Pathogenesis of Enterovirus 71 Brainstem Encephalitis in Pediatric Patients: Roles of Cytokines and Cellular Immune Activation in Patients with Pulmonary Edema

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Taiwan experienced several epidemics of enterovirus 71 (EV71) infections, which were associated with brainstem encephalitis (BE) and pulmonary edema (PE). To elucidate the role of immune mechanisms in the pathogenesis of BE caused by EV71 and its fatal complication, PE, we analyzed the laboratory findings, cytokine, and immunophenotypes of 73 EV71-infected patients with BE. Patients were stratified by disease: PE (n = 14), autonomic nervous system (ANS) dysregulation (n = 25), and isolated BE (n = 34). The mortality rate for PE was 64.3%. Leukocytosis and thrombocytosis were significantly more frequent among patients with PE. A significant elevation of plasma interleukin (IL)–10, IL-13, and interferon (IFN)–γ levels observed in patients with PE. Patients with PE also had lower circulating CD4+ T cells, CD8+ T cells, and natural killer (NK) cells. An extensive peripheral and central nervous system inflammatory response with abnormal IL-10, IL-13, and IFN-γ cytokine production and lymphocyte depletion appears to be responsible for the pathogenesis of EV71-associated PE.

Enterovirus 71 (EV71) is a major cause of hand, foot, and mouth disease in young children. After its initial identification in the United States in 1969, outbreaks have been reported in Australia, Southeast Asia, and Europe [1–4]. Taiwan experienced 3 major epidemics in 1998, 2000, and 2001. EV71 infection is usually self-limited but may progress to cause aseptic meningitis, brainstem encephalitis (BE), and acute flaccid paralysis that is indistinguishable from poliomyelitis. A remarkable feature of this disease is the high mortality associated with BE (26%) [4]. Most of the fatal cases occur in children aged <3 years. They develop rapidly progressive sympathetic hyperactivity, pulmonary edema (PE) and/or pulmonary hemorrhage, and cardiopulmonary collapse [4–9]. These complications cannot be explained by hemodynamic factors. The children have fairly normal cardiac function, normal pulmonary artery pressure measurements, and vascular resistance, and myocarditis is not present at autopsy [4, 9]. Therefore, we postulated that PE may be caused by increased pulmonary vascular permeability as the result of either brainstem lesions or a systemic inflammatory response caused by the excessive release of cytokines. Previous studies have shown that proinflammatory cytokines (interleukin [IL]–6, tumor necrosis factor [TNF]–α, and IL-1β) are associated with BE that is complicated by PE [10, 11]. The children with meningoencephalitis were found to have an altered cellular immune response associated with a high frequency of G/G genotype of the cytotoxic T lymphocyte antigen-4 polymorphism...
[12]. Humoral immunity does not appear to have a role, because patients with agammaglobulinemia can develop life-threatening complications from enterovirus infections [13]. In the present study, we provide further evidence that PE that occurs in children infected with EV71 is caused by an abnormal cytokine activation that produces a severe systemic inflammatory response that, in turn, causes increased pulmonary vascular permeability that is similar to acute respiratory distress syndrome.

MATERIALS AND METHODS

Case definition. EV71 infection was defined as the isolation and typing of the virus from at least 1 site (throat swab, stool, cerebrospinal fluid [CSF], or other) with a negative bacterial culture. BE was defined as an illness characterized by myoclonus, ataxia, nystagmus, oculomotor palsies, and bulbar palsy in various combinations, with or without neuroimaging. Autonomic nervous system (ANS) dysregulation was defined by the presence of cold sweating, mottled skin, tachycardia, tachypnea, and hypotension. PE was 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. Cardiopulmonary collapse was defined as the development of hypoxemia and hypotension, despite the administration of inotropic drugs.

Study population. The study population consisted of 73 children who met the case definition and 15 age-matched healthy control subjects. The patients were admitted to the National Cheng Kung University Hospital (Tainan, Taiwan) during the 3 major epidemics of EV71 in 1998, 2000, and 2001. Patients were divided into 3 categories: (1) isolated hand, foot, and mouth disease with BE, (2) BE with ANS dysregulation; and (3) BE complicated by PE and/or pulmonary hemorrhage that progressed to cardiopulmonary collapse.

