Cytokine Immunopathogenesis of Enterovirus 71 Infection

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1 Introduction

Humoral mediators including cytokines are the molecular proteins of the innate and specific immune response, play key roles in the pathophysiology of viral infection (Dinarello, 1997). Cytokines, pleiotropic immunological messengers that both boost and sequester inflammation, are produced in response to infections and other stimuli and extend a request to surrounding and/or distant cells for a specific response. Specific cytokines have autocrine, paracrine, and/or endocrine activity and, through receptor binding, can elicit a variety of responses, depending upon the cytokine and the target cell (Tisoncik et al., 2012). Systemic inflammatory response syndrome (SIRS) caused by infection, is a typical condition with in which pro-inflammatory mediators released from infected cells, and persistent hypercytokinemia may result in progression to multiple organ failure (Oda et al., 2005). It is known that activation of cytokine networks increases levels of various cytokines in blood. The burst of cytokine release that follows sepsis, toxin-mediated shock syndrome (eg, Streptococcus pyogenes and Staphylococcus aureus) (Nakane et al., 1995; Wang et al., 2008), some virus infections such as severe acute respiratory syndrome (SARS) coronavirus (Cameron et al., 2008), influenza virus (Skoner et al., 1999), dengue virus (Lei et al., 2001) and Epstein-Barr virus (Canna et al., 2012) induce an overwhelming stimulation of innate and/ or immune responses that storm the physiology of the body. The cytokine response is strong enough to flood beyond the site of infec-

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tion, it induces reactions on a systemic level, potentially causing life-threatening tissue damage (Nazinitsky & Rosentha, 2010).

2 Clinical Spectrum of EV71 Brain Stem Encephalitis

Human enterovirus 71 (EV71) is a member of the genus Enterovirus, family Picornaviridae, which consists of a non-enveloped capsid surrounding a core of single stranded, positive-polarity RNA approximately 7.5 kb in size and 27-30 nm in diameter (Hsiung & Wang, 2000; McMinn, 2002). EV71 produces a broad spectrum of clinical manifestations. The majority of infected individuals have asymptomatic infection. Mild cases characterized as cutaneous diseases such as hand-foot-and-mouth disease (HFMD) and herpangina. However, potentially life-threatening neurological complications such as brain stem encephalitis (BE) are of the greatest clinical and public concern (Wang et al., 1999; Ho et al., 1999; Huang et al., 1999; Lin et al., 2002). EV71 has been recognized as highly neurotropic and associated with a diverse range of neurological diseases, such as aseptic meningitis, BE, encephalomyelitis, acute flaccid paralysis (AFP) and post-infectious neurological syndromes. After the 1998 Taiwan epidemic several clinical stage categories of the disease were developed for the severity of BE to help monitor the clinical course of EV71 infection and to aid management. These systems, however, are not widely accepted, possibly because they are not always simple to follow by primary care physicians. In 2011, World Health Organization Regional Office (WHO) for the Western Pacific and the Regional Emerging Diseases Intervention (REDI) Centre documented guide for clinical management on Hand, Foot, and Mouth Disease, has proposed a simple clinical stages of disease manifestation to describe the disease severity (Wang et al., 2008; Wang et al., 2009). The EV71 BE was stratified into three important critical stages by disease severity, including uncomplicated BE, autonomic nervous system (ANS) dysregulation, and pulmonary edema (PE) (Figure 1), which resulted in high mortality rates (Ho et al., 1999; Huang et al., 1999; Ooi et al., 2010; Solomon et al., 2010; Wang et al., 2014) or long-term neurologic sequelae in survivors (Huang et al., 2006).

The EV71 BE is a continuous and dynamic disease sequence. It may be a reversible disease because each critical stage is a turning point in early period. Through this staging system the pathogenesis of BE was explored and then effective ways to manage the patients was developed. BE is defined as an illness characterized by myoclonus, ataxia, nystagmus, oculomotor palsies, and bulbar palsy in various combinations, with or without neuroimage evidences. ANS dysregulation is defined by the presence of cold sweating, mottled skin, tachycardia, tachypnea, and hypertension. PE is defined as respiratory distress with tachycardia, tachypnea, rales, and frothy sputum that developed after ANS dysregulation, together with a chest radiograph that showed bilateral pulmonary infiltrates without cardiomegaly (Figure 2).

