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Innate Immune Responses in Serum and Cerebrospinal Fluid From Neonates and Infants Infected With Parechovirus-A3 or Enteroviruses

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Background. Parechovirus (PeV)-A3 and enteroviruses (EV) are the most common viruses causing sepsis and meningoencephalitis in neonates and young infants. Clinical manifestations of PeV-A3 infection are more severe than those of EV infection, and no pleocytosis with a positive polymerase chain reaction (PCR) result for PeV-A3 in cerebrospinal fluid (CSF) are characteristic findings. We hypothesized that innate immune responses to PeV-A3 and EV are distinct in serum and CSF.

Methods. We evaluated 22 cytokines/chemokines in serum and CSF from PeV-A3- or EV-infected patients younger than 4 months in Niigata, Japan, from 2015 through 2018. Infection was diagnosed with real-time PCR followed by sequencing. Febrile neonates and infants with sepsis-like syndrome who had negative bacterial culture and viral PCR for both PeV-A and EV were also included (non-PeV-A/EV patients).

Results. Among 192 febrile patients, we evaluated 16 PeV-A3-infected, 15 EV-infected, and 8 non-PeV-A/EV patients. Serum pro-/anti-inflammatory cytokine/chemokine levels were higher in PeV-A3-infected patients than in EV-infected patients ($P < .02$). Although most cytokine/chemokine were elevated in CSF from EV-infected patients, levels were low or undetectable in PeV-A3-infected and non-PeV-A/EV patients ($P < .001$).

Conclusions. Distinct cytokine/chemokine patterns in serum and CSF may explain the different clinical manifestations of PeV-A3-infected and EV-infected neonates and young infants.

Keywords. cerebrospinal fluid; enteroviruses; innate immunity; parechovirus-A3; serum.

In neonates and infants younger than 4 months, sepsis and meningoencephalitis result in high morbidity and mortality. The well known causative pathogens are bacteria such as *Streptococcus agalactiae* and enteric bacteria; however, evidence collected after the development and distribution of molecular diagnostics for viruses suggests that viruses are also important causes of sepsis and meningoencephalitis [1]. Parechovirus (PeV)-A and the enteroviruses (EV) are the most commonly detected viruses in serum and cerebrospinal fluid (CSF) from neonates and young infants [2].

Parechovirus-A, previously referred to as human parechovirus, has 19 genotypes [3]. Parechovirus-A3, first discovered in Japan in 1999 [4], is the most important genotype in pediatrics because it causes sepsis and meningoencephalitis in neonates and young infants [5] and because it may result in severe neurological sequelae in encephalitis cases [6, 7].

Neonates and infants with sepsis and meningoencephalitis usually present with fever; however, the clinical presentations of PeV-A3 and EV infections differ. Previous studies reported that PeV-A3 infection is associated with more severe clinical manifestations, including higher fever, tachycardia, higher incidence of signs showing poor peripheral circulation, and higher admission rates to pediatric intensive care units [8–13]. In addition, patients with PeV-A3 infection tend to present with unique clinical signs, including palmar and/or plantar erythema [14] and abdominal distension with umbilical protrusion [15]. Cerebrospinal fluid pleocytosis is often absent, even though polymerase chain reaction (PCR) analysis of CSF is positive for PeV-A3 [16]. The reasons for the more severe clinical manifestations of PeV-A3 infection are unknown.

We hypothesized that innate immune responses to PeV-A3 and EV in neonates and young infants would be distinct and thus evaluated innate immune responses by measuring important cytokines and chemokines in serum and CSF. The results should increase understanding of the pathophysiology of clinical manifestations of PeV-A3 and EV infection in neonates and young infants.

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METHODS

Subjects and Sample Acquisition

The subjects were febrile neonates and infants younger than 4 months who required hospitalization at Niigata University Hospital or its 19 affiliated hospitals in the region during the period from January 2015 through December 2018. Samples of their serum and CSF were collected simultaneously on admission (within an interval of several hours), frozen, and sent to the laboratory at Niigata University for PCR analysis and genotype testing. Bacterial infection was excluded on the basis of the patients' clinical course and laboratory data and negative bacterial cultures for blood, urine, and/or CSF. In suspected cases, infection with herpes simplex virus (HSV) types 1/2 was excluded by real-time PCR [17]. Parechovirus-A and EV infections were diagnosed with a real-time PCR assay targeting the conserved 5'-untranslated region that used both serum and CSF specimens [18, 19]. Parechovirus-A genotype PeV-A3 was confirmed by sequence analysis of the VP1 region [20]. Sequence analysis of the VP1 was used to classify EV infection by genotype [21], after which the EV B species were selected because they were reported to be associated with septic syndrome in infants [22].

We selected PeV-A3- or EV-infected patients when PCR results for both serum and CSF samples, obtained simultaneously, were positive. We excluded patients unless PCR results were positive for both serum and CSF because we evaluated innate immune responses in serum and CSF simultaneously when virus was present at 2 anatomical sites. When PeV-A or EV was isolated from a patient, clinical information for the patient was collected from questionnaires. We also included a group of patients who were negative for bacterial culture and PCR for both PeV-A and EV in serum and CSF (non-PeV-A/EV patients). For comparison with PeV-A3-infected patients, we included patients from the period January 2016 through December 2018 because there were no PeV-A3 cases in 2015. Patients who received a diagnosis of aseptic meningitis were excluded because no PeV-A3-infected patient had CSF pleocytosis. The final diagnosis for all non-PeV-A/EV patients was sepsis-like illness.

