Implications of Age-Dependent Immune Responses to Enterovirus 71 Infection for Disease Pathogenesis and Vaccine Design

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Epidemics of enterovirus serotype 71 (EV71) infection in Asia appear to be increasing in size and severity, and there is increasing concern for pandemic spread. Efforts are underway to develop an effective EV71 vaccine. However, the immunologic correlates of protection against EV71 infection are not fully understood, and studies suggest that severe complications may result from a combination of pathological immune responses and direct viral effects. Severe disease and death typically occur only in young children, which is likely due in part to a lack of EV71-specific adaptive immunity but possibly also due to age-dependent hyperactive innate immune responses. Infants are the primary targets of EV71 vaccination strategies. Therefore, studies are needed to understand the interplay between age, immunopathology, and severity of EV71 infection to distinguish protective from harmful immune responses and to guide the development of effective EV71 vaccines. This review summarizes our current understanding and outlines the next steps forward.

In 1969, a novel enterovirus (EV) serotype, EV71, was first described in California, although it likely circulated much earlier based on retrospective studies [1, 2]. Subsequently, EV71 has been associated with periodic outbreaks of hand, foot and mouth disease (HFMD) in infants and children throughout Asia and in Eastern Europe as well as sporadic cases in North America and Western Europe (reviewed in [3, 4]). A small proportion of children infected with EV71 go on to develop severe disease manifestations, including brainstem encephalitis and pulmonary edema (PE), which result in high rates of neurologic sequelae or death [5]. Due to the increasing size of EV71 epidemics, and the number of associated deaths that have been reported across the Asia-Pacific region [3, 6], EV71 has become a public health problem of global importance. In this review of EV71 infection, we focus on emerging evidence suggesting that there is a complex interplay between age, immune response, and development of severe complications of EV71 infection.

Clinical Manifestations and Management

Hand, foot and mouth disease is a common viral exanthem of childhood and is characterized by fever that is usually low grade, vesicles on the buccal mucosa and tongue, and small, tender papulovesicular lesions on the hands and feet, as well as occasionally the buttocks and genitals (reviewed in [4, 5]). Herpangina is a related febrile viral exanthem of multiple oral ulcers that predominantly affect the posterior of the oral cavity, including the uvula, tonsillar fauces, and soft palate. Most EV71 infections are asymptomatic or induce HFMD or herpangina that is self-limited and mild, although orodynia and decreased oral intake may be problematic. However, more severe and potentially fatal central nervous system (CNS) manifestations also occur, most commonly brainstem encephalitis, which may be PE, cardiopulmonary failure, and sudden death [4, 5]. Clinical predictors of severe disease include young age, high temperature, lethargy, and cerebrospinal fluid pleocytosis [5].
There is no effective antiviral licensed for treatment of EV71 infection, and the management of severe disease is thus largely supportive. Intravenous immunoglobulin (IVIg) is commonly used for severe EV71 infection, based on the possible benefits reported from some case series [7–9], although controlled trials have not been performed and the potential mechanism of action is unclear (see Antibody Responses). A prophylactic EV71 vaccine is not yet available. However, driven by the alarming rise in cases and deaths, considerable efforts are ongoing to develop a safe and effective EV71 vaccine to prevent infection and disease in infants and young children. Multiple EV71 vaccine candidates are in development, including inactivated virus, virus-like particles, capsid protein VP1 peptide or recombinant protein-based constructs, VP1-expressing viral or bacterial vectors, VP1 DNA constructs, as well as live, attenuated EV71 [10]. Currently, several inactivated EV71 candidates have entered early clinical testing (ClinicalTrials.gov identifiers: NCT01313715, NCT01273246, NCT01268787) [11], and 1 construct may soon be entering Phase III clinical trials [12]. The approach to vaccine evaluation is proceeding using a traditional “empirical” approach with the primary goals of immunogenicity and safety; ie, these trials aim to generate high titers of neutralizing antibodies (NAb) while minimizing adverse reactions. These strategies are well founded for guiding early clinical development of EV71 vaccines. However, the development of vaccine candidates would benefit from a greater understanding of EV71 epidemiology and pathogenesis, particularly the role of the immune response to EV71 in young children (Figure 1) [13–18].

