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Biological Plausibility of a Link Between Arterial Ischemic Stroke and Infection with Varicella-Zoster Virus or Herpes Simplex Virus

Charles Grose, MD

Division of Infectious Diseases/Virology Laboratory, Children's Hospital, University of Iowa, Iowa City, IA

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There are now 9 human herpesviruses; the increase from 8 to 9 was caused by a recent decision to split human herpesvirus (HHV) strains 6A and 6B into distinct species. The 9 species are subdivided into three subfamilies, called *alpha*: herpes simplex virus (HSV) types 1 and 2 and varicella-zoster virus (VZV); *beta*: cytomegalovirus (CMV), HHV6A, HHV6B and HHV7; *gamma*: Epstein-Barr virus (EBV) and Kaposi's sarcoma-associated herpesvirus (sometimes called HHV8). These same herpesviruses have evolved with humankind around the world since the great migration of modern humans across the Mandeb Strait out of Africa around 60 to 100 thousand years ago¹. In the current report in this issue of *Circulation*, the authors sought to determine whether any of 5 herpesvirus infections—HSV1, HSV2, VZV, CMV or EBV—increased the risk of arterial ischemic stroke (AIS) in children 18 years of age or younger². They conclude that HSV1 infection and to a lesser extent VZV infection may act as triggers for childhood AIS. This Editorial will examine the biological plausibility of that assertion, based on both a literature review and the known pathogenesis of infection with HSV, VZV and also the live attenuated varicella vaccine virus.

VZV is the virus of greatest interest because the data linking VZV to arteritis and stroke are the strongest among all herpesviruses (Figure 1). The largest review of VZV infection and AIS in children includes 70 cases³. The median age was 5 years (range from 6 months to 14 years). Stroke occurred after varicella in 90% and herpes zoster in 10%; in 2 children, stroke followed varicella vaccination. The median interval between varicella or herpes zoster and subsequent stroke was 18 weeks. The most common clinical presentations were hemiparesis, speech disorders, facial palsy and headache. In the current report, the association of AIS with VZV was not as strong. The reason that a stronger link was not found may be twofold: (i) the study was conducted mainly in a population of children immunized with varicella

Address for Correspondence: Charles Grose, MD, Professor and Director of Pediatric Infectious Diseases Division, University of Iowa, 2501 JCP, 200 Hawkins Drive, Iowa City, IA 52242, Phone/Fax: 319-356-2270, charles-grose@uiowa.edu.

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vaccine and (ii) a relatively insensitive commercial varicella ELISA antibody screening assay was selected. Children were enrolled between 2010–2014 from centers in 9 countries; however, the majority of children were enrolled from the United States, Canada and Australia, countries with universal varicella vaccination programs. Had this study been conducted before 1995 (year of approval of varicella vaccine in United States), wild-type varicella (chickenpox) almost certainly would have been the most common herpesvirus infection associated with AIS in children.

VZV and HSV antibody testing

There is a major problem with the varicella antibody testing in this study. The authors selected a commercial VZV ELISA test to measure serum IgM and IgG antibodies. The commercial ELISA kits have been proven to be too insensitive to detect varicella antibody induced by varicella vaccination, since the original clinical trials of varicella vaccination conducted in the United States in the 1980's⁴. The commercial IgM testing kits are even more insensitive than the commercial IgG testing kits. Since many immunized children never develop antibodies detectable by the commercial kits, the numbers (and percentages) of children with negative VZV titers in tables 2 and 3 are not valid; in other words, many of the immunized children with negative VZV titers would have positive titers by more sensitive assays. Here is a specific example: there were 274 AIS cases from North America and Australia. Because of universal varicella immunization, at least 247 of these cases should have been positive for VZV antibody, assuming a 90% seroconversion rate after varicella vaccination (especially in children who had received 2 varicella vaccines). Yet, the authors found VZV IgG antibody in only 182 AIS cases from their entire cohort of 326 cases from the 9 countries (table 2).

In future studies, varicella antibodies must be measured by more sensitive assays; the two that are available are called (i) FAMA (fluorescent antibody against membrane antigen) and (ii) glycoprotein ELISA assay. Titers by both assays correlate with neutralization titers^{5, 6}. Although neither assay is commercially available, the Centers for Disease Control and other virology research laboratories have adapted these assays to measure anti-VZV antibody titers in children in the United States. For all these reasons relating to their ELISA testing methodology, the authors may have missed any association between live attenuated varicella vaccination and AIS, or conversely, any protection of varicella vaccination against AIS.