This study was approved by the Ethics Committee of Niigata University (No. 2018-0289), and informed consent was obtained for all patients. The study was conducted in accordance with the human experimentation guidelines of Niigata University, Japan.

Laboratory Analysis

Cytokine/Chemokine Analysis

Cytokine and chemokine levels in serum and CSF were measured with a MILLIPLEX MAP human cytokine/chemokine magnetic bead panel (Merck Millipore, Darmstadt, Germany). The 22 cytokine/chemokine levels measured were C-X3-C motif chemokine legend 1 (CX3CL1), granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon (IFN)-α2, IFN-γ, interleukin (IL)-1α, IL-1β, IL-1Rα, IL-2, IL-4, IL-6, IL-10, IL-12p40, IL-15, IL-17, C-X-C motif chemokine ligand 1 (CXCL1), CXCL8, CXCL10, C-C motif chemokine legend 2 (CCL2), CCL3, CCL4, and tumor necrosis factor α (TNF-α). All samples were assayed according to the manufacturer's instructions.

In brief, standards, controls, and samples were loaded into 96-well plates, after which antibody immobilized beads were added. Plates were incubated overnight at 4°C on a plate shaker and then washed twice with wash buffer. Detection antibodies were then added to the wells and incubated for 1 hour. Streptavidin-phycoerythrin was added to each well and incubated for 30 minutes. Plates were washed twice before resuspending the beads in sheath fluid, followed by analysis on a Luminex 200 (Merck Millipore). Using xPONENT software version 3.1 (Luminex Corporation, Austin, TX), we generated a standard curve with a 5-parameter logistic curve for each mediator, after which cytokine/chemokine concentrations were calculated.

Quantitation of Viral Ribonucleic Acid

To quantify viral ribonucleic acid (RNA) copy numbers of PeV-A3 and EV, a 2-step real-time reverse transcription PCR assay was used. In this assay, 10^3 copies/μL plasmids were included in triplicate and corrected for quantification with a standard curve generated from serial dilution of known numbers of copies of plasmids (10^2 to 10^6 copies/μL) produced by a TA-cloning technique with a Mighty TA-cloning Kit (TaKaRa, Tokyo, Japan). In the assays, the upper and lower limits of quantification (LOQ) were 2×10^6 and 2×10^2 copies/reaction, respectively. The upper and lower limits of detection (LOD) were 3.8×10^7 copies/reaction and 1 copy/reaction, respectively. For the 1 copy/reaction limit, the coefficient of variation was 3.0%. When the viral load was between the upper LOQ and upper LOD, the sample was diluted until it was less than the LOQ.

Statistical Analysis

Cytokine and chemokine levels in serum and CSF specimens were compared after log transformation of data, to increase statistical validity. Data are expressed as median (range), and copy numbers of viral RNA are expressed by using a logarithmic scale. The 2-tailed χ^2 test or Fisher exact test was used to analyze categorical variables. The Kruskal-Wallis test was used for among-group comparisons of cytokine/chemokine concentrations. The Mann-Whitney test was used for comparisons of 2 groups. Correlations of viral RNA levels in serum and CSF between PeV-A3- and EV-infected patients, and correlations of cytokine/chemokine levels with clinical and laboratory data, were calculated by using Spearman rank correlation. Data ware analyzed with the IBM SPSS Statistics 25.0 package (IBM SPSS, Chicago, IL). Hierarchical clustering of the log-transformed data matrix was performed to classify patient groups with different cytokine/chemokine types by means of Euclidian distance

and Ward's clustering method. The pheatmap library (https://cran.r-project.org/web/packages/pheatmap/index.html) for R (https://www.R-project.org/) was used to produce the heatmap figure, and the default color setting was used for the clustering. $P < .05$ was considered to indicate statistical significance.

RESULTS

Clinical Characteristics of Parechovirus-A3 and Enterovirus-Infected Patients and Non-PeV-A/EV Patients

Patient flow is shown in Figure 1. Among a total of 192 patients, we selected 142 patients for whom simultaneous real-time PCR assays for serum and CSF samples were performed: 23 (16.2%) had a positive result for PeV-A3 in serum and/or CSF, 46 (33.3%) had a positive result for EV in serum and/or CSF, and 73 (51.4%) had a negative result for both PeV-A3 and EV. Subsequently, we excluded patients with a negative PCR result for serum or CSF, namely, 3 of 23 (13.0%) patients for PeV-A3 infection and 24 of 46 patients (52.1%) for EV infection. In addition, 1 patient with EV A species infection was excluded. Last, 4 PeV-A3-infected and 6 EV-infected patients were excluded because remaining volumes of serum or CSF leftover samples were insufficient for cytokine/chemokine assay. Among non-PeV-A/EV patients—who had negative PCR results for both PeV-A and EV—8 patients were selected because (1) clinical data were available, (2) clinical course was uncomplicated and the final diagnosis was sepsis-like illness (no sepsis, meningitis, or other significant diseases), and (3) remaining volumes of leftover serum and CSF samples were sufficient for cytokine/chemokine assay. In total, data from 16 PeV-A3-infected patients (PeV-A3 group), 15 EV-infected patients (EV group), and 8 non-PeV-A/EV patients were analyzed.