If the diagnosis of EV71 BE once delayed, usually because of the clinical symptoms are not recognized in the early time. Myoclonic jerks are seen more often in EV71 than in other serotypes of the enterovirus in infected patients, and could be an early indicator of brain stem involvement. Diagnostic work-up of EV71 BE should include the
**Figure 1:** Clinical stages of enterovirus 71 infection by disease severity.

**Figure 2:** The clinical manifestations of autonomic nervous system (ANS) dysregulation and pulmonary edema (PE) of enterovirus 71 (EV71) infection. Severe vasoconstriction (left upper), tachycardia and hyperthermia (left lower) in a patient with EV71 associated ANS dysregulation. Radiological evidence (right upper) and frothy tracheal aspirates (right lower) in a patient with EV71 associated PE.
search for one or more neurological symptoms, especially myoclonus jerk and limb paralysis, and the measurement of disease biomarkers, such as peripheral white blood cell count, platelet count, glucose level, inflammatory cytokines and chemokines, immune cell subsets and cerebrospinal fluid analysis (Lin et al., 2002; Chang et al., 1999; Wang et al., 2003; Lin et al., 2003; Wang et al., 2007; Wang et al., 2008).

In the 2008 outbreak of Taiwan, 238 virologically and clinically confirmed severe cases identified, include 41% uncomplicated BE, 44% ANS dysregulation and 15% PE (Wang et al., 2012). The EV of different serotypes has been co-circulated in Taiwan yearly. The EV and EV71 isolates distribution by year since 1998 at National Cheng Kung University Hospital was illustrated (Figure 3). EV71 plays a cardinal role among the EV epidemics in 1998, 2000, 2001, 2008 and 2012.

![Figure 3: Yearly distribution of enteroviruses and enterovirus 71 isolates of National Cheng Kung University Hospital, Tainan, Taiwan, 1998-2014.](image)

3 Pathogenesis of Complicated EV71 Brain Stem Encephalitis

Both innate and adaptive immune mechanisms are important for host defense against viral infection. The innate immune system provides the first line of defense against virus by activation of adaptive immunity through antigen presentation as well as secretion of pro-inflammatory cytokines. The pathogenesis of PE and hemorrhage in EV71 infections has been studied, with some important findings. Destruction of vasomotor and respiratory centers in the medial, ventral, and caudal medulla by EV71 leads to
ANS dysregulation and PE. It is similar to that observed in bulbar poliomyelitis, produces sympathetic hyperactivity, with surge catecholamine and autonomic dysfunction (Wang et al., 2003; Kao et al., 2004). Abundant catecholamines and strong central nervous system (CNS) inflammatory responses aggravate cytokine storm and pulmonary vascular permeability, causing PE. Catecholamines are among the neurotransmitters that affect immune responses humorally through circulating epinephrine, as well as locally through neuronal release of norepinephrine (NE) (Haskó, 2001). Plasma concentrations of NE and epinephrine (EP) were significantly higher in EV71-infected patients with ANS dysregulation and PE than they were in EV71-infected patients merely with uncomplicated BE. Both α1A- and β2-adrenergic receptors were expressed on A549, RD, SK-N-SH, HL-60, THP-1, Jurkat cells and hPBMCs. NE and EP treatment elevated the percentages of EV71-infected cells in THP-1 and Jurkat cells. α- and β-blockers reduced the percentages of EV71-infected cells with NE or EP treatment. NE and EP may play a role in the pathogenesis of EV71 BE complicated with ANS dysregulation and PE. IL-6 production was enhanced in EV71-infected hPBMCs at a concentration of 10^2 pg/mL NE (Liao et al., 2015). Local release of neuroendocrine mediators coupled with specific receptor expression in immune cells establishes a functional neuroimmune connection capable of modulating various responses, including cytokine production. The excessive sympathetic activation plays a vital role in the pathogenesis and the progression from EV71 BE to PE.

