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Enterovirus Genotype Specific Immune Response in Cerebrospinal Fluid of Infected Infants

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ABSTRACT (Revised)

Background

Non-polio Enteroviruses (EV) are important neonatal CNS pathogens. Multiple EV genotypes have been detected in pediatric CSF, e.g. Echovirus (E)6 and E30. CSF innate immune responses to EV genotypes remain poorly defined. Most data are from EV-A71 or E30 CNS infections and do not compare responses between these or other EV types. We sought to better define innate immune responses to EV genotypes in CSF.

Methods

Salvaged standard of care CSF samples from ≤6 month olds (real time EV PCR(+) or EV PCR(-) controls) from Jan 2010 - Dec 2020 were tested in duplicate on a 21-cytokine bead panel (MilliporeSigma). EV positive samples were previously genotyped by sequencing the viral capsid gene. Cytokine levels calculated from the standard curve were compared by Kruskal-Wallis and post-hoc analysis (GraphPad Prism 8.4.3). Natural partitioning of participants was explored using principal component analysis (IBM SPSS v27) and cluster analysis (BioNumerics v6). The utility of cytokine signatures in predicting EV status was explored using discriminant analysis (IBM SPSS v27).

Results

Data from 149 CSF with CVA9 (N=13), E6 (N=20), E9 (N= 27), E18 (N=25), E30 (N=21) and PeV-A3 (N=10) showed significant differences among EV genotypes vs. controls for 19 cytokines. Significant differences in cytokine levels in EV CSF vs controls were seen: CVA9 for 9 cytokines, E6 for 18 cytokines (not RANTES), E9 for 10 cytokines, E18 for 9 cytokines, and E30 for 18 cytokines (not RANTES). PCA revealed only minor overlap of controls and EV positives; EV types overlapped, except E30, differing most from CVA9, E9 and E18 but overlapping E6. The most important type-differentiating cytokines by PCA were IP-10 and MCP1. Patterns in DA resembled PCA; controls clearly separated from EV CSF, E30 being the most distinct. Overall, the discriminant model correctly classified EV type or controls at a 49% rate - highest for controls (94.4%), E9 (69.6%), and E30 (62.5%). In the DA model, the most important cytokines were IL-2, IL-13, IL-5, and IP-10.

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INTRODUCTION

- Non-polio Enteroviruses (EV) and human Parechoviruses (PeV) are two of the most important causes of aseptic meningitis in neonates and infants[1,2].
- In a recent study of CNS infections in children over 30 EV genotypes have been identified, including Echovirus (E) 6 and E30, Coxsackievirus (CV) A9, and CV B genotypes, along with PeV-A types 3 and 4[3,4].
- Previous studies of immune response to CSF EV and PeV infections suggest EV CNS infections cause global elevation in cytokines compared to PeV[5,6,7].
- Relatively few studies have characterized EV or PeV genotype specific responses, and those that have focused on investigating the response to EV-A71 or E30[8, 9, 10].
- Those studies have shown elevated IFN-γ, IL-1R2, IL-2, IL-6, IL-8, and TNFα in E30 as compared to control CSF samples[9, 10].
- However, more work is needed to elucidate responses to other common EV infections.

METHODS

- 149 salvaged standard of care CSF samples from ≤6 month olds collected between January 2010 and December 2020 were identified as either:
  - real time EV and PeV PCR negative control samples,
  - EV PCR positive experimental samples, or
  - PeV PCR positive experimental samples
- EV or PeV PCR positive samples were previously genotyped by sequencing the viral capsid gene[11].
- EV positive samples from the 5 most prevalent EV genotypes were included in this study[3], along with the most prevalent PeV genotype, PeV-A3.
- Samples were tested in duplicate on a 21-cytokine bead panel (MilliporeSigma).
- Cytokine levels calculated from the standard curve were compared by Kruskal-Wallis and post-hoc analysis (GraphPad Prism 8.4.3).
- GM-CSF and IL-17a were excluded from subsequent analysis due to frequent levels below the assay limit of detection.
- Natural partitioning of participants was explored using principal component analysis (IBM SPSS v27) and cluster analysis (BioNumerics v6).
- The utility of cytokine signatures in predicting EV status was explored using discriminant analysis (IBM SPSS v27).

RESULTS

Table 1: Sample Demographics

Group	Sample size (n)	Average age (d)	% Female
Controls	33	70.30	33.33
CVA9	13	33.23	53.85
E6	20	63.09	40.00
E9	27	30.97	48.15
E18	25	36.80	32.00
E30	21	32.59	71.43
PeV-A3	10	29.94	30.00