Virological studies. All specimens were collected in viral transport medium and were inoculated onto monolayers of A549 cells, green monkey kidney cells, and Vero cells within 24 h. The cultures were incubated at 37°C and inspected daily for a minimum of 14 days for viral cytopathic effect (CPE). When typical enteroviral CPE was observed, EV71 identification was performed by 2 EV71 type-specific monoclonal antibodies (MAbs 3323 and 3324; Chemicon International) with immunofluorescence stains. Because EV71 MAb 3323 cross-reacts with coxsackievirus A16 and MAb 3324 does not cross-react with coxsackievirus A16, EV71 was identified by positive staining of both MAbs. These isolates were further confirmed by use of the neutralization test with polyclonal antibodies produced in rabbit serum against EV71.

Determination of cytokine levels. Plasma for cytokine determination from patients and control subjects were harvested within 30 min at 37°C of venipuncture from EDTA-anticoagulated venous blood samples and were stored at −70°C until analyzed. An ELISA for quantitative determination of IL-13 (Quantikine; R&D Systems) was used. The BD Human Th1/Th2 Cytokine CBA Kit (BD PharMingen) was used to measure IL-2, IL-4, IL-5, IL-10, TNF-α, and IFN-γ protein levels by flow cytometry in a particle-based immunoassay, which allows for simultaneous measurement of 6 cytokines with 50 μL of samples. The assays were performed according to the instructions of the manufacturers. The limits of detection of these immunoassays were 2.6 pg/mL for IL-2, 2.6 pg/mL for IL-4, 2.4 pg/mL for IL-5, 2.8 pg/mL for IL-10, 2.8 pg/mL for TNF-α, 7.1 pg/mL for IFN-γ, and 32 pg/mL for IL-13.

Flow cytometric analysis. The immunophenotypes of peripheral blood lymphocytes obtained from the patients and control subjects were determined as follows. All blood specimens were drawn by venipuncture into Vacutainer-EDTA tubes (Becton Dickinson). Whole blood (150 μL) was incubated on ice with 10 μL of each antibody for 15 min in the dark. Two milliliters of FACS lysing solution (Becton Dickinson) was added and incubated at room temperature for 10 min. The cells then were washed with PBS and fixed with 0.5 mL of 0.1% glutaraldehyde solution in PBS. Stained lymphocytes were analyzed by flow cytometry (Becton Dickinson Immunocytometry Systems). Data were acquired and analyzed by Cell Quest software (Becton Dickinson). The following fluorescent MAbs were used: peridinin chlorophyll protein–conjugated Leu 4 (CD3; pan T), phycoerythrin-conjugated Leu-3a (CD4 T cells), Leu-2a (CD8 T cells and NK cells), Leu-11c (CD16; NK lymphocytes), and Leu-19 (CD56; NK lymphocytes and T lymphocyte subset). All MAbs were purchased from Becton Dickinson. IgG isotype control antibody conjugates were included in all assays to determine background fluorescence.

Statistical analysis. Proportional data were tested by use of χ² or Fisher’s exact test. Continuous data were tested by Student’s t test. The Mann-Whitney U test was used for non-parametric data that did not have a normal distribution. All analyses were performed by use of SPSS software (version 10.0; SPSS). Data are presented as the mean ± SD. P<.05 was considered to be significant.

RESULTS

Patient characteristics and clinical and laboratory findings. Seventy-three patients with hand, foot, and mouth disease or herpangina complicated by BE were enrolled in the study. The demographic and clinical data are summarized in table 1. The patients were subdivided into 3 groups, as described above. All
34 patients with isolated BE and the 25 patients with ANS dysregulation survived. Nine (64.3%) of the 14 patients with PE died. The median age did not differ significantly among patients with isolated BE, ANS dysregulation, or PE, but, in children aged <3 years, 73.5% of isolated BE, 80% of ANS dysregulation, and 92.3% of pulmonary edema. High fever (temperature >39°C) was more frequent in patients with PE (53.9%) than in those with isolated BE (23.5%; P = .003). Mothers of patients with PE reported a longer duration of fever before hospitalization than those with isolated BE (P = .043).