**Epidemiology**

Small and medium-sized outbreaks of EV71 have been described over the last few decades, primarily in Asia [3, 19]. In 2008, however, the global landscape of EV71 epidemiology changed dramatically when an outbreak of HFMD numbering approximately half a million cases occurred throughout the Peoples Republic of China (PRC) [6]. Large numbers of cases were observed in Fuyang of Anhui Province, which subsequently spread to provinces throughout the PRC [20]. Even during EV71 outbreaks, not all cases of HFMD are attributable to EV71, but the proportion of severe HFMD attributable to EV71 since 2008 in the PRC has exceeded 80%. Furthermore, HFMD cases that came to clinical attention were associated with higher rates of morbidity and mortality among young children than any previous EV71 outbreaks including outbreaks in Malaysia, Taiwan, Australia, Singapore, and Brunei [3]. Since 2008, and in contrast to historical patterns, both the incidence and case fatality of HFMD and EV71 infections in PRC has increased annually according to national surveillance data from the Chinese Centers for Disease Control and Prevention.

![Figure 1](image-url). A model of human enterovirus 71 pathogenesis. A, EV71 infection may either be prevented by high titers of preformed neutralizing antibody (NAb) in young infants due to maternal antibody or in older individuals with a history of exposure to EV71 or perhaps other cross-reactive EV isolates. If infection does occur, NAb and/or a strong type 1 T helper (Th1) antiviral response limits viral replication (1). Protective immune responses result in minimal immunopathology (2) and limit viral replication, which could reduce the likelihood of central nervous system infection and direct cytopathic effects (3). B, Due to age-dependent immunologic biases, EV71 infection may be less likely to evoke strong Th1 responses in young children, who would also be expected to lack high NAb titers, resulting in ineffective control of viral replication (1); the presence of low NAb titers may also result in antibody-dependent enhancement of EV71 replication. Th2/Th17 responses (resulting from high levels of interleukin (IL)-18 and IL-6 production) to EV71 dominate in these children, which results in excessive inflammation and pathology (2) that may be exacerbated by direct viral destruction of tissues (3). Abbreviations: CNS, central nervous system; NAb, neutralizing antibodies; Th, T helper.

<table>
<thead>
<tr>
<th>Year</th>
<th>Incident Cases</th>
<th>Severe Cases</th>
<th>Deaths</th>
<th>Case Fatality Rate (per 10 000)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2008</td>
<td>489 540</td>
<td>1194</td>
<td>127</td>
<td>2.59</td>
</tr>
<tr>
<td>2009</td>
<td>1 155 575</td>
<td>13 834</td>
<td>353</td>
<td>3.05</td>
</tr>
<tr>
<td>2010</td>
<td>1 774 669</td>
<td>27 908</td>
<td>905</td>
<td>5.10</td>
</tr>
</tbody>
</table>

aData derived from the Chinese Centers for Disease Control and Prevention (CDC). HFMD cases were defined as shown in Wang et al [6]. In approximately 4% of cases, confirmatory laboratory testing was also performed using 1 or more of the following laboratory methods: isolation of EV71 in culture; detection of EV71-specific RNA; a titer of EV71-specific neutralizing antibody ≥ 256, or ≥ 4-fold increase in the titer between the acute phase and the recovering phase. EV71 infection was confirmed in approximately 50% of all HFMD cases, in >80% of severe cases, and in >90% of deaths [6] and unpublished data).