4 Cytokine in the Systemic Inflammatory Response of EV71 Infection

There is increasing evidence that pro-inflammatory and anti-inflammatory cytokines may play a central role in EV71 BE. PE might be the result of increased pulmonary vascular permeability caused by the brain stem lesions and/or a systemic inflammatory response syndrome produced by the release of cytokines and chemokines. The clinical presentation of EV71 PE is caused by a hyperinflammatory syndrome resulting from hypercytokinemia and central nervous system inflammation of various inflammatory mediators. Some studies have shown that pro-inflammatory cytokines (interleukin [IL]-6, tumor necrosis factor [TNF]-α, and IL-1β) are associated with BE that is complicated by PE (Lin et al., 2002; Lin et al., 2003). A significant elevation of plasma IL-10, IL-13, and interferon (IFN)-γ levels are observed in patients with PE (Wang et al., 2003). CD4⁺ and CD8⁺ T cell, and NK cells were depleted in patients with PE. The depletion of CD4⁺ and CD8⁺ T cell subsets and NK cells may cause the release of specific cytokines, contributed to the immunopathogenesis of EV71 BE. Human P-selectin glycoprotein ligand-1 (PSGL-1; CD162), a sialomucin membrane protein expressed on leukocytes that has a major role in early stages of inflammation, tethering and rolling of leukocytes on vascular endothelium, was proved as a functional receptor for EV71 infection (Nishimura et al., 2009). The interaction of EV71 with PSGL-1 on lymphocytes may induce production of the inflammatory cytokines involved in BE with PE (Patel & Bergelson, 2009). In an animal study, monkeys were intravenously inoculated with cDNA-derived PSGL-1-
binding (EV71-02363-EG) and PSGL-1-nonbinding (EV71-02363-KE) strains of EV71, respectively. Mild neurological symptoms, transient lymphocytopenia, and inflammatory cytokine responses, were found predominantly in the 02363-KE-inoculated monkeys. In general, cytokine responses were more evident in 02363-KE-inoculated monkeys than those in EG-inoculated monkeys (Kataoka et al., 2015). Mononuclear phagocytic cells are the most important source of IL-6; however, IL-6 is also produced by T and B lymphocytes and numerous other cells (Akira et al., 1993). Elevated plasma level of IL-6 was detected in EV71-infected patient with ANS dysregulation, the priming stage of PE (Wang et al., 2006). The IL-1β, IL-6 and TNF-α levels in fatal patients with encephalitis plus PE were significantly higher than those of uncomplicated patients. Elevated IL-6 may represent the net effect of IL-1β and TNF-α biological actions. IL-6 > 70 pg/ml was suggest as the best predictor of EV71 encephalitis with PE (Lin et al., 2002; Lin et al., 2003).

EV71 infection significantly increased the release of IL-6 from dendritic cells (Lin et al., 2009). IL-6 and T cells are shown to reduce mortality of EV71-infected mice by reducing tissue viral loads in a previous study (Lin et al., 2009). However, Khong and coworkers reported that administration of anti-IL-6 neutralizing antibodies, at day 3 or 6 post-infection, after the onset of the clinical symptoms successfully improved the survival rates and clinical scores of the EV71 infected mice (Khong et al., 2011). Compared to untreated infected controls, anti-IL-6-treated mice displayed reduced tissue damage, absence of splenic atrophy, increased CD4+, CD8+ T cells and B cells activation and markedly elevated systemic levels of IL-10. Further, the anti-IL-6 antibody-mediated protection is independent of the virus load. These findings are justified in treating EV71-infected patients complicated with ANS dysregulation with intravenous immunoglobulin (IVIG) (Lin et al., 2009). However, anti-IL-6 treatment at the time of infection is detrimental to the mice. It means that IL-6 production is beneficial to the host early post-infection to trigger the antiviral host response through attraction of various immune cells; however, sustained high levels of IL-6 may cause tissue damage and immunopathology.