Figure 1a-i: Average Cytokine/Chemokine Levels by Genotype

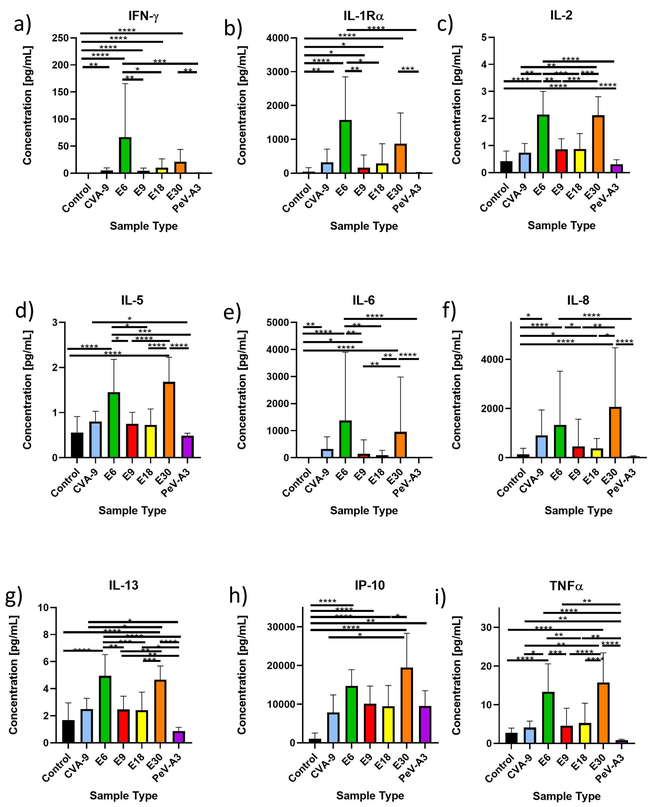


Figure 1a-i: Average levels of selected cytokines per group compared using Kruskal-Wallis with post-hoc Dunn's test. **** p < 0.0001; *** p <0.001; ** p < 0.01; * p < 0.05

SUMMARY & CONCLUSIONS

- EV genotypes demonstrate distinct but overlapping cytokine /chemokine production patterns.
- E6 and E30 were found to have higher average levels of cytokines compared to other genotypes.
- Discriminant analysis illustrated significant overlap between CVA9, E9, and E18, whereas E6 and E30 were more distinguishable.
- PeV-A3 demonstrated lower levels of cytokines compared to EV, and it was difficult to distinguish the pattern compared to CVA9, E9 and E18, likely due to small sample size.

Figure 2: Discriminant Analysis by Genotype

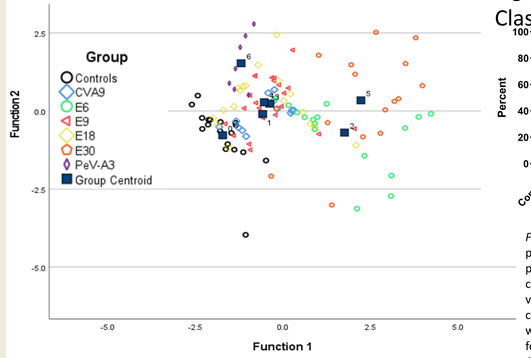


Figure 3: DA Rate of Correct Classification (RCC)

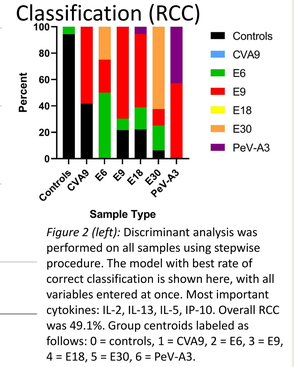
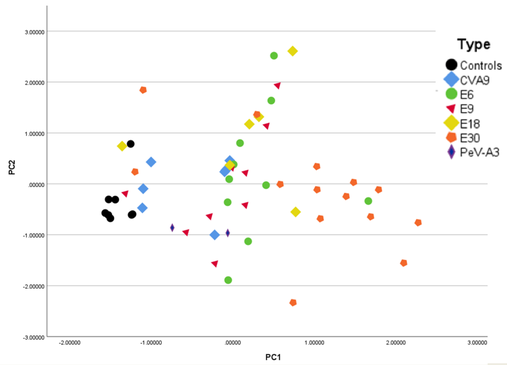


Figure 2 (left): Discriminant analysis was performed on all samples using stepwise procedure. The model with best rate of correct classification is shown here, with all variables entered at once. Most important cytokines: IL-2, IL-13, IL-5, IP-10. Overall RCC was 49.1%. Group centroids labeled as follows: 0 = controls, 1 = CVA9, 2 = E6, 3 = E9, 4 = E18, 5 = E30, 6 = PeV-A3.

Figure 4 and Table 2: Principal Component Analysis by Genotype



	PC1		PC2	
IP-10	0.992	MCP-1	0.804	
IFN-α2	0.747	Fractalkine	0.327	
IL-4	0.699	IL-10	-0.31	
TNFα	0.695	IL-8	0.282	
IL-13	0.693	IL-13	0.196	
IL-12p40	0.676	IL-1Rα	-0.142	
IL-2	0.655	TNFα	0.127	
IL-5	0.644	IP-10	-0.123	
MCP-1	0.593	RANTES	-0.107	
IL-1β	0.59	IL-15	0.106	
IL-15	0.578	IL-5	-0.102	
IL1-Rα	0.539	IL-1β	0.071	
IL-6	0.537	IFN-α2	0.058	
MIP-1a	0.513	MIP-1a	0.047	
Fractalkine	0.429	IL-12p40	0.031	
IL-8	0.4	IL-4	0.025	
IL-10	0.359	IFN-γ	-0.013	
IFN-γ	0.308	IL-6	0.009	
RANTES	-0.135	IL-2	0	

Figure 3 (left) and Table 3 (above): PCA with component matrices.

Figure 4: Cytokine Expression Pattern in Controls and EV/PeV Infected Children