Potential mechanisms by which adeno-associated virus type 2 causes unexplained hepatitis in children

To the Editor,

On May 6, 2022, the UK Health Security Agent first reported the detection of large amounts of adeno-associated virus (AAV) type 2 (AAV2), which is considered harmless to humans, in the blood and liver tissue of patients with pediatric hepatitis of unknown cause (PHUC). AdVs require a helper virus such as adenovirus (AdV). Starting in April 2020, reports of positive AdV tests using fecal or gastrointestinal specimens from children in the United Kingdom, almost exclusively from those under 4 years of age, were markedly decreased compared to the previous 5 years. However, since November 2021, reports of positive tests have increased greatly. The timing of this increase and the patients’ age range coincided with the occurrence of cases of PUHC. The prevalence of serum neutralizing antibodies to AdV41 and AAV2 was diminished in children older than 3 years. Countries where cases of PHUC were seen, such as Europe, the United States, and Japan, tend to have good sanitary conditions. In countries with good sanitation, a large proportion of children likely already had low immunity to AdV41 and AAV2, and this proportion may have been increased by the measures taken to prevent COVID-19. We hypothesized that infection with AdV41, which has been reported to cause liver damage when administered as a recombinant vector at medium to high doses for gene transfer to hepatocytes, could cause hepatitis in children with low immunity to both viruses.

Subsequent preprints of observational studies showing that AAV2 was detected in large quantities at a frequency >90% in cases of PHUC and was rare in controls or AdV-infected individuals without liver damage supported our hypothesis. AAV2 can proliferate without helper viruses due to the damage caused to infected cells. However, it is not considered a marker of liver damage because it is rare or not present in cases of hepatitis of other causes, many of which were already thought to be infected with AAV2. Possible genetic predisposition to PHUC susceptibility has also been reported. When children are infected with both AAV2 and its helper virus AdV41 at approximately the same time, the viruses may replicate in the same place, such as the intestinal tract. AdV41 is an intestinal AdV that is transmitted via the fecal-oral route—where AAV2 proliferates is unknown, but since it is related to sanitary conditions and was detected in feces from both AdV41-positive cases and controls and one PHUC case, it is possible that it also proliferates in the intestinal tract, where the helper virus AdV41 can cause a greater effect.

AAV2 can proliferate more than AdV41 because of the synergistic effect of the lack of immunity and the fact that its helper virus, AdV41, can also multiply to a high degree due to lack of immunity. AdVs, especially AdV41, are thought to rarely infect hepatocytes and are not known to cause hepatitis in children with healthy immune systems. However, when AAV2 is present in high amounts, it can enter the bloodstream and infect hepatocytes because of its hepatotropic nature.

Since no viral proteins or particles were detected in liver biopsies, it is difficult to assume that cases of PHUC are caused by direct lytic infection. Therefore, if AAV2 causes liver damage, it is presumably through immunological mechanisms. Around 1 week after AdV41 and AAV2 infection, adaptive immune responses, including antibodies and CD8+ cells, begin to eliminate the virus. After another week or more, accumulative tissue damage should become apparent. Most patients with PHUC had developed gastroenteritis symptoms consistent with AdV41 infection 1–11 weeks before the onset of hepatitis. This may explain why AdV41 was not always detected in the blood or is present at low titers in patients with PHUC. AAV2 was detected in large quantities in the blood, even in the presence of an adaptive immune response, and in the liver, despite unlikely helper virus co-infection in the liver; besides being present in large quantities, it may have further replicated without helper virus due to damage to hepatocytes or other infected cells. Indeed, an AAV2 RNA pattern suggestive of viral replication has been detected in cases of PHUC. Large amounts of AAV2 may elicit a stronger AAV2-specific immune response, and simultaneous immune response to AdV41 may further increase cytokine levels, inducing and further aggravating hepatitis. Co-infection with SARS-CoV-2 could also exacerbate hepatitis by increasing cytokine levels. Even under normal circumstances, a child with a genetic predisposition to develop hepatitis who is infected with AAV2 and AdV41 at the same time could develop hepatitis, but the probability may be low. Pre-existing immunity to AAV2 may explain why humans who received medium and high doses of AAV2 vector showed only asymptomatic transaminase elevations and a decrease in transgene products after 2–3 weeks. In these cases, analysis of interferon-γ (IFN-γ) in peripheral blood mononuclear cells using an IFN-γ enzyme-linked immunospot (IFN-γ ELISPOT) showed a response to the AAV type 2 capsid but not the transgene product. This suggests the...
destruction of hepatocytes by CD8+ T cells.\textsuperscript{4,5,7,8} Since no cellular immune response to the transgene product was observed,\textsuperscript{7,8} and the recombinant AAV2 gene therapy vector contained only the inverted terminal repeat sequence associated with genomic integration and no other viral sequences,\textsuperscript{6} it is unlikely that recombination is the cause of its hepatotoxicity. If IFN-γ ELISPOT of AAV2 in PHUC patients yields similar results, it should be possible to estimate CD8+ destruction in AAV2-infected hepatocytes. These are the potential mechanisms by which AAV2 causes unexplained hepatitis in children.

**AUTHOR CONTRIBUTIONS**

Daisuke Miyazawa contributed to the conception/design of the work; the acquisition, analysis, and interpretation of data; and the drafting and revision of the manuscript. Daisuke Miyazawa approved the current version of the manuscript and agrees to be accountable for all aspects of the work.

**CONFLICT OF INTEREST**

The author declares no conflict of interest.

**DATA AVAILABILITY STATEMENT**

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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