bThe Chinese CDC definition of severe infection differed between 2008 and subsequent years: the 2008 definition required complications such as myoclonia, acute flaccid paralysis, encephalitis, cardiopulmonary failure, or pulmonary edema; beginning in 2009, the definition was broadened to include increased peripheral white blood cells, increased blood sugar, abnormal cerebrospinal fluid, or any abnormality in electroencephalogram, cerebrospinal magnetic resonance imaging, chest x-ray, or echocardiogram.
Disease Control and Prevention (Table 1) [6]. Case definitions of severe infections across the sites collecting these data changed somewhat from the time surveillance began in 2008 to 2009, limiting the quantitative precision of these analyses during that period. However, the magnitude of increased incidence is so large that it is unlikely to be due solely to methodologic bias, especially between 2009 and 2010 when case definitions were uniform, indicating important qualitative trends. The number of cases reported in each of the nadirs between epidemic seasons, typically near zero, increased as well, raising the fear of a shift from an epidemic to an endemic pattern of occurrence in PRC [4]. Annual incidence of EV71 appears to continue to increase in PRC, and large recent outbreaks have occurred elsewhere in Asia, including Japan, Vietnam, Cambodia, and Singapore [21, 22].

EV infections are generally more common and more severe in infants and young children compared to older children or adults [23]. This increased susceptibility to EV infections during early life is likely attributable to some combination of inadequate levels of preformed specific antibody as well as innate and cellular immune responses that are ineffective or pathogenic. Analyses and modeling PRC national surveillance data (2008–2010) for EV71 HFMD indicate the complexity of the relationship between age and severity of infection. Although infants within 6 months of birth have a relatively low attack rate (comparable to that among children 6–10 years of age), they have a more than 5-fold greater severity rate and a 25-fold greater rate of fatality (Table 2) [6]. The relative attack rate increases precipitously (>11-fold) for infants 6–12 months of age and is highest for children 1–3 years of age, yet the case severity and fatality rates decline monotonically with age at infections. Thus, although infants appear to acquire EV71 infection less frequently than older children, if they do become infected they are at the highest risk of complications and death. Similar patterns of disproportionately highly severe complications of EV71 infection during infancy have also been reported in Taiwan [24–26]. Animal models of EV71 infection suggest the possibility that the stage of immunologic development may have an important impact on outcome, as similar to human infection, only very young mice develop severe disease and brainstem inflammation [27].

### Virology

Enteroviruses are members of the Picornaviridae family, and they comprise a large genus of nonenveloped, positive-sense, single-stranded RNA viruses. Like other EVs, EV71 virions are small (~30 nm) icosahedral particles that contain a genome of approximately 7.4 kb in length. The virus capsid is made up of 60 identical subunits (protomers); each protomer comprises 1 copy of the 4 structural proteins, named VP1, VP2, VP3, and VP4. Due to the absence of a lipid envelope, virions remain infectious for prolonged period in the environment, and they can survive passage through the acidic gastric environment. Based on current understanding, EV71 appears to be transmitted primarily by the fecal-oral route, although other routes such as via respiratory droplet and fomites may also occur [3, 23]. Two receptors for EV71 binding and entry into host cells have been identified, including human scavenger receptor class B, member 2 (SCARB2), which is ubiquitously expressed [28], and human P-selectin glycoprotein ligand-1 (PSGL1; CD162), which is expressed on myeloid, lymphoid, and dendritic cells (DCs) [29]. Enterovirus serotype 71 may use additional receptors that have yet to be identified [30].

After EVs bind host cell surface receptors, the capsid undergoes structural changes that result in the formation of pores in the plasma membrane, allowing entry of viral genome into the cytoplasm [3, 30]. The positive-sense, polyadenylated viral RNA is translated directly into a single, large polypeptide, which is then cleaved by the viral proteases 2A and 3C into 11 mature structural and non-structural proteins. The viral genome is replicated by the RNA-dependent RNA polymerase in the cytoplasm, within a “replication vesicle,” after which it is packaged into a virus capsid to form a mature virion. Cell lysis then results in the release of infectious virus particles.

