Enterovirus D68 Visualized in the Anterior Horn of the Spinal Cord of a Pediatric Patient with Flaccid Paralysis

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Outstanding Questions

Does enterovirus D68 have neurotropism in humans?

Does viral infection vs. para-infectious process cause AFM?

Abstract

Background: Acute flaccid myelitis (AFM) is a polio-like paralyzing illness of children. AFM incidence is increasing during every other year outbreaks that occur in the United States simultaneously with outbreaks of enterovirus D68 (EV-D68) infection. Demonstrating that EV-D68 directly causes AFM has been challenging due to rare detection of the virus in the cerebrospinal fluid (CSF) of patients despite frequent detection at nonsterile sites. Murine studies have shown that EV-D68 can infect spinal cord anterior horn motor neurons and cause paralysis, similar to poliovirus. However, a key outstanding question is whether EV-D68 causes AFM in humans by direct viral pathogenesis or by indirect host immunopathogenesis.

Methods: We investigated the pathogenesis of AFM using tissues from a previously reported case of a 5-year-old boy who presented in fall 2008 with four days of progressive limb and voice weakness followed by incontinence, apnea, and death. He had a CSF pleocytosis of 2094/μL with EV-D68 identified in the CSF by sequencing of the VP1 gene. We designed probes for in situ hybridization (ISH) based on this sequence to stain formalin fixed paraffin embedded tissues from his autopsy. For immunohistochemistry (IHC) we used both commercial polyclonal anti-EV-D68 antibodies and our own human monoclonal antibodies that stain virus infected cells in vitro. Immunophenotyping was done by IHC.

Results: We identified EV-D68 in the anterior horn of the patient's spinal cord, corresponding to the location of motor neuron cell bodies. This area was highly inflamed, with an infiltrate of few CD8 T cells and many macrophages. Viral RNA and viral protein was visualized in motor neurons but not supporing cells using ISH and IHC, respectively. Viral RNA but not viral protein was detected rarely in the lungs in macrophages, which had extensive inflammatory infiltrate. The infiltrate was predominantly composed of macrophages with a CD8 T cell component as well. The transcriptome of cells in the inflamed tissue was enriched for genes involved in antigen presestation on MHC.

Conclusions: Deaths in AFM patients are rare and often distant from initial presentation, but this patient died four days after onset of weakness, allowing us to directly demonstrate that EV-D68 can infect the human spinal cord. Motor neurons but not neural support cells are directly infected by EV-D68 with a corresponding infiltrate of macrophages. Antigen presentation processes are upregulated in inflamed tissues. Therefore, both direct viral pathology and immune factors likely contribute to AFM disease in EV-D68 infection.

Case Presentation

5 yo male, fall 2008

Day 1 - upper respiratory symptoms (many classmates, too)

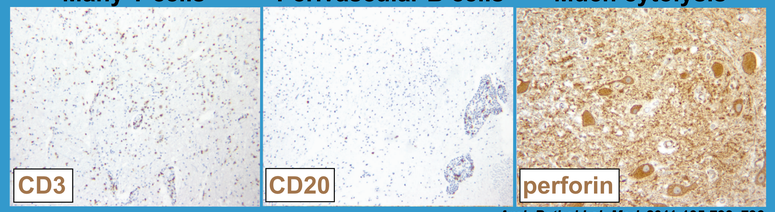
Day 3 - asymmetric upper extremity weakness, voice change