Complete blood, differential white blood cell (WBC), and platelet counts, and C-reactive protein (CRP) levels are shown in table 1. Patients with PE tended to have higher total WBC counts (P = .008), absolute neutrophil counts (P = .002), and absolute bandform counts (P = .003) than those with isolated BE. Patients with PE had lower absolute monocyte counts than those with ANS dysregulation (P = .04) and isolated BE (P = .02). Although the absolute lymphocyte count did not differ between patients with ANS dysregulation and PE, there was a difference in subpopulations that is presented below. Patients with PE had higher platelet levels than those with ANS dysregulation (P = .029) and isolated BE (P = .005). The platelet count decreased more in patients with PE than with ANS dysregulation during hospitalization (P = .004). The median CRP levels were similar in patients with isolated BE, ANS dysregulation, and PE. However, the CSF lactate levels were significantly elevated in patients with PE than in patients with ANS dysregulation (P = .001) or with isolated BE (P < .001).

Depletion of CD4 T cells, CD8 T cells, and NK cells in EV71-associated PE. The absolute lymphocyte count in patients with PE did not significantly differ from those with ANS dysregulation, but there was a difference in immunophenotypes after further analysis. As shown in table 2, CD4+ and CD8+ T cell counts and NK cell counts decreased as the patients advanced from ANS dysregulation to PE. The CD4+ T cell counts of patients with PE were significantly lower than those with ANS dysregulation (P = .01). This also was found for CD8+ T cell counts (P = .04) and NK cell counts (P = .04). Nine (90%) of 10 patients with PE, compared with only 1 (11.1%) of 9 patients with ANS dysregulation, had CD4+ T cell counts <500 cells/μL. All patients with PE, but only 4 of those with ANS dysregulation (44.4%), had CD8+ T cell counts <500 cells/μL. There was

### Table 1. Clinical and laboratory measurements of enterovirus 71 (EV71)–infected patients after diagnosis at admission, by disease.

<table>
<thead>
<tr>
<th>Category</th>
<th>BE (n = 34)</th>
<th>ANS dysregulation (n = 25)</th>
<th>PE (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, months</td>
<td>33.6 ± 39.1 (21.5)</td>
<td>20.5 ± 14.8 (14.2)</td>
<td>17.3 ± 13.3 (17.6)</td>
</tr>
<tr>
<td>Fever day at admission</td>
<td>2.7 ± 1.2</td>
<td>3.0 ± 1.5</td>
<td>3.4 ± 1.2a</td>
</tr>
<tr>
<td>Fever &gt;39°C at admission, %</td>
<td>23.5</td>
<td>32</td>
<td>53.9a</td>
</tr>
<tr>
<td>WBC count, cells/mm³</td>
<td>11,958 ± 3786 (11,300)</td>
<td>14479 ± 4677 (15,300)</td>
<td>18881 ± 7284 (19,900)a</td>
</tr>
<tr>
<td>WBC count &gt;20000 cells/mm³, %</td>
<td>2.9</td>
<td>16</td>
<td>38.5b</td>
</tr>
<tr>
<td>ANC, cells/mm³</td>
<td>6905 ± 3324 (6150)</td>
<td>9338 ± 4858 (8580)</td>
<td>13209 ± 6371 (12510)b</td>
</tr>
<tr>
<td>ASC, cells/mm³</td>
<td>6458 ± 322 (5321)</td>
<td>8774 ± 4879 (7896)</td>
<td>10985 ± 5745 (10547)a</td>
</tr>
<tr>
<td>ABC, cells/mm³</td>
<td>844 ± 1080 (226)</td>
<td>703 ± 443 (410)</td>
<td>2717 ± 1363 (2610)b</td>
</tr>
<tr>
<td>AMC, cells/mm³</td>
<td>1005 ± 487 (954)</td>
<td>849 ± 431 (620)</td>
<td>806 ± 714 (488)c</td>
</tr>
<tr>
<td>ALC, cells/mm³</td>
<td>3779 ± 2092 (3430)</td>
<td>3929 ± 2660 (3034)</td>
<td>4546 ± 4289 (2587)</td>
</tr>
<tr>
<td>Platelet count, ×10⁹ cells/mm³</td>
<td>320 ± 88 (313)</td>
<td>375 ± 87 (372)</td>
<td>443 ± 115 (443)c</td>
</tr>
<tr>
<td>Platelet count &gt;400 ×10⁹ cells/mm³, %</td>
<td>20.6</td>
<td>44</td>
<td>61.5b</td>
</tr>
<tr>
<td>Maximum decreased platelet count, ×10⁹ cells/mm³</td>
<td>NA</td>
<td>111 ± 107 (90)</td>
<td>317 ± 148 (311)d</td>
</tr>
<tr>
<td>CRP level, mg/L</td>
<td>11.6 ± 31.7 (3.8)</td>
<td>9.3 ± 23.7 (2.6)</td>
<td>19.7 ± 25 (4)</td>
</tr>
<tr>
<td>IVIG treatment, %</td>
<td>61.8</td>
<td>84.6</td>
<td>92</td>
</tr>
<tr>
<td>CSF/WBC, cells/mm³</td>
<td>122 ± 133 (65)</td>
<td>224 ± 177 (180)a</td>
<td>210 ± 182 (176)</td>
</tr>
<tr>
<td>CSF/CSF lactate, mmol/L</td>
<td>1.8 ± 0.8 (1.7)</td>
<td>1.8 ± 0.6 (1.7)</td>
<td>5.9 ± 3.5 (5.2)b,d</td>
</tr>
</tbody>
</table>