The patients' demographic data and clinical and laboratory characteristics are summarized in the Table 1. There was no significant difference in sex or age among the 3 groups ($P = .53$ and $P = .73$, respectively). The PeV-A3 group had significantly higher heart rates (tachycardia) and lower white blood cell (WBC) counts (leukopenia) than the EV group ($P = .018$ and $P = .012$, respectively) and non-PeV-A/EV group ($P = .035$ and $P = .011$, respectively). Most patients in the EV group (53%) had CSF pleocytosis; in contrast, no CSF pleocytosis was present in the PeV-A3 or non-PeV-A/EV group ($P = .002$).

Serum and Cerebrospinal Fluid Cytokine/Chemokine Profiles in Parechovirus-A3 and Enterovirus-Infected Patients

The levels of dominant cytokine/chemokine in the 3 groups are summarized in the Supplementary Table 1.

Serum

In serum, some cytokine/chemokine levels—such as IFN-α2 (Figure 2A, left), CXCL8 (Figure 2C, left), CX3CL1 (Figure 2F, left), IL-15 (Figure 2G, left), IL-10 (Figure 2H, left), and IL-1Rα (Figure 2I, left)—were significantly higher in the PeV-A3 group than in the EV ($P < .02$) and non-PeV-A/EV

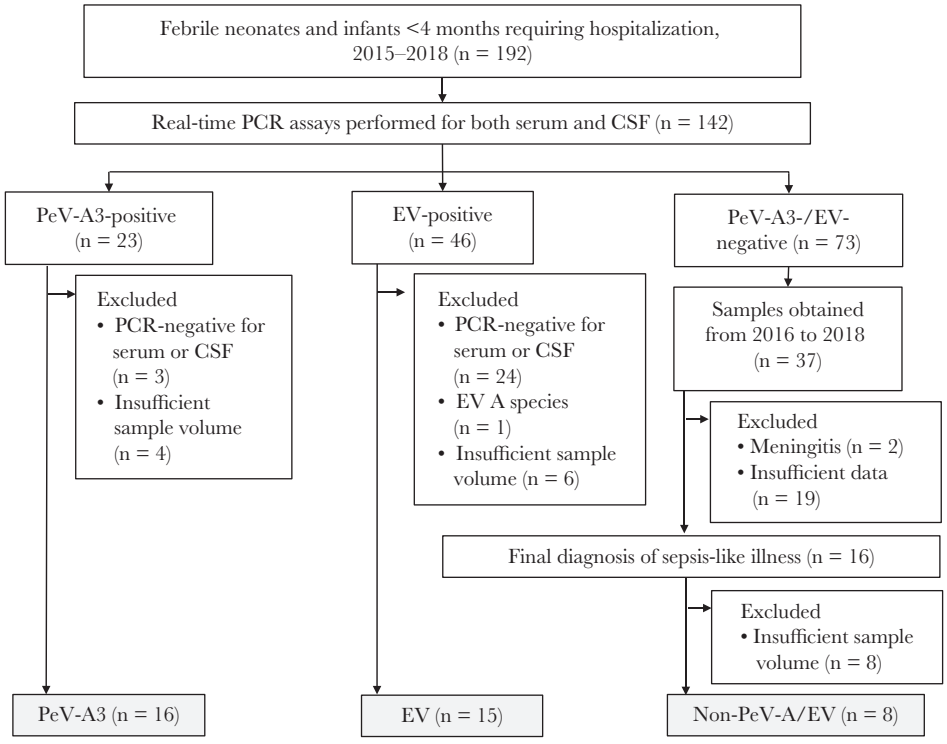


Figure 1. Flow chart of the enrollment of parechovirus (PeV)-A3-infected patients, enterovirus (EV)-infected patients, and non-PeV-A/EV-infected patients. CSF, cerebrospinal fluid; PCR, polymerase chain reaction.

