsy of a fatal case revealed mild cerebral edema with shallow sulci and a flattened gyrus, and cerebrovascular hyperemia was observed grossly. No obvious hemorrhage or necrosis was observed in the brain parenchyma [76]. In the spinal cord, the primary pathological feature was widespread inflammation in the central gray matter, predominantly affecting the upper anterior horn of the spinal cord and extending to involve the entire gray matter across all levels of the spinal cord [74,77]. Brainstem encephalitis represents the primary manifestation of EVA71 involvement in CNS, with affected regions potentially extending to the midbrain, thalamus, and cerebellum as the disease progresses [78]. Pathological examinations have revealed slight lymphocytic infiltration of the meninges, parenchymal infiltration, diffuse or nodular hyperplasia of macrophages/microglia, and cuffs of dense inflammatory cells infiltrating areas around the vessels [79]. In the brainstem and spinal cord, multiple or focal encephalomalacia with foam cells has been observed, although less inflammation is typically noted in the cerebral motor cortex. Some cases also exhibit swollen astrocytes and microabscess-like formations associated with extensive neuronal degeneration and necrosis [74].

Each member of the HEV family exhibits a distinct tropism for different regions of the CNS, likely influenced by a combination of cellular receptors and virus-specific factors [80,81]. Nonetheless, the mechanisms by which HEVs target specific regions and cell types within the brain and spinal cord remain unclear.

7.3. Immunopathology of CNS injury

The CNS is safeguarded by intricate multi-layer barriers that regulate access to nutrients and interactions with peripheral tissues. Apart from neurons, three main types of non-neuronal cells types, astrocytes, oligodendrocytes, and microglia, play indispensable roles in maintaining homeostasis. Furthermore, viral genomic loads have been observed to be positively correlated with the severity of HFMD. In fatal cases, viral antigens were predominantly observed in neurons undergoing neuronophagia or degeneration, as well as in astrocytes, microglia cells, and inflammatory cells [2,74,77]. This suggests that the direct neuronal damage resulting from viral replication is a crucial mechanism in tissue injury. In mouse models, viruses were also found to directly infect neurons and astrocytes, thereby inducing neurological disease [82]. Further investigations have demonstrated that HEVs directly induce neuronal impairment by triggering apoptosis and autophagy through various mechanisms. For instance, EVA71 3Dpro can increase ROS production by suppressing acyl-CoA oxidase 1 protein expression and peroxisome numbers, thereby inducing apoptosis and autophagy in neurons [83]. EVA71 also causes severe neurological damage by upregulating the expression of cyclooxygenase-2, which mediates the activation of AKT, MAPK, NF-κB, and AP-1 pathways [84]. Additionally, the absent in melanoma 2 protein, which is associated with EVA71 replication, plays a critical role in pyroptotic cell death following EVA71 infection through the upregulation of Caspase-1 and IL-1β [85].

Many researchers assert that immunopathology, rather than direct damage to the gray matter of the brainstem, is the primary factor in the pathogenesis of neurological diseases. Autopsy results demonstrate that inflammatory infiltrates consist primarily of $CD68^+$ macrophages/microglia and $CD15^+$ neutrophils, and this is particularly evident in microglia nodules and perivascular cuffs [86]. The heightened activation of microglia, astrocytes, and neurons may lead to the temporary and spatially dysregulated release of neurotoxic substances. Ultimately, this phenomenon decreases neuronal activity, disrupts synaptic connectivity, and contributes to neuron death. Activated astrocytes play a crucial role in modulating catecholamine release by secreting cytokines, particularly IL-6, which can contribute to neuronal damage [15]. Experiments demonstrated that intracerebral injection of IL-6 and adrenaline exacerbated the severity of EVA71 infection, while counteracting this effect with an IL-6-neutralizing antibody or an adrenergic antagonist reversed the lethal effect of EVA71 in neonatal mice [16,87]. Moreover, M1-polarized microglial cells exhibit the dysregulated secretion of cytokines, including IL-1β, IL-6, and TNF-α, along with the generation of ROS and NO [88]. Furthermore, severe CVA6 infections in mice have been associated with the infiltration of neutrophils and monocytes into the brain [89]. Neutrophil infiltration can inflict damage on neuronal systems through the release of cytokines, ROS, and other substances.

Toll-like receptors (TLRs) play pivotal roles as the immune system's frontline defense. TLR7 and TLR8 exhibit significant expression in the brain tissues of fatal EVA71 infection cases, suggesting their potential involvement in the pathogenesis of neuronal damage [90]. EVA71 infection triggers neurodegeneration by activating TLR7 signaling, leading to the enhanced production of IL-6 [87]. Similarly, the engagement of EVA71 with intracellular TLR9 induces a neurotoxic glial response through IL12p40-iNOS/NO signaling, thereby contributing to the neurological manifestations associated with EVA71 infection [91]. Nonetheless, the innate immune escape linked to the dysfunction of TLR receptors could also account for the serious consequences of the infection. TLR3 deficiency might underlie severe EVA71 infection with encephalitis [92]. Additionally, a proteomic analysis of infected mouse brains suggested the involvement of complement factors, coagulation cascades, innate and adaptive immune responses, platelet activation, and nitrogen metabolism in the progression of severity [38].

Despite the extensive research on neurological pathogenesis, the identification of key molecules that could serve as the theoretical basis for drug development and preventive measures remains elusive. Understanding the complex interactions between neurons and immune cells post-infection can provide new insights for future research into HFMD pathogenesis.