**NOTE.** Data are mean ± SD (median), unless otherwise indicated. ABC, absolute bandform count; ALC, absolute lymphocyte count; AMC, absolute monocyte count; ANC, absolute neutrophil count; ANS, autonomic nervous system; ASC, absolute segment count; BE, brainstem encephalitis; CRP, C-reactive protein; CSF, cerebrospinal fluid; IVIG, intravenous immunoglobulin; NA, not applicable; PE, pulmonary edema; WBC, white blood cell.

a P < .05 vs. BE group.
b P < .005 vs. BE group.
c P < .05 vs. ANS dysregulation group.
d P < .005 vs. ANS dysregulation group.
no significant change of T cell subsets during the early stages of BE.

**Changes of cytokines in patients with PE.** The Cytokine Bead Array assay was used to simultaneously quantify the Th1/Th2 cytokine profile (IL-2, IL-4, IL-5, IL-10, TNF-α, and IFN-γ; table 3). IL-10 was significantly higher in patients with PE than in those with ANS dysregulation ($P < .001$) or isolated BE ($P < .001$). IFN-γ levels also were significantly higher in patients with PE than in those with ANS dysregulation ($P = .048$), although patients with PE also exhibited a high level of IFN-γ. The levels of IL-4 and IL-5 in each group were about at the detection limit; thus, no significant differences among different groups were noted.

Because IL-13 has been reported to be involved in airway hyperresponsiveness and mucus production in asthma and atopic dermatitis [14, 15], we postulated that it also might affect pulmonary vascular permeability and play a role in the pathogenesis of PE. Therefore, serum IL-13 levels were determined by ELISA. Patients with PE were found to have higher IL-13 levels than those with isolated BE ($P = .048$).

**Kinetic changes of leukocytes and cytokines in EV71-induced PE.** The response of the immune system to EV71 is a dynamic process. Therefore, cross-section analysis may not adequately delineate the evolving picture of EV71-caused disease. We had the opportunity to monitor the progressive changes in several hospitalized patients. A representative case is shown in figure 1. The total WBC count, absolute segment count, absolute monocyte count, and absolute lymphocyte count all decreased dramatically after hospital admission, whereas the absolute bandform and absolute neutrophil counts increased at day 2 after admission. The leukocyte counts gradually returned to normal at day 8. CD4+ and CD8+ T cells and NK cells also were depleted after admission, but the loss of CD4+ T cells was greater than that of CD8+ T cells (figure 2). The decrease in T cells was transient and rebounded after day 8. The change of Th1/Th2 cytokine profile also was examined. IL-10 levels peaked at 24 h after admission, whereas, in contrast, IFN-γ levels surged at 48 h after admission (figure 3). There were no significant changes in the levels of the other cytokines (IL-2, IL-4, IL-5, and TNF-α).

**DISCUSSION**

EV71 produces a spectrum of clinical manifestations. These vary from mild hand, foot, and mouth disease or herpangina, to BE, ANS dysregulation, and PE. The primary site of attack is the central nervous system, particularly the brainstem. There is no apparent evidence of viral myocarditis or pneumonia. Overwhelming PE is the leading cause of death [4–8].

PE could result from the interactions of several factors: (1) an increase in the pulmonary capillary hydrostatic pressure, (2) an increase in endothelial permeability induced by the increase in the capillary hydrostatic pressure and/or release of cytokines, (3) pulmonary microembolization secondary to intravascular thrombosis and platelet aggregation, and (4) lymphatic obstruction caused by sympathetic activation [16, 17]. Hemodynamic studies of patients with PE revealed normal cardiac function and normal pulmonary hydrostatic pressure [9]. These findings suggest that PE might be the result of increased pulmonary vascular permeability caused by the brainstem lesions and/or a systemic inflammatory response syndrome produced by the release of cytokines. In the present study, we provide evidence that PE is associated with extensive inflammatory responses, including elevated levels of IL-10, IL-13, and IFN-γ, as well as the depletion of CD4+ and CD8+ T cells and NK cells.