Table 1. Demographic Data and Clinical and Laboratory Characteristics of Patients With Parechovirus-A3, Enteroviruses, or Neither (Non-Parechovirus-A/Enteroviruses)

Parameters	PeV-A3-Infected Patients (N = 16)	EV-Infected Patients (N = 15)	Non-PeV-A/EV Patients (N = 8)
Male, %	69	60	36
Age, days (median [IQR])	30.5 (18–42)	28.0 (16–30)	21.5 (13–35)
Maximum temperature, °C (median [IQR])	38.6 (38.4–39.1)	38.5 (38.3–38.8)	38.6 (38.3–38.9)
Heart rate, /min (median [IQR])	189 (179–196)	162 (160–180)	166 (149–178)
Respiratory rate, /min (median [IQR])	40 (40–43)	45 (40–55)	35 (32–36)
Blood			
WBC, cells/mm^3 (median [IQR])	4700 (4220–6775)	8600 (7500–10 300)	9280 (7975–11 678)
CRP, mg/dL (median [IQR])	0.3 (0.2–0.5)	0.3 (0.1–0.65)	0.5 (0.1–1.6)
LDH, IU/mL (median [IQR])	298 (270–343)	295 (268–346)	277 (246–332)
Cerebrospinal fluid			
WBC, cells/mm^3 (median [IQR])	1 (1–3.8)	66 (6.5–168.5)	3 (1.8–4.3)
Pleocytosis, %	0	53	0
Glucose, mg/dL (median [IQR])	59 (52.3–68)	46 (41–49)	58 (47–59.5)
Protein, mg/dL (median [IQR])	41 (32–50)	68 (52.5–81)	55 (40–70)

Abbreviations: CRP, C-reactive protein; EV, enteroviruses; IQR, interquartile range; LDH, lactate dehydrogenase; PeV, parechovirus; WBC, white blood cells.

groups ($P < .03$). However, IL-6 level in the PeV-A3 group was higher than that in the EV group and similar to that of the non-PeV-A/EV group (Figure 2B, left). Interleukin-1α level in the PeV-A3 group was significantly higher than that of the non-PeV-A/EV group, but not higher than that of the EV group (Figure 2E, left). The pattern was similar for CXCL10 and CCL2 (Supplementary Table 1). No significant differences among the 3 groups were observed in G-CSF, GM-CSF, IFN-γ, CXCL1, IL-1β, IL-2, IL-4, IL-17, CCL3, CCL4, or TNF-α ($P > .05$ for all; data not shown).

To clarify the cytokine/chemokine patterns of the 3 groups, a heatmap was used to display the dominant cytokine/chemokine profiles in serum (Figure 3A). Hierarchical clustering showed a trend that separated the PeV-A3 group from the EV and non-PeV-A/EV groups.

Cerebrospinal Fluid

Levels of most cytokine/chemokine in CSF were significantly higher in the EV group than in the PeV-A3 and non-PeV-A/EV groups (Supplementary Table 1). Significant cytokines/chemokines in CSF are shown in Figure 2. Levels of some cytokine/chemokine, such as IL-6 (Figure 2B, right), IL-10 (Figure 2H, right), and IL-1Rα (Figure 2I, right), were high in the EV group but undetectable in the PeV-A3 and non-PeV-A/EV groups ($P < .001$), whereas some cytokines/chemokines, such as IFN-α2 (Figure 2A, right), CXCL8 (Figure 2C, right), TNF-α (Figure 2D, right), and IL-15 (Figure 2G, right), were detected in all 3 groups but were significantly higher in the EV group ($P < .001$). The level of IFN-α2 was significantly higher in the EV group than in the PeV-A3 ($P = .03$) and non-PeV-A/EV groups ($P < .001$). Levels of IL-1α (Figure 2E, right) and CX3CL1 (Figure 2F, right) were significantly higher in the PeV-A3 and EV groups than in the non-PeV-A/EV group ($P < .001$ and $P < .001$, respectively) but did not differ between the PeV-A3 and EV groups ($P = .096$ and $P = .106$, respectively). No significant differences among the 3 groups were observed in IL-12p40, CXCL10, or CCL2 (data not shown).

The heatmap and hierarchical clustering of significant cytokine/chemokine profiles in CSF are shown in Figure 3B. Compared with serum, the cluster of the PeV-A3 and EV groups was distinct. The cytokine/chemokine pattern in CSF was similar in the PeV-A3 and non-PeV-A/EV groups.

Viral Ribonucleic Acid Levels in Serum and Cerebrospinal Fluid From Parechovirus-A3- and Enterovirus-Infected Patients

When we compared viral RNA levels in serum and CSF samples from the PeV-A3 and EV groups, RNA levels in serum and CSF were strongly correlated in the PeV-A3 group ($r = 0.714$, $P = .004$); however, there was no such correlation in the EV group ($r = 0.064$, $P = .82$). In serum, the median level of viral RNA in the PeV-A3 group (5.06 $\log_{10}$ copies/mL) was significantly higher than that in the EV group (3.24 $\log_{10}$ copies/mL) ($P = .004$). In contrast, the median level of viral RNA in CSF was significantly higher in the EV group (2.92 $\log_{10}$ copies/mL) than in the PeV-A3 group (1.75 $\log_{10}$ copies/mL) ($P = .020$) (Figure 4).

Correlations Between Cytokine/Chemokine Levels and Clinical and Laboratory Findings

We then focused on representative cytokines/chemokines, which were significant in previous analyses, including IFN-α2, IL-6, CXCL8, TNF-α, IL-1α, CX3CL1, IL-15, IL-10, and IL-1Rα. When we evaluated correlations of these cytokine/chemokine levels with serum and clinical data in PeV-A3-infected patients, TNF-α and IL-1Rα were positively correlated with respiratory rate ($r = 0.70$, $P = .01$ and $r = 0.69$, $P = .01$, respectively).