There is also a potential problem with the HSV ELISA testing kits. In table 3, the authors report that 32 cases had positive IgM antibodies to HSV1, while no case had positive IgM antibodies to HSV2. What is puzzling about the subsequent HSV data is that 48 children had IgM antibodies that were indeterminate between HSV1 and HSV2. In Supplemental Materials, the authors state that they used an HSV antibody testing kit called HerpeSelect (Focus Diagnostics), which can differentiate IgG antibodies to HSV1 from HSV2. The HerpeSelect IgG test also may be more sensitive than standard HSV IgG ELISA testing kits⁷. Yet, even with the respected HerpeSelect assay, they were not able to detect low levels of IgG antibody to either HSV1 or HSV2 in the 48 children with indeterminate HSV IgM antibody. Among a total enrolled population of 326 AIS children and 115 controls (up to age

19 years), it is noteworthy that not a single child or adolescent had a positive HSV2 IgM test by their ELISA assays (table 3).

VZV and HSV neuropathogenesis and conclusions

The neuropathogenesis of stroke following VZV infection has been investigated more than that following HSV infection (Figure 2). The basic pathogenesis of varicella includes a viremia, by which the virus travels in lymphocytes to the skin to cause the characteristic exanthem^{8, 9}. Even though the varicella vaccine virus is greatly attenuated, there is a brief viremia in some recipients¹⁰. Thus both wild-type and vaccine virus could cause an arteritis by direct invasion of arteries. However, since the average length of time between wild-type varicella and stroke is 18 weeks, the most likely mechanism involves establishment of a latent VZV infection in the trigeminal ganglion after varicella, followed by reactivation (herpes zoster with or without a rash) and viral spread to the arteries in the brain via sensory fibers from the trigeminal ganglion¹¹. Of note, varicella vaccine virus can also establish latency in and reactivate from the trigeminal ganglion.⁴ Because there are very few deaths following stroke in childhood, few cerebral arteries have not been examined for VZV DNA and antigens. However, many temporal arteries have been examined in biopsies from adults with Giant Cell Arteritis; both VZV DNA and VZV antigens have been detected (Figure 1)¹². Furthermore, the serological response to herpes zoster definitely can include both IgM and IgG antibodies against VZV proteins¹³. Thus, the biological plausibility of VZV infection as a cause of cerebral arteritis appears highly likely (Figure 2)^{14, 15}.

Primary HSV1 infection presenting as gingivostomatitis has been associated with a viremia¹⁶. After causing HSV gingivostomatitis, the virus enters the trigeminal ganglion and establishes a latent state. Since HSV also reactivates in the trigeminal ganglion, HSV could also travel via efferent nerves into cerebral and temporal arteries. However, the data about HSV infection of arteries after HSV reactivation in humans are much more conflicting^{17, 18}. As with VZV, HSV specific IgM antibody responses after HSV reactivation have been seen¹⁹. (As with VZV also, the sensitivity of commercial ELISA IgM testing kits may vary.) Since there were few parental reports of a facial rash in the current report, that fact may suggest that subclinical reactivation rather than mild primary HSV1 infection is the more likely infection being discovered by their IgM analyses.

In conclusion, the authors of the current article certainly have provided serologic evidence that HSV infection (probably either recent or reactivated) is associated with an increased risk of AIS. But they have not shown from this study that HSV infection acts as a trigger for AIS. Thus, the biologic plausibility of HSV infection as a cause of arteritis needs further investigation. While it may not yet be timely to start prolonged treatment with acyclovir, it certainly is timely to pursue further translational studies of this intriguing association between herpesvirus infection and AIS.

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Figure 1.

Electron micrograph of several VZV particles at the cell surface. VZV has the structure of a typical herpesvirus. The viral particle includes a central capsid enclosing a double-stranded DNA genome, which codes for ~70 genes. The capsid is surrounded by an inner tegument and an outer envelope. The diameter of the complete virion is ~200 nanometers. The structure of HSV is very similar to VZV.

Figure 2.

Varicella-zoster virus infection, the trigeminal ganglion and arteritis. **A.** Varicella in child. During primary varicella (chickenpox), virus first replicates in the tonsillar area, after which virus spreads in lymphocytes throughout the body, leading to the characteristic vesicular rash. Virus subsequently travels retrograde in sensory nerves from the varicella vesicles on the face to the trigeminal ganglion (red arrow). After establishing latency in the ganglion, the virus can reactivate later in childhood and travel anterograde from the trigeminal ganglion via sensory fibers to arteries in the brain (blue arrow) or to the temporal artery

(green arrow). **B.** Example of VZV infection of a temporal artery. The confocal micrograph shows an artery with giant cell arteritis immunolabeled with anti-VZV monoclonal antibody and a secondary fluorescent probe. A single VZV-positive giant cell (green color) is designated by an arrow. **C.** Giant cell. This panel includes an enlarged image of the same giant cell produced by merging several confocal micrographs. Again, VZV proteins are labeled green (arrow).