Day 5 - incontinent, cannot walk, later found apneic

Autopsy - EV-D68 in cerebrospinal fluid by RT-PCR

Prior Spinal Cord Pathology

Many T cells | Perivascular B cells | Much cytolysis



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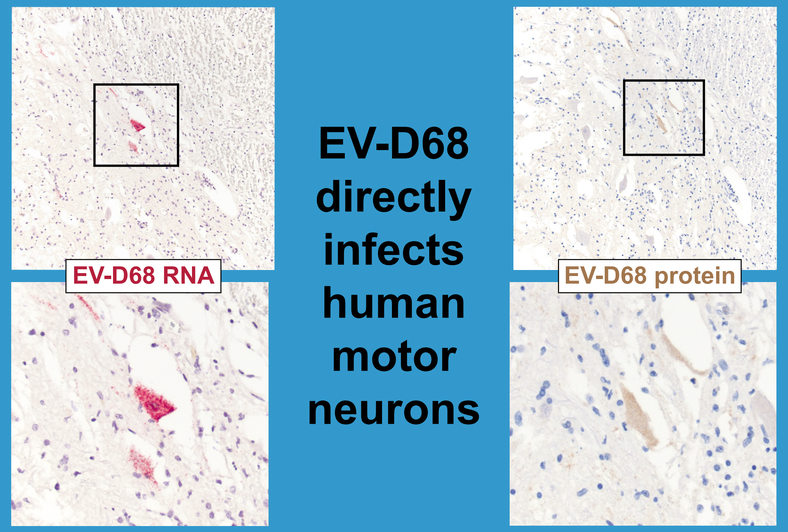
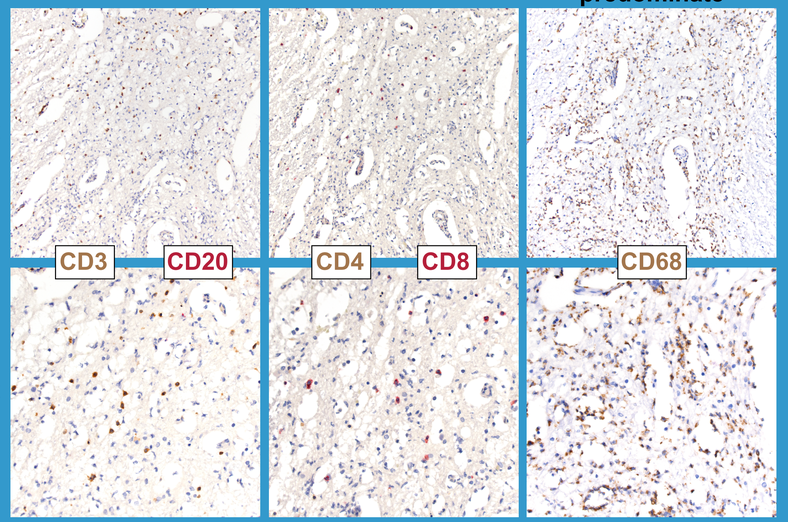
New Spinal Cord Pathology



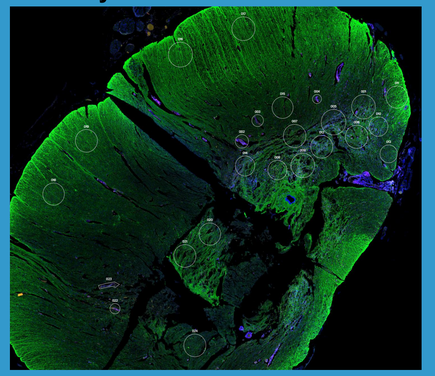
EV-D68 directly infects human motor neurons

T cells >>> B cells | CD8 >>> CD4 T cells | Macrophages predominate



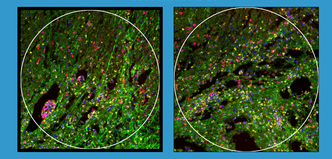
Spatial Transcriptomic Characterization

Asymmetric inflammation

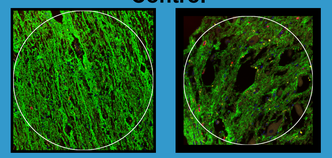


Example regions of interest:

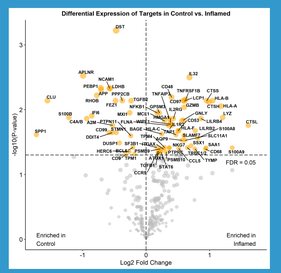
Inflamed



Control



Morphology markers: GFAP CD68 CD3E DNA



Ontology of biological processes enriched in inflamed tissue (vs. control):

++Antigen presenation on MHC
++Neutrophil aggregation
+Zinc sequestration
+IL-4 mediated signaling
+Negative regulation of macrophage apoptosis
+Protein nitrosylation
+Protection from NK cell cytotoxicity
+Membrane fusion involved in viral entry

Conclusions

EV-D68 infects human motor neurons early in disease.

AFM could be a consequence of both direct viral pathogenesis and inflamatory damage from macrophages and CD8 T cells.

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