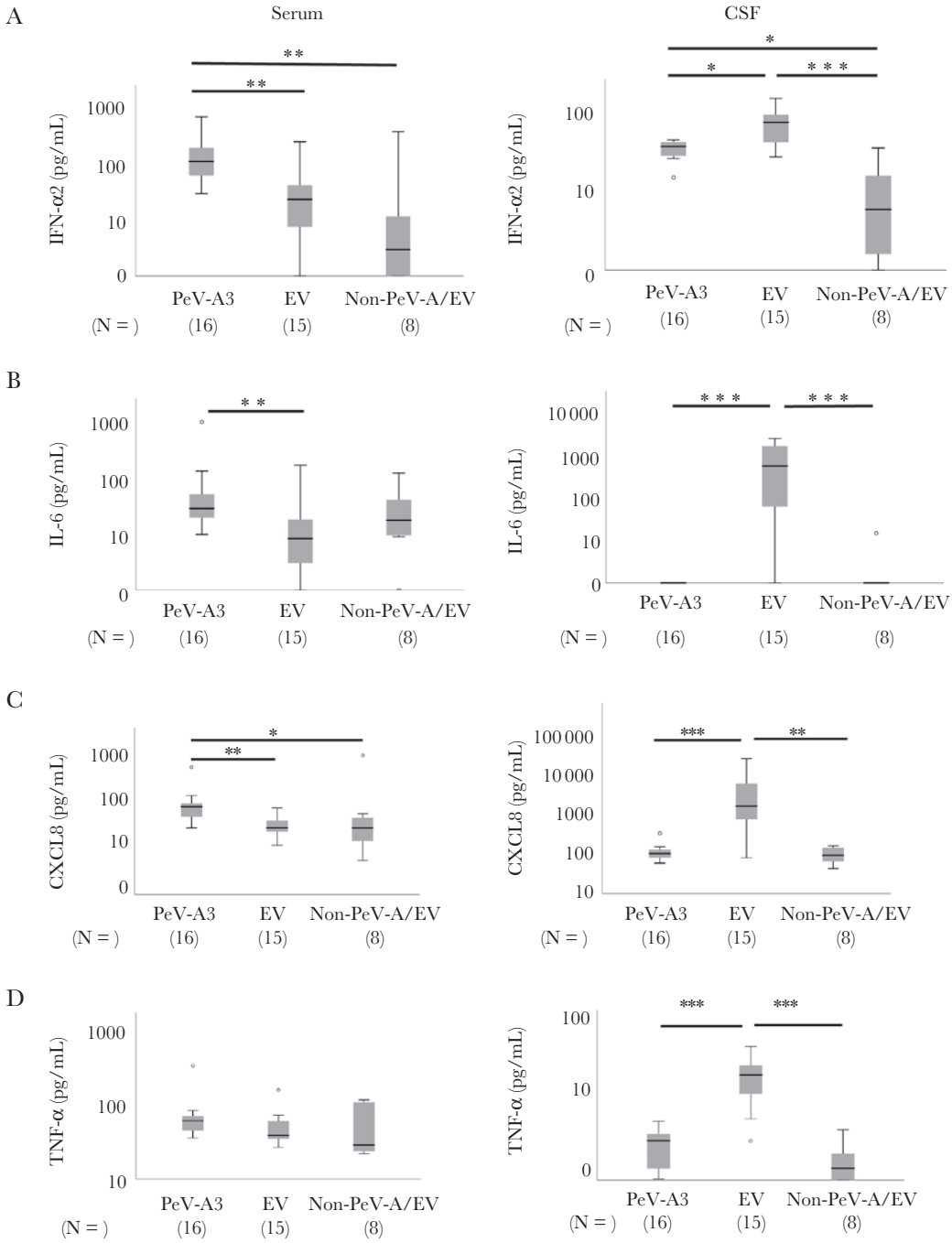
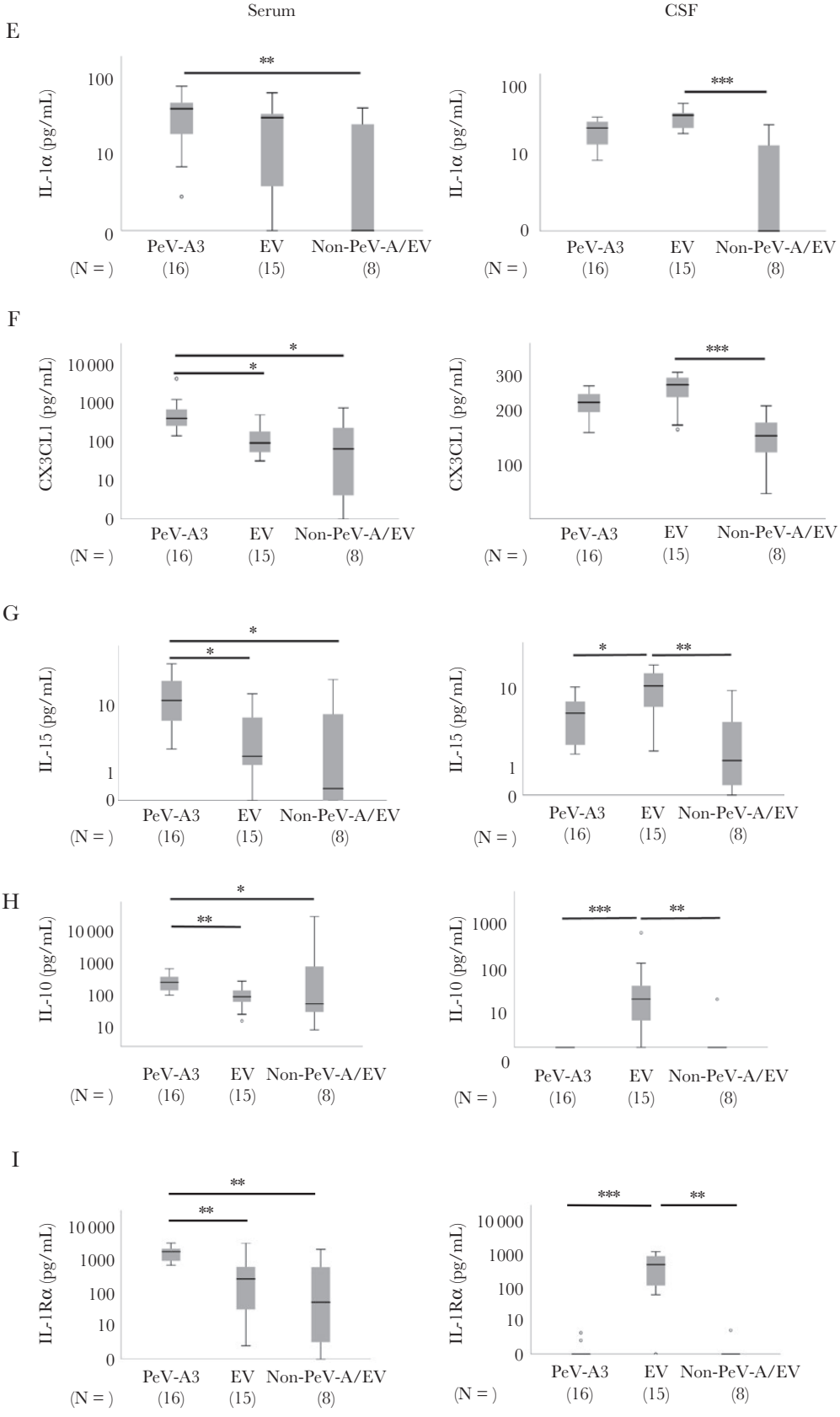