IL-10 initially described as a product of Th2 cells that inhibited cytokine synthesis in Th1 cells. It has emerged as an important immunoregulatory cytokine with multiple biologic effects known to be sourced principally from monocytes/macrophages, dendritic cells, CD4+, CD8+ and Treg lymphocytes during or shortly after antigen presentation (Duell et al., 2012). IL-10 can both impede pathogen clearance and ameliorate immunopathology. Numerous human viral pathogens have been shown to induce IL-10 in various studies. IL-10 was significantly higher in EV71-infected patients with PE than in those with ANS dysregulation or uncomplicated BE (Wang et al., 2003). A close relationship exists between catecholamine and IL-10 release (Grilli et al., 2000). Systemic IL-10 release triggered by sympathetic activation may be an important neuroimmunological mechanism contributing to immunodepression after injury and stress. Woieciechowsky and coworker showed surge of systemic IL-10 and immunodepression closely dependent on brain stem involvement and sympathetic activation (Woieciechowsky et al., 1998). IL-10 can be modulated in several acute and chronic neuropathological conditions. This suggests that IL-10 plays a role in the immune-regulatory functions of the CNS. Thus,
the systemic IL-10 increase in patients with PE appears to be triggered by persistent sympathetic activation as a consequence of direct brain stem destruction by the virus. IL-10 inhibits production of several pro-inflammatory mediators, including IL-1, IL-6, IL-8, granulocyte colony stimulating factor and TNF-α, and upregulates the expression of the naturally occurring IL-1 receptor antagonist (Duell et al., 2012). In an animal study of EV71 infection, anti-IL-6 treatment resulted in dramatically increased IL-10/IL-6 ratios, reflecting the upregulation of IL-10 production in the treated animals. Therefore, upregulation of IL-10 likely helped balance between pro-inflammatory and anti-inflammatory cytokines and subsequent tissue damage (Khong et al., 2011). Furthermore, low levels of IL-10 in the bronchoalveolar lavage fluid of patients with acute respiratory distress syndrome were associated with a poor prognosis (Donnelly et al., 1996). Upregulated IL-10 levels may have a protective effect in the development of PE by influencing the pulmonary capillary permeability. IL-10 may play a double-edged role, appears to function as both sword and shield in the response to EV71 infection in the pathogenesis of PE (Wang et al., 2003).

Interferon (IFN)-γ plays a central role in the immune response against infection. IFN-γ, a pleiotropic cytokine, is secreted by both cells of the innate immune system (NK cells, γδ T-cells) and the adaptive immune system (CD8+ T cells and Th1 CD4+ T cells). NK cells and γδ T cells provide early sources of IFN-γ during infection (Boehm et al., 1997). Elevated plasma levels of IFN-γ in patients with uncomplicated BE and PE was found. Moreover, the kinetic analysis in patients with PE showed that the production of IFN-γ occurred 24 h after IL-10 production. Increased pulmonary vascular permeability may play a pivotal role in PE. IFN-γ can exhibit enhanced vascular permeability (Martin et al., 1988). IFN-γ-mediated microvascular leakage occurs as a result of the reduced endothelial barrier and tight junction (Corada et al., 1999). IL-10 is a cytokine synthesis inhibitor that will terminate the production of IFN-γ. IFN-γ production appeared later than that of IL-10 in PE patients, which suggests that IFN-γ might play an important role in the development of PE. Recently, IFN-γ was found to be significantly elevated in infected AG129 mice, which lack type I and II interferon receptors, are susceptible to infection with a non-mouse-adapted EV71 strain via both the intraperitoneal and oral routes. The defect in IFN signaling may lead to some compensatory changes in the pattern of immune responses, which implies this model may not accurately reflect the immunopathogenesis seen in immunocompetent patients (Khong et al., 2012). Liao and colleagues demonstrated that the IFN-γ receptor is likely to be critical for protection from EV71 induced paralysis and death by using an IFN-γ receptor knockout (ifngr KO) mouse model. ifngr KO mice, defective only in IFN-γ, not in IFN-α, displayed paralysis and death rates around 80% (Liao et al., 2014). Severe EV71-infected patients with PE have lower numbers of peripheral circulating leukocytes, including natural killer (NK) cells, CD4+ and CD8+ T cells, in comparison with patients of mild disease (Wang et al., 2003). Whereas, invariant natural killer T (iNKT) cells are a distinct subpopulation of T cells that express an invariant αβ T cell receptor (TCR) and share many cell surface markers in common with NK cells. NK and iNKT cells both produced IFN-γ after mouse-adaptive EV71 infection. Zhu et al. display that EV71 infection primarily activates iNKT cells, and in return, presumably promotes the activation of NK cells. The
mouse-adaptive EV71-infected macrophages dramatically induced IFN-γ production by iNKT cells (Zhu et al., 2015).