### Viral Pathogenesis

The severity of EV71 infection is likely determined by a combination of viral and host factors, which may result in

<table>
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<tr>
<th>Table 2. Relative Attack, Case Severity, and Fatality Rates of Hand, Foot and Mouth Disease During 2010</th>
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<tbody>
<tr>
<td>Age (yrs)</td>
</tr>
<tr>
<td>0–0.5</td>
</tr>
<tr>
<td>0.6–&lt;1</td>
</tr>
<tr>
<td>1–&lt;3</td>
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<tr>
<td>3–&lt;6</td>
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<tr>
<td>6–&lt;10</td>
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<td>≥10</td>
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*Data derived from the Chinese Centers for Disease Control and Prevention. Hand, foot and mouth disease (HFMD) cases were defined as shown in Wang et al [6].

*Attack rate is defined as number of HFMD cases among age-matched population, and relative attack rate is defined as attack rate relative to (6–<10) year group.

*Case severity rate is defined as percentage of severe cases over all cases at the age group, and relative case severity rate is defined as case severity rate relative to (6–<10) year group.

*Case fatality rate is defined as percentage of deaths over all cases at the age group, and relative case fatality rate is defined as the death rate relative to (6–<10) year group.

*Reference group.
greater direct viral cytotoxic effects or detrimental host responses to infection (Figure 1). Many details of EV71 and other EV infections are incompletely understood, even for poliovirus, the prototype EV infection. For example, it is unknown which cells are initially infected in vivo, the routes of CNS entry have not been identified, and the determinants of neurovirulence are not known [23, 31]. After gastrointestinal entry, EV71 multiplies initially in Peyer’s patches and mucosal lymphoid tissue, resulting in a minor viremia and dissemination throughout the reticuloendothelial system in lymph nodes, bone marrow, spleen, and liver [3]. Although a subsequent major viremia has been associated with invasion of the CNS and other tissues for poliovirus and other enteroviral infections, this has not been documented in human EV71 infection. Autopsy and neuroimaging studies of children with severe EV71 infection suggest somewhat stereotypical involvement of the spinal cord gray matter, brainstem, basal ganglia, thalamus, and cerebellum, which could indicate viral entry into the CNS via peripheral nerves rather than through direct hematogenous spread across the blood-brain barrier [32, 33]. Retrograde invasion of the CNS through peripheral nerves has also been implicated in poliovirus and other EV infections. Viremia and high levels of systemic EV71 replication might still predispose to CNS entry, either directly, or via hematogenous spread to the muscle and resident peripheral nerve axons [34]. Notably, EV71 is not readily detected in cerebrospinal fluid (CSF) or blood even in severe cases ([35, 36] and unpublished data). Thus, the importance of systemic EV71 replication or viremia and development of severe disease is currently unknown. Additional studies to characterize EV71 viremia, especially early in the course of infection, may help to clarify these issues.

Some studies suggest that EV71 disease severity may be partially attributable to viral strain differences. EV71 isolates from cases of encephalitis have been reported to display greater neuronal tropism and cytotoxicity in vitro [37–39]. In addition, specific VP1 and VP2 mutations have been found to be associated with greater replication in neuronal cell lines and a lower 50% lethal dose in mice [40, 41]. The CNS pathology of severe EV71 infection appears to be due at least in part to direct viral effects based on animal studies as well as post mortem identification of virus from brains of children with encephalitis [42–45]. The extent of viral replication in the brain in these studies appears relatively minor compared with the amount of inflammation observed, which raises the possibility that pathogenic immune responses could play an important role in severe disease [3, 33]. The pathogenesis of severe PE and heart failure is also unclear, but it is partly or wholly neurogenic, developing as a complication of brainstem encephalitis [3]. Viral replication is only detectable in the lung or heart in a minority of cases of EV71 PE [46–49], which is consistent with a model of severe cardiopulmonary disease resulting from uncontrolled systemic inflammation or hydrostatic effects of neurogenic dysregulation [3, 50–52]. Cardiac histopathology in such cases has demonstrated characteristic features of catecholamine-associated cardiotoxicity [47, 53], suggesting that high catecholamine levels due to brainstem encephalitis could cause cardiac dysfunction and pulmonary hypertension. This process, therefore, in combination with excessive vascular permeability and systemic inflammation, might lead to PE in severe EV71 infections [3, 54].