IL-10 is perhaps the most well-known anti-inflammatory cytokine [18, 19]. It inhibits the secretion of proinflammatory cytokines, suppresses phagocytosis, the oxidative burst, and intracellular killing, inhibits antigen presentation to T cells and

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Table 2. T cell subpopulations and NK cell counts of enterovirus 71 (EV71)-infected patients and healthy control subjects, by disease

<table>
<thead>
<tr>
<th>Cell category</th>
<th>Control subjects (n = 15)</th>
<th>BE (n = 8)</th>
<th>ANS dysregulation (n = 9)</th>
<th>PE (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4+ T cells</td>
<td>1001 ± 561</td>
<td>1066 ± 633</td>
<td>1160 ± 930</td>
<td>249 ± 243a,b</td>
</tr>
<tr>
<td>CD8+ T cells</td>
<td>482 ± 232</td>
<td>533 ± 316</td>
<td>582 ± 398</td>
<td>249 ± 154a,c</td>
</tr>
<tr>
<td>CD3+ T cells</td>
<td>1572 ± 553</td>
<td>1756 ± 876</td>
<td>1814 ± 1198</td>
<td>684 ± 434a</td>
</tr>
<tr>
<td>NK cells</td>
<td>276 ± 184</td>
<td>294 ± 204</td>
<td>288 ± 357</td>
<td>107 ± 75c</td>
</tr>
</tbody>
</table>

**NOTE.** Data are mean ± SD cells/mm³. ANS, autonomic nervous system; BE, brainstem encephalitis; PE, pulmonary edema.

* $P < .005$ vs. BE group.
* $P < .05$ vs. ANS dysregulation group.
* $P < .05$ vs. BE group.
causes T cell anergy. We found markedly enhanced IL-10 levels only in patients with ANS dysfunction who progressed to PE. A close relationship exists between catecholamine and IL-10 release [20]. 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 brainstem destruction by the virus. Eppinger et al. [21] reported that IL-10 reduced lung permeability by 53% in a rat model of pulmonary ischemia-reperfusion injury. Donnelly et al. [22] found that low levels of IL-10 in the bronchoalveolar lavage fluid of patients with acute respiratory distress syndrome were associated with a poor prognosis. Up-regulated IL-10 levels may have a protective effect in the development of PE by influencing the pulmonary capillary permeability. The protective effects of IL-10 are mediated through mechanisms that inhibit TNF-α, IL-1, IL-6, and IL-8 and reduce the secretion of reactive oxygen intermediates [23].

Occlusion of pulmonary vessels by microembolism also can contribute to PE [24, 25]. Our patients with PE and ANS dysfunction usually had increased platelet counts on admission. The platelet counts rapidly decreased during the course of hospitalization. IL-10 is reported to induce thrombocytopenia [26]. The formation of pulmonary intravascular microthrombi caused by platelet aggregation needs to be considered as a potential mechanism of PE. Intravascular coagulation could increase pulmonary vascular permeability [27]. Therefore, IL-10 may play a double-edged role in the pathogenesis of PE.

IL-13, as with IL-4 and IL-10, is another cytokine produced by T cells that has potential anti-inflammatory activity and suppresses the cytotoxic functions of monocytes/macrophages [14, 15]. An exaggerated production of IL-13 was observed in our patients and usually peaked during the early phase of hospitalization. High levels of IL-13, which are affected by endogenous IL-4 and required for airway hyperresponsiveness and mucus production, are found in patients with asthma and atopic dermatitis [28, 29]. We did not detect changes in IL-4 in EV71-infected patients, but IL-13 levels were consistently elevated in all 3 groups. Because IL-13 can act alone in the pulmonary model [29], we considered that overproduction of IL-13 might contribute to the pathogenesis of PE by increasing pulmonary vascular permeability and smooth muscle hyperplasia and cause airway hyperresponsiveness [30].