Polymorphism of some inflammatory mediators associated with the complications and disease severity. Yang et al. reported that IFN-γ + 874 A allele and IL-10-1082 A allele was observed with significantly higher frequency in patients with EV71 encephalitis compared with HFMD patients without complications (Yang et al., 2012). Macrophages, NK cells, dendritic cells and fibroblasts were reported to produce IFN-1 (α and β) in response to viral infection or exposure to other microbial pathogens. The early induction and action of IFN-1 result in cellular resistance to viral infection, inhibition of viral replication and impediment of viral dissemination (Samuel, 2001). Liu and coworkers showed that polyriboinosinic : polyribocytidylic acid [poly(I:C)], a potent IFN inducer, improved the survival rate and decreased the tissue viral titers after EV71 challenge, which correlated with an increase serum concentration of IFN-α, the percentage of dendritic cells, the expression of major histocompatibility complex class II molecule and IFN-α in spleen of mice (Liu et al., 2005). Type I IFNs represent an essential innate defense mechanism for controlling EV71 infection in mice.

IL-13 is a T-helper type 2 (Th2) cytokine produced predominantly by Th2-polarized CD4+ T cells that has potential anti-inflammatory activity and suppresses the cytotoxic functions of monocytes/macrophages (de Vries et al., 1999). Patients with PE were found to have higher IL-13 levels than those with uncomplicated BE (Wang et al., 2003). High levels of IL-13, which are affected by endogenous IL-4 and required for airway hyper-responsiveness and mucus production, are found in patients with asthma and atopic dermatitis (Brightling et al., 2010). Plasma level of IL-4 was not changed in EV71-infected patients, but IL-13 levels were consistently elevated in all groups, uncomplicated BE, ANS dysregulation and PE. Though IL-13 and IL-4 have partially overlapping effector profiles, that IL-13 may be the more important mediator of the effector at sites of Th2-induced inflammation. IL-13 can act alone in the pulmonary models, overproduction of IL-13 might contribute to the pathogenesis of PE by increasing pulmonary vascular permeability (Wills-Karp, 2004). Further, IL-13 can facilitate the selective recruitment of inflammatory cells from the bloodstream and induces the expression of a myriad of chemokines (Wills-Karp, 2004). Huang and coworkers showed exogenous treatment of IL-6, IL-13, and IFN-γ at day 3 after intracranial infection could induce mild PE and exacerbate pulmonary abnormality of EV71-infected mice. A synergistic pro-inflammatory cytokine responses and damage to specific brain regions may be necessary for the development of EV71-induced PE (Huang et al., 2011). IL-13 upregulation is associated with severe disease of EV71 infection.

Chemokines, a group of small (8-12 kd) proteins, are key regulators of leukocyte migration and involve in numerous aspects of cell growth, differentiation, and activation. Chemokines are distinguished from other cytokines by their ability to act on the superfamily of G-protein-coupled serpentine receptors. Chemokines are classified as homeostatic/constitutive (developmentally regulated) or inducible (inflammatory) by the contexts in which they function. Chemokines are characterized by the presence of 3 to 4 conserved cysteine residues and can be subdivided into 4 families, CC, CXC, CX3C and XC family, based on the positioning of the N-terminal cysteine residues (Rot A &
von Andrian, 2004).