**Immune Response and Immunopathogenesis**

As detailed below, preformed NAb, innate immunity, and EV71-specific cellular responses may each contribute to protection against severe HFMD. In contrast, there is also evidence that each of these immune responses may be pathogenic and increase the morbidity and mortality of EV71 infection. Several studies have reported qualitative differences in the innate and adaptive immune responses in those children with EV71 brainstem encephalitis and PE compared to cases with uncomplicated milder presentations (rather than simply higher levels of inflammation), suggesting that certain types of immune responses may contribute to the development of severe disease [15, 50, 51, 55–57]. Animal models of EV71 lend further support to the importance of immunopathogenesis [58]. However, such studies have limited ability to identify immune responses as a cause or consequence of severe disease. Thus, to date, the precise nature of protective versus pathologic immune responses has not been defined, nor is it understood what determines which type of response an individual will mount. Furthermore, little is known about the quality of immune responses early in EV71 infection before the development of complications. Such information is likely central to EV71 pathogenesis and the ability to predict and ultimately prevent severe disease. As outlined above, symptomatic infection in older children is virtually always limited to uncomplicated HFMD; younger children—especially those under 12 months old—more frequently develop meningitis, brainstem encephalitis, and PE, with a high rate of permanent sequelae [24, 59]. Developmental studies demonstrate that specific patterns of innate immune responses are expressed early in life and that adult-type patterns are only progressively established during childhood. These age-dependent patterns of innate immune response appear to correlate with well described “windows of vulnerability” to specific infectious pathogens [60, 61]. In particular, age-dependent immune responses to EV71 infection could potentially explain the
higher rates of severe disease and death in early childhood through either ineffective control of viral replication, preferential generation of pathologic immune responses, or a combination of the 2. An understanding of the complex effects of the immune response to EV71 on disease outcome in children, and an awareness of how these effects vary with age, may therefore be extremely valuable for developing an effective and safe prophylactic vaccine, as well as for the design of prognostic and therapeutic strategies.

**Innate Immune Responses.** Based on in vitro and animal studies [62–69], innate sensing of EV71 and picornaviruses by pattern recognition receptors (PRRs) appears to occur through Toll-like receptor 3 (TLR3), melanoma differentiation-associated gene 5 (MDA5), and the retinoid acid-inducible gene I (RIG-I) receptor. Of note, EV71 has evolved mechanisms to evade some of these responses; the structural protein 3C inhibits type 1 interferon (IFN) production in response to TLR3 and RIG-I signaling in vitro [65, 67], and the 2C protein blocks tumor necrosis factor (TNF)-α activation of nuclear factor-xB signaling [30, 70]. Recently, the 2B protein from EV71 and other picornaviruses was found to activate the NOD-like receptor (NLR) family, pyrin domain-containing 3 (NALP3) inflammasome, which is required for the caspase-1-dependent release of interleukin (IL)-1β, IL-18, and IL-33 [71].

Small clinical studies have described markedly higher levels of IL-1β and IL-6 in blood in children with EV71 encephalitis and PE compared to those with only encephalitis or uncomplicated infection [15–17, 50, 51]. Associations between increased levels of other cytokines and immune factors in blood and CSF with EV71 disease severity have also been reported, including TNF-α, IL-8, IL-10, IL-13, IFN-γ, IFN-γ-induced protein-10, monocyte chemoattractant protein 1, monokines induced by IFN-γ, IL-1 receptor antagonist (IL-1Ra), and granulocyte colony-stimulating factor [17, 50, 51]. Furthermore, plasma levels of IL-6 and other cytokines in EV71-infected children with PE were observed to fall dramatically after administration of IVIg, whereas no change in serum NAb titer was detected, supporting the possibility that IVIg may improve outcomes by suppressing pathologic inflammation [72].