IFN-γ, a pleiotropic cytokine, is produced principally by CD4⁺ Th1 cells, cytotoxic CD8⁺ T cells, and NK cells. It is essential for both innate and adaptive immunity [31]. Virus infection stimulates IFN production and cell-mediated immunity, including macrophage activation, costimulation of

### Table 3. Plasma cytokine levels in enterovirus 71 (EV71)–infected patients and healthy control subjects, by disease

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Control subjects (n = 15)</th>
<th>BE (n = 34)</th>
<th>ANS dysregulation (n = 29)</th>
<th>PE (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-4</td>
<td>3.8 ± 1.7 (4.1)</td>
<td>4.5 ± 3.2 (3.1)</td>
<td>3.2 ± 1.6 (2.6)</td>
<td>3.4 ± 3.0 (2.4)</td>
</tr>
<tr>
<td>IL-5</td>
<td>3.2 ± 1.5 (3.0)</td>
<td>3.5 ± 2.7 (2.7)</td>
<td>3.9 ± 3.2 (3.1)</td>
<td>6.9 ± 12.9 (2.1)</td>
</tr>
<tr>
<td>IL-10</td>
<td>103.3 ± 4.1 (9.1)</td>
<td>12.1 ± 18.8 (7.6)</td>
<td>12.8 ± 16.1 (7.3)</td>
<td>109.3 ± 149.9 (109.3)</td>
</tr>
<tr>
<td>IL-2</td>
<td>2.3 ± 1.1 (2.4)</td>
<td>6.9 ± 3.7 (8.2)</td>
<td>4.3 ± 1.7 (3.4)</td>
<td>39.3 ± 95.8 (6.9)</td>
</tr>
<tr>
<td>TNF-α</td>
<td>31.8 ± 46.5 (10.8)</td>
<td>39.1 ± 122.4 (8.05)</td>
<td>39.9 ± 74.2 (6.6)</td>
<td>12.5 ± 12.9 (5.4)</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>41.5 ± 24.7 (32.6)</td>
<td>100 ± 193.1 (34.1)</td>
<td>18.5 ± 57.3 (25.3)</td>
<td>137.7 ± 149.7 (82.1)</td>
</tr>
<tr>
<td>IL-13</td>
<td>96.0 ± 14.5 (95.4)</td>
<td>113.7 ± 39.8 (118.3)</td>
<td>137.0 ± 59.7 (116.6)</td>
<td>139.4 ± 83.7 (98.7)</td>
</tr>
</tbody>
</table>

NOTE. Data are mean ± SD picograms per milliliter (median). ANS, autonomic nervous system; BE, brain-stem encephalitis; IFN, interferon; IL, interleukin; PE, pulmonary edema.

* a P < 0.05 vs. BE group.
* b P < 0.05 vs. ANS dysregulation group.
* c P < 0.05 vs. BE group.
* d P < 0.05 vs. ANS dysregulation group.

### Figure 2

Kinetic changes of T cell subsets (CD4, CD8, and CD3) and natural killer cells (NK) in a patient with enterovirus 71 infection complicated with pulmonary edema.
antigen-presenting cells, and promotion of differentiation of naive CD4⁺ T cell to the Th1 subset, and promotes switching of various IgG subclasses to combat viral infection. We found elevated serum levels of IFN-γ in patients with BE and PE. 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 [32]. IFN-γ–mediated microvascular leakage occurs as a result of the reduced endothelial barrier and tight junction [33]. IL-10 is a cytokine synthesis inhibitor that will terminate the production of IFN-γ. We found that IFN-γ production appeared later than that of IL-10, which suggests that IFN-γ might play an important role in the development of PE.

Lymphocyte homeostasis requires a precise balance between the rates of cellular proliferation and programmed cell death (apoptosis). Lymphocytes depend on extracellular ligands, such as cytokines, antigens, and cell adhesion receptors, to maintain their viability [34]. We found that CD4⁺ T cells, CD8⁺ T cells, and NK cells were depleted in patients with PE, which might impair the clearance of EV71. In unpublished studies (H. Y. Lei, personal communication), we found that EV71 can infect lymphocytes and cause apoptosis. A high EV71 load perhaps results from impaired T cell–mediated response after diminution of T cells. Moreover, the depletion of CD4⁺ and CD8⁺ T cell subsets and NK cells may cause the release of specific cytokines [35, 36], which is not uncommon in acute viral infection. We also have reported a transient depletion of CD4⁺ T cells during acute dengue infection [37].

A better understanding of the complexity of cell-mediated immunity may shed light on improving the outcome of EV71 infection–induced PE and developing therapeutic strategies.

Acknowledgments

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