Figure 2. Levels of cytokine/chemokine in serum and cerebrospinal fluid (CSF) from parechovirus (PeV)-A3-infected patients, enterovirus (EV)-infected patients, and non-PeV-A/EV-infected patients. (A) Interferon (IFN)-α2, (B) interleukin (IL)-6, (C) CXCL8, (D) tumor necrosis factor (TNF)-α, (E) IL-1α, (F) CX3CL1, (G) IL-15, (H) IL-10, and (I) IL1-receptor α (IL-1Rα). Cytokine/chemokine levels are expressed as bars representing median values and interquartile ranges. The whiskers on either side represent the lowest and highest quartiles of the data. Individual dots beyond the whiskers represent outliers in the data set. *, $P < .05$, **, $P < .01$, ***, $P < .001$. CXCL, C-X-C motif chemokine ligand; CX3CL, C-X3-C motif chemokine ligand.

Likewise, correlations of CSF cytokine/chemokine levels with CSF parameters, including WBC count and glucose and protein level, were analyzed in EV-infected patients. Several cytokines/chemokines, including IFN-α2, IL-6, CXCL8, and IL-10 were significantly correlated with WBC count ($P < .05$) and protein level ($P < .01$) in CSF (Supplementary Table 2).

Correlations of Cytokine/Chemokine Levels With Viral Ribonucleic Acid Levels in Serum and Cerebrospinal Fluid in Parechovirus-A3- and Enterovirus-Infected Patients

Next, we analyzed correlations of cytokine/chemokine levels with viral RNA levels in serum and CSF in PeV-A3- and EV-infected patients. In serum, there were no correlations