In response to signals of infection early in life, the innate immune system is known to produce higher levels of certain proinflammatory cytokines, specifically IL-6, IL-1β, IL-23, and demonstrate a bias toward types 2 and 17 T helper (Th2 and Th17) responses, but lower levels of the Th1 cytokines IFN-α and IL-12p70 that are important for control of intracellular (ie, viral) pathogens [73, 74]. This bias has been extensively characterized for TLR responses in peripheral blood monocytes and DCs, which change profoundly with age (reviewed in [75] and [76]). Compared to adults, conventional DCs from cord blood produce significantly less IFN-α and IL-12 upon stimulation with the TLR3 agonist polyinosinic-polycytidylic acid, and cord blood DCs show lower expression of CD40 and CD80 [77]. Indeed, IFN-α and IL-12p70 (and consequently IFN-γ) responses to most TLR agonists, including 3M-003, which mimics single-stranded RNA signaling through TLR7/8, appear to be relatively weak in conventional and plasmacytoid DCs as well as monocytes from cord blood, whereas IL-1β, IL-6, IL-23, and especially IL-10 responses are as high or often much higher than those in adult peripheral blood mononuclear cells [74–76]. The expression and function of other PRRs (including RIG-I-like receptors, NLRs, IFIG6, etc.) have not been nearly as well described, but they are also likely to change during early life. Studies investigating NALP3 inflammasome responses during early life have not yet found a consistent age-dependent pattern [76, 78, 79]. Of note, age-dependent patterns of innate immune response may differ between races and perhaps between high- and low-income settings [73, 78], and in-depth studies of Asian infants have not yet been published.

This propensity to produce higher IL-6 and IL-1β early in life is of particular interest given that these cytokines are associated with increased severity of EV71 infection [15–17, 50, 51, 80]. In the recent study by Griffiths et al [50], IL-1β levels tended to be higher among young children with fatal EV71 infection and cardiopulmonary failure, and the authors note that IL-1β has been associated with the acute respiratory distress syndrome and has negative inotropic effects on cardiac contractility [81, 82]. Furthermore, this association with IL-1β suggests the possible benefit of anakinra, a synthetic IL-1Ra with cardioprotective effects, for the treatment of EV71-associated cardiopulmonary failure [83]. The importance of IL-6 in EV71 pathogenesis indicated by cohort studies is further supported by recent data from mouse models [58, 84]. In a study by Khong et al [58], treatment of EV71-infected neonatal mice with anti-IL-6 antibody resulted in significantly reduced mortality and tissue destruction compared with untreated controls. More importantly, the protective effect of anti-IL-6 antibody in these mice was independent of viral load. The developmentally determined propensity to produce high levels of IL-6 may therefore be causally related to morbidity and mortality during EV71 infection. Interleukin-6 signaling could represent a novel target for therapy of severe EV71 disease and merits further study. Although Th17 responses are induced by EV71 infection [80] and are also disproportionately induced in...


early life [75], more studies are needed to understand the relevance for progression to severe disease.

**Antibody Responses.** Neutralizing antibodies appear to be important for protection against acquisition of poliovirus and other EV infections [23]. Likewise, in mouse models of EV71 infection, passive administration of NAb can protect against lethal challenge [44, 85]. The development of effective NAb responses after EV71 infection may partially explain the periodicity of HFMD epidemics in many areas outside of PRC and the high attack rates among young children beginning at approximately 6 months of age, a point at which maternal antibody levels have substantially declined [24, 25, 86]. There appears to be significant cross-reactive NAb activity between EV71 genotypes [41, 87, 88]. Sterilizing immunity after EV71 infection is not universal, however, and repeated EV71 infections with different or similar genotypes may occur [13, 89]. This result is consistent with the finding that immunocompetent individuals are readily reinfected by repeated doses of oral poliovirus vaccine strains and shed virus in the stool, despite being protected against paralytic polio [31]. As such, EV71 epidemiology reflects at least partial cross-neutralizing activity between EV71 isolates, as well as non-71 EV serotypes [3, 24, 41].