IL-8 was identified as a neutrophil-specific chemotactic factor and later classified as a member of the CXC chemokine family. The major effector functions of IL-8 are activation and recruitment of neutrophils to the site of infection or injury (Kunkel et al., 1991). Patients with ANS dysregulation had higher plasma levels of IL-8 than patients with PE. IL-8 and other cytokines have been proposed to induce alterations in pulmonary permeability. IL-8 is considered the main neutrophil chemoattractant in acute respiratory distress syndrome (ARDS). The levels of IL-8 in ARDS pulmonary edema aspirates and bronchoalveolar lavage fluid (BALF) also correlate with survival and disease severity, as IL-8 levels are lower in survivors compared with nonsurvivors (Baughman et al., 1996). This might account for the elevation of IL-8 in EV71-infected patients with ANS dysregulation and PE. In animal models of acute lung injury, neutralizing IL-8 reduced the severity of lung inflammation and tissue damage (Sekido et al., 1993). Treatment of the rabbits that showed extensive edema in the alveolar lumina with a humanized anti-IL-8 antibody prevented neutrophil infiltration in the lung in association with alleviated acute lung injury syndrome (Bao et al., 2010). These studies provide important information regarding the role that IL-8 play in the pathogenesis of EV71 infection complicated with PE. Because of the known increased expression of IFN-γ in Th1 diseases in children with EV71-associated BE, IFN-γ-induced protein-10 (CXCL10/IP-10), monokine induced by IFN-γ (CXCL9/MIG), and IFN-inducible T cell α chemoattractant were studied. Plasma levels of IP-10, monocyte chemoattractant protein (MCP)-1, and MIG were significantly higher in patients with PE than in those with uncomplicated BE (Wang et al., 2008). IP-10 is recognized as a biomarker that predicts severity of various diseases. IP-10 expression also can be up-regulated by the Th1 cytokine IFN-γ during acute lung inflammation. This is consistent with previous findings that both circulating IFN-γ (Wang et al., 2003) and IP-10 levels were increased in patients with EV71 PE. MCP-1 in the systemic inflammatory response has been suggested. The concentration of MCP-1 has been shown to increase in the plasma of patients with persistent ARDS (Puneet et al., 2005). This may provide an explanation for the increased in MCP levels in plasma when the disease progresses from uncomplicated BE to PE. MIG plays a role in host defense after viral infection (Salazar-Mather et al., 2000). Increased level of MIG in patients with EV71 PE supports the notion that MIG contributes to host defense by promoting a protective Th1 response. Overexpression of the chemokine cascade in the systemic compartment appears to play an important role in the elicitation of the immune response to EV71. Pre-existing antibodies may play a critical role in controlling viral infection or in aggregating the disease severity. Mice that received subneutralizing anti-EV71 IgG before EV71 administration had significantly high serum levels of IFN-γ and monocyte chemoattractant protein-1 (MCP-1) (Chen et al., 2013). These findings support the concept that subneutralizing antibodies directed enhance some cytokines and chemokines production in EV71 infection through antibody-dependent enhancement (ADE) of infectivity pathway in newborn mice model. IL-17A, is a pro-inflammatory cytokine that plays an essential role in host defense against microbial infections and is implicated in various inflammatory conditions. Chen and coworkers reported high expression of acid-related orphan nuclear receptor gamma t (ROR t) in peripheral blood mononuclear
cells and elevated serum concentrations of IL-17 and IL-23 of EV71 infections. Further, the frequencies of Th17 cells in blood from EV71-infected children were significantly higher in comparison with controls (Chen et al., 2012).

5 Cytokine in Central Nervous System of EV71 Infection

Cytokines are constitutively expressed in the CNS. Normally, the cellular expression of cytokines in the CNS is highly integrated and under tight regulatory control. However, in certain pathological conditions, cytokine production may become spatially and temporally dysregulated, leading to inappropriate production. Although the blood-brain barrier (BBB) is relatively impermeable to cytokines owing to their size and hydrophilicity, in EV71 BE the integrity of the BBB may be compromised permitting cytokine action within the CNS. The neuroinflammatory cascade activated in response to EV71 BE is mediated by the release of pro- and anti-inflammatory cytokines and chemokines, and the primary source of these inflammatory mediators is in the brain. In the inflammatory process of echovirus 30 meningitis, cytokine network shifts from production of pro-inflammatory cytokines (IL-6, IL-8, and IFN-γ) to that of anti-inflammatory cytokines (IL-10 and TGF-β1) during or after the period when the virus is eliminated from the cerebrospinal cavity (Sato et al., 2003).

Lin and coworkers examined the relationship between level of IL-6 in the cerebrospinal fluid (CSF) and the CNS involvement of EV71 infection (Lin et al., 2003). The median CSF level of IL-6 was significantly higher during the first or second day of CNS involvement. Whereas, CSF levels of IL-6 were not significantly different among EV71-infected patients with different clinical syndromes, PE, encephalitis and/or poliomyelitis-like syndrome and aseptic meningitis during the acute stage of CNS involvement (Lin et al., 2003). However, another study demonstrated the mean CSF concentrations of IL-6 were elevated significantly in children with PE, and ANS dysregulation as compared to children with uncomplicated BE (Wang et al., 2007). The CSF concentrations of IL-6 were elevated by the diseases severity of EV71 infection. The source of IL-6 appears to be the brain, since CSF levels of IL-6 exceed those in plasma. This suggests that IL-6 may contribute to the overwhelming disease process.