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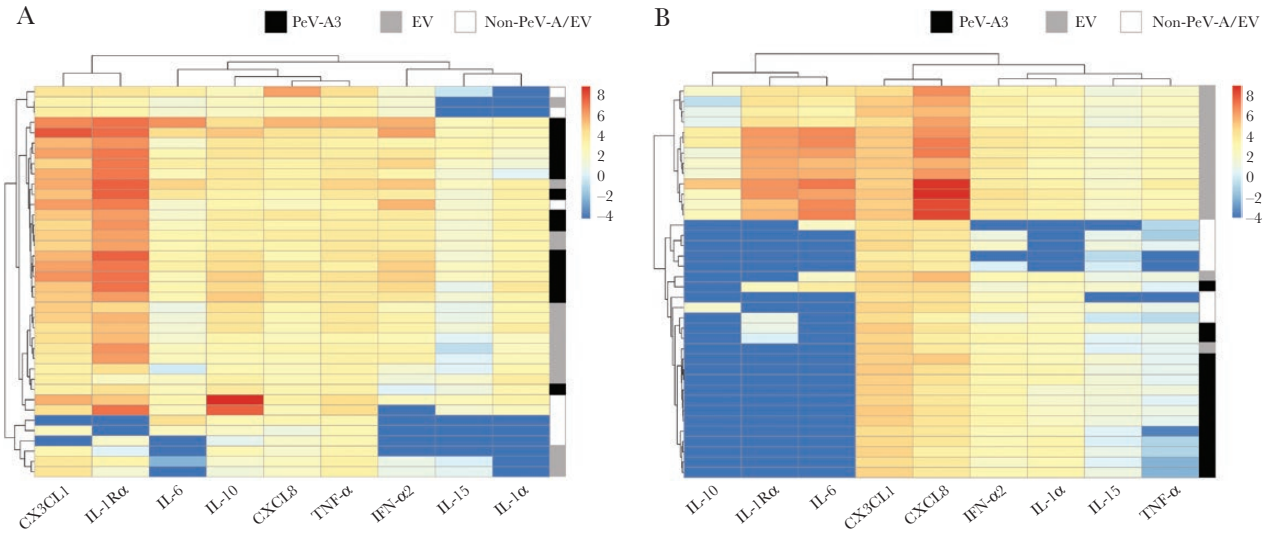

Figure 3. Hierarchical clustering and heatmap analysis of cytokine/chemokine expression profiles for patients infected with parechovirus (PeV)-A3 (black), enteroviruses ([EV] gray), and non-PeV-A/EV (white). (A) Serum (B) cerebrospinal fluid (CSF) cytokine/chemokine levels are depicted by using a color scale: red, yellow, and blue represent high, intermediate, and low concentrations, respectively. CXCL, C-X-C motif chemokine ligand; CX3CL, C-X3-C motif chemokine ligand; IFN, interferon; IL, interleukin; IL1-Rα, interleukin1-receptor α; TNF, tumor necrosis factor.

between cytokine/chemokine levels and viral RNA levels in PeV-A3- and EV-infected patients ($P > .12$ and $P > .42$, respectively). In CSF, IL-15 was negatively correlated with viral RNA levels in PeV-A3-infected patients ($r = -0.54$, $P = .047$), and IFN-α2 was positively correlated with viral RNA levels in EV-infected patients ($r = 0.60$, $P = .02$). The detailed results are summarized in Supplementary Table 3.

DISCUSSION

This is the first study to evaluate cytokines/chemokines in simultaneously obtained serum and CSF samples from neonates and young infants with PeV-A3 and EV infection. The cytokine/chemokine profiles in serum and CSF were distinct in PeV-A3- and EV-infected patients. The higher serum levels of proinflammatory cytokine/chemokine in PeV-A3-infected patients than in EV-infected patients might explain the more severe clinical manifestations and unique clinical presentation in the former. The absence of CSF pleocytosis in PeV-A3-infected patients may be attributable to minimal or nonexistent cytokine/chemokine response in CSF.

Few studies have investigated cytokine/chemokine in infants with PeV-A3 infection [21, 22]. In studies using only CSF samples, levels of cytokine/chemokine in CSF were generally higher in EV-infected patients than in PeV-A-infected patients. This difference in CSF cytokine/chemokine profiles between PeV-A and EV infection might be due to distinct tissue tropisms of viruses in the central nervous system (CNS), ie, the smooth muscle cells of leptomeningeal blood vessels in PeV-A3 [23] and CNS parenchymal cells, including neurons, in EV [24], which may mute or weaken innate immune response in CSF during PeV-A infection. However, the findings of these studies cannot explain the more severe clinical manifestations in PeV-A-infected patients, and distinct tissue tropisms in the CNS are not likely linked to converse cytokine/chemokine profiles in serum from PeV-A3-infected patients, as demonstrated in the current study.

The neonatal immune system is usually biased toward type 2 helper T-cell responses, which decreases secretion of proinflammatory cytokines, including TNF-α, IFN-γ, and IL-1β [1]. This inappropriate immune response causes inflammation but inhibits viral clearance. In the present study, serum levels of these cytokines were comparable in the 3 groups, which reflects the immature immune response of neonates and young infants. In contrast, serum levels of IL-6 and IL-10 were higher in PeV-A3-infected patients than in EV-infected patients (Figure 2B and 2H). These cytokines are produced through the Toll-like receptor (TLR)-mediated pathway, which is believed to be responsible for viral sepsis [1]. During HSV infection, cytokine production through the TLR2 pathway was higher in neonates than in adults [25]. The exaggerated inflammatory response through the TLR-mediated pathway may be related to the more severe clinical manifestations of PeV-A3 infection. Serum levels of type 1 IFN, such as IFN-α2, were higher in the present PeV-A3-infected patients than in the EV-infected and non-PeV-A/EV-infected patients (Figure 2A). A feature of the neonatal immune system is reduced production of type 1 IFNs, which can hamper viral clearance [1]. Elevated serum IFN-α2 levels in PeV-A3-infected patients suggest enhanced viral clearance. Furthermore, serum levels of anti-inflammatory cytokines such as IL-10 and IL-1Rα were significantly higher in the PeV-A3-infected patients than in EV-infected and non-PeV-A/