Antibody responses may also modulate the severity of EV71 infection once it occurs. B cell depletion increases disease severity in EV71 mouse models [44], and patients with X-linked agamaglobulinemia frequently suffer persistent and morbid EV infections that are sometimes ameliorated with IVlg [31, 90]. There is also some evidence that suggests a clinical benefit of IVlg for severe neonatal EV infections [91]. As mentioned, IVlg may be beneficial for the treatment of severe EV71 infections, which could be mediated in part by the presence of EV71-specific NAb present in many IVlg preparations [7, 72]. Identifying the mechanisms by which IVlg may modify severe EV71 infections is complicated by the potent anti-inflammatory and immunomodulatory properties of IVlg and variability of antigen-specificity between preparations [92]. Notably, in the small series that have been reported to date, no difference in acute or convalescent NAb titers was measured in children with severe versus uncomplicated EV71 infections [55, 56].

It should be noted that in vitro and murine EV71 experiments have demonstrated a paradoxical enhancement of infection with sub-NAb titers (antibody-dependent enhancement [ADE]) [13, 14]. The importance of ADE is best understood in dengue virus infections, but it has also been implicated in the pathogenesis of several other viral infections, including coxsackievirus B myocarditis and cardiomyopathy (reviewed in [93]). It is plausible that ADE may contribute to the high risk of severe EV71 infection observed during infancy, when titers of maternal NAb begin to wane [24–26, 86]. If ADE was shown to occur in human EV71 infection, it would have a profound implication for vaccine design. However, it is reassuring to note that ADE has not been reported with poliovirus vaccines.

**T Cell Responses.** Several studies have reported associations between severe EV71 infection and T cell responses, although the evidence is limited and somewhat conflicting [15, 17, 55–57]. Reduced lymphocyte proliferation to both EV71-specific and nonspecific stimuli was found among a small number of Taiwanese children with acute brainstem encephalitis and PE compared with uncomplicated cases [55]. However, a separate study from Taiwan reported no difference in mitogen-induced lymphocyte proliferation between children with and without EV71 CNS involvement [56]. Also of note, unlike individuals with humoral immunodeficiencies, disorders of impaired cellular immunity do not appear to confer a disproportionate risk of severe EV infections [23]. Thus, the possible protective and pathogenic effects of the adaptive cell-mediated immune response to EV71 infection require additional study.

**Conclusion and Perspectives For Future Research**

Investigations focusing on aspects of the immune response to EV71 infection could have profound implications for the “rational” design and evaluation of EV71 vaccines in infants. There is evidence that the pathogenesis of severe EV71 disease may be at least partly immune-mediated. Furthermore, the immunopathology of severe EV71 infection may be dependent in part on the stage of immunologic development, such that early in life there is a predisposition to generate not only ineffective but harmful responses to EV71. Because the target population for any EV71 vaccine would primarily be for infants, there is a possibility that an EV71 immunogen mimicking natural infection could result in a higher rate of untoward side effects. This type of age-dependent immunopathology in young children has been described in other severe viral infections, such as respiratory syncytial virus (RSV) [94]. An inactivated RSV vaccine developed in the 1960s aimed at generating protective antibody responses in infants resulted in more severe disease in ~80% of infected vaccines. This paradoxical adverse vaccine effect appears to have resulted from priming of Th2 type responses to RSV. Thus, the possibility of ADE of EV71 infection must be investigated. If results from in vitro and animal models are predictive for human infection, low-level NAb to EV71 could in fact potentiate viral replication. If this result is confirmed, a critical reevaluation of current EV71 vaccine strategies would be required.
Given the apparent trends of increasing incidence of EV71 infection and severity of disease and potential for pandemic spread, priority should be given to improve our understanding of the immunologic basis of prevention and pathology of EV71 disease in children. Studies aimed at identifying immune response signatures that are predictive of decreased versus increased risk for severe disease would be of great clinical benefit. Putative mediators of immunopathology in young children with EV71 infection, such as IL-1β and IL-6, may represent novel therapeutic targets for severe disease and merit more study. Such information would also be invaluable when defining immunogenicity endpoints in early-phase clinical trials of EV71 vaccine candidates and in the design and interpretation of vaccine safety evaluations, preferably before large numbers of infants at risk for EV71 infection are exposed to vaccine candidates.

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