8. Conclusions and perspectives

Collectively, this review has provided an overview of recent findings regarding the severity of HFMD. Cytokine storm, RAS, regional immunity, endothelial dysfunction, catecholamine storm, and the cellular molecular mechanisms of neurological damage have been reviewed and discussed (Fig. 5). It is speculated that the domino effect of multiple systems triggered by damage to the CNS are key mechanisms of HFMD severity. Neurogenic PE, resulting from the sympathetic activation associated with CNS damage, is widely recognized. Within a short time, severe illness occurs as a result of the comprehensive effects of CNS involvement and subsequent multi-system responses. The early identification of signs indicating severe disease, anticipating the progression of HFMD severity, and providing appropriate multidisciplinary preventive interventions are key pursuits for reducing HFMD mortality. Currently, we still are unable to accurately predict which patients will develop severe disease, making it challenging to assign treatments to those most in need. Therefore, identifying reliable biomarkers associated with CNS in-

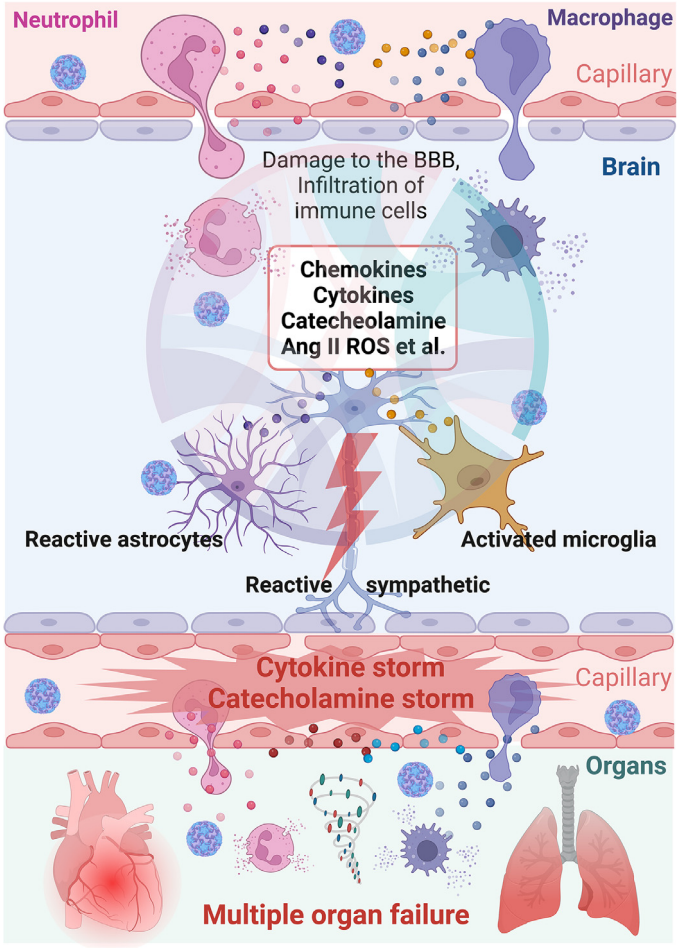

Fig. 5. Theoretical hypothesis diagram: multi-system dysfunction resulting from systemic hyperinflammatory response contributes to HFMD severity. HEVs invade the CNS through multiple routes, leading to the activation of glial cells. Lesions in the brainstem, thalamus, and medulla oblongata, as well as other CNS tissues, stimulate sympathetic nerves and release large amounts of neuroactive substances, such as catecholamines. Excessive catecholamines have a direct impact on related receptors, resulting in hemodynamic changes that can lead to cardiopulmonary dysfunction. Catecholamines further affect cardiopulmonary function by interacting with the RAS, exacerbating cytokine storms and aggravating regional immunity. A variety of harmful factors combine to disrupt vascular endothelial barrier function. Created with BioRender.com.

volvement in the early stages holds potential for predicting the development of severe disease, offering considerable clinical utility for patient management. The individualized immune system plays a crucial role in determining a patient's initial susceptibility to HFMD severity. A mild and appropriate immune response is capable of eliminating viruses in a timely manner, thereby preventing severe secondary viremia and subsequent sympathetic activation. Therefore, the development of high-potency antiviral drugs or vaccines remains the most promising treatment research field. Given the spectrum of pathogenic mechanisms implicated in the progression of severe HFMD, it is unlikely that a solitary treatment will be effective once the viruses attack the CNS. Multiple avenues of treatment may be required, and significant effort should be invested in determining the most dominant pathogenic processes. Understanding how these multi-system responses are generated and how they interact with each other is crucial. Further studies are especially needed to clarify the mechanism of CNS damage and the important roles of both catecholamine-related cardiopulmonary toxicity and hemodynamic changes arising from multiple system participation in the occurrence and development of HFMD severity. Addressing the scope and burden of HFMD morbidity and mortality will have significant implications for clinicians and medical researchers. Because prophylactic and therapeutic agents are not yet well-established globally, studies focusing on the development of new antivirals are critically important to reduce disease burden from pediatric infections. We have summarized several potential representative therapeutic drugs and existing pharmacological modulators in Table S1. Translating the current theories into clinical practice will be a high priority for years to come.

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Author contributions

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Declaration of competing interest

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Data available statement

Data sharing is not applicable to this article, as no datasets were generated or analyzed during the current review.

Ethics statement

Ethics approval was waived for this review because no patient data were reported.

Informed consent

Not applicable.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.imj.2024.100124.

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