Children with EV71 ANS dysregulation or PE had higher IFN-γ levels in CSF than those with isolated BE and echovirus meningitis. This suggests that IFN-γ is responding maximally to severe infection (Wang et al., 2007). IFN-γ is not normally present in the brain parenchyma. IFN-γ appears to promote gliosis and inflammation by its effect on astrocytes. Inflammation protects the brain from infection, but it aggravates injury. This suggests a detrimental effect of IFN-γ on the CNS (Olsson et al., 1994).

IL-1 activates microglia and vascular endothelial cells to recruit peripheral leukocytes and produce neuroinflammation. Elevated IL-1β levels were found only in the CSF and not in the plasma of EV71-infected patients with PE (Wang et al., 2007). Hosoi et al. found increased levels of IL-1β mRNA in the brain in the absence of an increase in circulating IL-1β in rats (Hosoi et al., 2000). These findings support the notion that IL-1β is probably synthesized in the CNS in response to severe EV71 infection. IL-1 can also
regulate neurotransmission mediated by amines such as NE and dopamine. The activity of the locus ceruleus, which is the most important source of NE inside the brain, was increased after in vivo IL-1β microinjection in this area; this effect was blocked by IL-1β receptor antagonist (Borsody & Weiss, 2002-2003). This may provide explanation of elevated of IL-1β level in CSF of PE patients, following the stage of ANS dysregulation.

IP-10 and its receptor, CXCR3, are expressed by the CNS and by CNS infiltrating lymphocytes, respectively, only in patients with ongoing CNS inflammation, suggesting an important role for these molecules in the pathogenic process. Increased IP-10 concentration in CSF in patients with enteroviral meningitis was observed (Lahrtz et al., 1998). CSF levels of IP-10 and IL-8 in patients with EV71 BE were significantly higher than the plasma levels in the control subjects (Wang et al., 2008). IP-10 is prominently expressed within the CNS of mice with viral encephalitis (Asensio & Campbell, 1997). Early expression of IP-10 within the CNS after virus infection is important in initiating and maintaining a protective Th1 immune response. This is characterized by high level production of the antiviral cytokine IFN-γ (Asensio & Campbell, 1997; Hoffman et al., 1999). Early expression of IP-10 appears to be beneficial by attracting Th1 T lymphocytes into the CNS, which participate in viral clearance. Shen and colleagues showed that IP-10 deficiency significantly reduced serum levels of MIG, and levels of IFN-γ and the number of CD8 T cells in the mouse brain. Absence of IP-10 significantly increased the mortality of infected mice by 45 %, along with decrease virus clearance in several vital tissues in IP-10 gene knockout mice (Shen et al., 2013).

CSF levels of MIG to be significantly more elevated in patients with EV71 PE than in those with EV71 ANS dysregulation and uncomplicated BE. The CSF to plasma ratio for MIG tended to increase with increasing severity of disease (Wang et al., 2008). In a study of murine brain endothelial cells, MIG was induced following treatment with a cytokine cocktail containing IFN-γ, TNF-α and IL-1β (Ghersa et al., 2002). The coordinate regulation of IP-10 and MIG was mediated by IFN-γ in cultured murine astrocytes and microglia (Carter et al., 2007). This may bolster the previous findings, the increased CSF level of MIG may relate to the increased CSF level of IL-1β and IFN-γ in patients with PE.