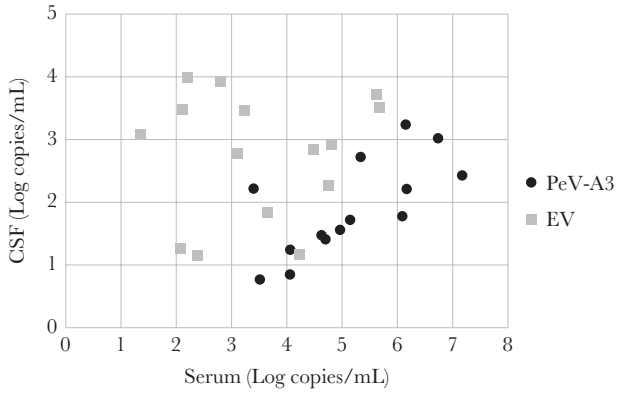

Figure 4. Correlation between serum and cerebrospinal fluid (CSF) viral ribonucleic acid levels in patients infected with parechovirus (PeV)-A3 (circle) and enteroviruses ([EV] square).

EV-infected patients (Figure 2H and 2I). In fact, viral RNA levels rapidly decreased during sepsis in neonates and young infants with PeV-A3 infection [16]. Exaggerated inflammatory response through the TLR-mediated pathway and elevated secretion of cytokines for viral clearance may be important in the pathogenesis of PeV-A3 infection in neonates and young infants. This may explain why clinical manifestations were more severe than those of EV infection and why fever resolved after a median duration of 3 days, without specific antiviral therapy [16].

Immune evasion of EV may explain in part why immune responses differ between PeV-A3 and EV infection. Enteroviruses interfere with or avoid initial pattern recognition receptor (PRR) recognition or downregulate downstream cytokine signaling pathways [1]. Toll-like receptors are one of the PRR families. This interference may have reduced the TLR-mediated pathway in EV-infected patients. In contrast, PeV-A3 may not interfere in this way with innate immune signaling. The difference in viral load between serum and CSF samples from PeV-A3- and EV-infected patients (Figure 4) may be another reason for the distinct cytokine/chemokine profile. The viral RNA level in serum was generally higher in PeV-A3-infected patients, whereas the viral RNA level in CSF was often higher in EV-infected patients. When we evaluated correlations of representative cytokines/chemokines with viral RNA levels, INF-α2 was positively correlated with viral RNA levels in CSF in EV-infected patients. In contrast, there was a negative correlation between IL-15, which is secreted by virus-infected phagocytes inducing proliferation of natural killer cells [26], and viral RNA levels in CSF in PeV-A3-infected patients. Parechovirus-A3 in CSF might induce immune suppression rather than elicit an immune response. Thus, a future study should focus on the effects of these specific cytokines/chemokines on immune responses in serum and CSF.

In the current study, we were able to visualize serum and CSF cytokine/chemokine patterns in PeV-A3- and EV-infected patients and the non-PeV-A/EV patients by using heatmap analysis with 9 representative cytokines/chemokines (Figure 3). Although cytokine/chemokine profiles in the groups varied slightly, serum and CSF cytokine/chemokine patterns were distinct in the 3 groups, especially those for CSF. Our understanding of clinical manifestations will likely improve when we compare these findings with patient clinical information so that clinical manifestations can be explained in relation to variations in cytokine/chemokine profiles.

We included a group of febrile neonates and infants younger than 4 months who required hospitalization, had negative PCR results for both PeV-A and EV, and had received a diagnosis of sepsis-like illness without CSF pleocytosis (the non-PeV-A/EV patients). Although this group was small, we identified distinct differences in serum and CSF cytokine/chemokine patterns between this group and PeV-A3- and EV-infected patients. This was helpful in understanding cytokine/chemokine levels in patients infected with PeV-A3 and EV and in those without such infections.

This study has some limitations. First, blood and CSF samples were obtained when patients were admitted to hospital, so we were unable to control the timing of sample collection with respect to disease onset. Fever in neonates and young infants is a pediatric emergency, and parents need to bring their children to a clinic or hospital immediately. Thus, we believe that the interval from fever onset to sample collection was relatively short in the current study. Second, cytokine/chemokine profile was measured by using samples obtained on admission. Analysis of changes in cytokine/chemokine profile in samples collected at multiple time points during hospitalization might better clarify the characteristics of immune responses. Last, patients with encephalitis were not included in this study cohort because there were no cases of encephalitis during the study period. Evaluating samples from patients with the most severe clinical manifestations of PeV-A3 infection may deepen our understanding of pathogenesis.

CONCLUSIONS

In conclusion, innate cytokine/chemokine responses to PeV-A3 and EV are distinct in serum and CSF. These findings improve our understanding of the different pathophysiological characteristics of PeV-A3 and EV infection in neonates and young infants.

Notes

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Potential conflicts of interest. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

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