6 Modulation of Systemic Inflammatory Response of EV71 Infection

IVIG is a polyclonal immunoglobulin derived from large pools of human serum. IVIG are used not only in replacement therapy in immunodeficient individuals who are unable to mount their own effective immune responses, but also constitute a therapeutic option in patients with autoimmunity. IVIG is being used as a therapeutic modality (in doses higher than for replacement therapy) in certain bacterial or viral infectious diseases. Advantages for IVIG treatment has been demonstrated in patients with sepsis syndrome associated with systemic inflammatory immune responses and organ damage, presumably mediated via an anti-inflammatory effect (Turgeon et al., 2007). The plausible mechanisms of action of IVIG that have been reported to cause an amelioration of
inflammatory processes include interaction with Fc receptors, induction of apoptosis, blockade of co-stimulatory molecules, interference with the cytokine network, and neutralization of pathogenic antibodies (Elovaara & Hietaharju, 2010).

IVIG has been used prophylactically and therapeutically against neonatal enterovirus infections and in immunocompromised hosts (Abzug et al., 1995). IVIG injection decreases plasma catecholamines in coxsackievirus B3 myocarditis, suggesting that immunoglobulin exerts its cardioprotective effect through sympathetic modulating actions (Kishimoto et al., 2000). There is considerable evidence linking cytokine-mediated severe systemic inflammatory responses to PE and other adverse outcomes in patients with EV71-associated BE (Lin et al., 2002; Wang et al., 2003; Wang et al., 2007). Modulating cytokine expression by IVIG may offer a strategy for clinical practice. A previous study demonstrated a decrease in the plasma concentration of IL-6, IL-8, IL-10, IL-13, and IFN-γ following administration of IVIG in patient with ANS dysregulation and PE (Wang et al., 2006). These changes may be responsible for the rapid improvement in symptoms in some treated patients. Patient with ANS dysregulation is the critical timing to receive IVIG infusion. It is possible that a more favorable survival might have been obtained by modulating cytokine storm and reducing sympathetic activity.

Without milrinone treatment, patients with PE have a fatality rate as high as 80-90%. Most patient fatalities occurred within 6-12 hours without prompt care. Milrinone, a bipyridine phosphodiesterase (PDE) III inhibitor, is a member of both inotropic and vasodilatation characters. Milrinone increases cardiac output, and reduces systemic vascular resistance and pulmonary capillary wedge pressure without excessive increases in myocardial oxygen consumption (Shipley et al., 1996). Inhibition of cyclic adenosine 3′, 5′-monophosphate (cAMP) degradation by intracellular PDE3 may attenuate inflammation, reduce edema formation, improve endothelial function and induce pulmonary vasodilation (Hayashida et al., 1999). A pilot study was designed to evaluate the potential therapeutic effect of milrinone in the treatment of patients with EV71-induced PE (Wang et al., 2005). The mortality was lower in the milrinone-treated than non-treated group. Sympathetic tachycardia, white blood cell and platelet counts were decreased. There was a significant decrease in plasma level of IL-13 in milrinone-treated patients compared to controls. The effectiveness and efficacy of milrinone treatment in patients with EV71-related PE has been proven in historically controlled (Wang et al., 2005) and randomized controlled studies (Chi et al., 2013). Milrinone therapy not only increased the expression frequency of CD4+Foxp3+ in severe EV71 infection but also reduced the plasma levels of cytokines, IL-6, IL-8 and IL-10. Plasma concentrations of cAMP were significantly decreased in patients with ANS dysregulation or PE compared with patients with HFMD or BE; however, cAMP levels increased after milrinone treatment (Wang et al., 2014). Milrinone therapy provides a useful therapeutic approach for treating life threatening EV71 infections.

7 Conclusions

The production of inflammatory cytokines and chemokines is a unique aspect of the
immune responses in the CNS and systemic arms to EV71 infection. Cytokines and chemokines released by EV71 infected immune cells contribute directly or indirectly to the disease severity. IVIG and milrinone treatment represents an appropriate approach to the inflammatory responses elicited by severe EV71 infection. Alternative modalities for controlling the cytokine network have been explored experimentally. A better understanding of the fundamental mechanisms and the engaged inflammatory signal-transduction pathways of the cytokines production will be expected to be of value in the treatment of inflammatory responses of EV71 infection.

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References


Duell, B. L., Tan, C. K., Carey, A. J., Wu, F., Cripps, A. W., & Ulett, G. C. (2012). Recent insights into microbial triggers of interleukin-10 production in the host and the impact on infectious disease pathogenesis. FEMS Immunology and Medical Microbiology, 64, 295–313.


