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COMMENTARY

The Link Between Alzheimer Disease and Herpes Simplex Virus Infection: Better Late Than Never, or Better Never Than Late?

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In 1913, Noguchi and Moore [1] used the Levaditi silver staining method to identify *Treponema pallidum* in 12 of 70 postmortem brains of patients with general paresis from the Central Islip State Hospital in New York. Although a potential syphilitic etiology for general paresis had been postulated as early as the mid-nineteenth century, this landmark study provided direct evidence for the link between an infectious pathogen and a dementia. The demonstration 53 years later by Gajdusek et al. [2] that kuru could be transmitted from human brain material to chimpanzees added further evidence for the role of "transmissible" agents in causing dementia. Examples of viral links to dementia or neurodegenerative disease subsequently grew to include measles and subacute sclerosing panencephalitis (SSPE) [3, 4] and most recently HIV-associated neurocognitive disorders [5].

In the early 1980s, Ball [6] commented on the anatomical overlap in pathological areas affected in Alzheimer disease (AD) and herpes simplex encephalitis and speculated that reactivation of latent herpes simplex virus (HSV) from the trigeminal ganglion and its centripetal spread, "Might be the cause of "degenerative" lesions typical both of Alzheimer's disease and of the normal aged human brain." This ushered in an era of searching for direct evidence of CNS HSV infection in AD brains using immunohistochemistry (IHC) for HSV-encoded proteins, and first in situ hybridization (ISH) and later polymerase chain reaction (PCR) to detect HSV nucleic acids. Early studies with both IHC and ISH failed to find consistent evidence of HSV protein or nucleic acid in AD brains [7, 8]. Using the more sensitive technique of PCR, HSV genes, including those encoding the viral thymidine kinase, were detected in AD brains, but also in nearly equivalent frequency in non-AD controls including "normal" aged brains [9, 10]. Studies of the prevalence of CSF HSV intrathecal IgG antibody synthesis similarly found no significant difference in the prevalence of intrathecal HSV antibody synthesis in normal elderly patients (69%) compared to AD cases (52%) [11]. A subsequent refinement to the PCR approach was to look for differences in the pattern of HSV DNA deposition within brains of AD and normal individuals. At a more cellular level, Wozniak et al. [12] reported that the majority of HSV1 DNA detected by PCR appeared to be preferentially plaque-associated in AD brains (72%) compared to aged normal individuals (24%).

The high frequency of detection of HSV nucleic acid in brains and HSV antibody in CSF of patients without AD suggested that a simple causal link between HSV CNS infection and AD was untenable and led to the revised hypothesis that host factors might be critical in determining whether HSV CNS infection contributed to AD pathology or not. These host factors could include the frequency of HSV CNS reactivation or the nature of the host response to that reactivation. Itzhaki et al. [13] summarized this by noting, "We postulated that in the central nervous system, periodic mild reactivation of HSV1 (in response to such factors as stress and immunosuppression) results in damage that is more severe in those people destined to develop AD, because of a difference in viral or host characteristics". An obvious candidate for a host factor were APOE alleles. Although the frequency of HSV1 PCR-positive specimens from temporal, frontal, or hippocampal brain regions was similar in 44 elderly non-AD controls compared to 46 AD patients, differences emerged in the associated distribution of APOE allele frequency among HSV-1 PCR-positive and HSV PCR-negative patients. APOE-E4 was present in 53% of HSV-1-PCR-positive AD cases versus only 10% of HSV PCR-negative AD cases. The odds ratio (OR) for APOE-E4

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allele presence in HSV-1-PCR-positive AD patients was 17 (95% CI 3.6–78) compared to 1.7 for HSV-PCR-negative AD patients, each compared to age- and sex-matched controls. It was proposed that the host APOE-E4 allele could act to augment HSV1 infection by increasing the number of cells infected, the number of virions released per cell, or the frequency of viral reactivations. Alternatively, APOE-E4 might act subsequent to infection to influence factors such as the extent of repair after HSV1-induced CNS damage. The potential for APOE-E4 to influence HSV CNS pathogenesis is supported by studies in APOE-E4 transgenic mice that showed higher viral loads in brain in these mice compared to APOE knockout and APOE-E3 transgenic mice [14]. Unfortunately, the linkage between APOE-E4 and HSV-1 DNA in the brain has not been consistently replicated [15, 16]. Beffert et al. [15] examined 73 elderly individuals with neuropathologically confirmed AD and found that there was no significant difference in the frequency of APOE-E4 among those with and without HSV-1 + PCR (37% v. 24%, $p = 0.19$).

A more recent genetic approach to searching for viral links to AD involves examining the pattern of RNA expression ("transcriptome") in selected brain regions of AD and control brains. Using this "RNA-Seq" approach, Readhead et al. [17] found evidence for over-expression in pre-symptomatic AD brain of certain promoters that "suggested a potential role for virus-mediated network activities." This prompted them to search for and analyze expression of nucleic acid sequences from 515 known human viruses using a metagenomic approach. The most consistent difference they found was an overabundance of transcripts linked to Human Herpesviruses-6A and 7 (*Roseoloviruses*). In this survey, they also found evidence for over-expression of the HSV-1 latency-associated transcript (LAT) in some tissues and some cohorts, although the result was neither as consistent nor as robust as for HHV6A and 7. The authors suggested that, "AD biology is impacted by a complex constellation of viral and host factors acting across different timescales and physiological systems" and they proposed based on their data several "top candidate" molecular mediators in this process including mucosal and innate immunity, cytoskeletal organization, mitochondrial respiration, protein and tRNA synthesis, nucleotide pool maintenance, kinase and transcription factor networks, as well as neuronal loss and amyloid processing. These analyses helped provide a broad conceptual framework for thinking about how viral infections could contribute to the pathogenesis of AD.

Several studies have now established with more specificity biologically plausible mechanisms by which HSV-1 infection could contribute to canonical AD pathogenesis pathways including by enhancing amyloid beta accumulation and amyloid plaque generation, and by increasing tau hyper-phosphorylation and the generation of paired helical filaments and neurofibrillary tangles (reviewed in [18]). These results are consistent with studies suggesting that beta-amyloid may serve as an innate immune protein whose antimicrobial properties are mediated by fibrillization. For example, mice transgenically over-expressing human beta-amyloid (5XFAD mice) show delayed mortality compared to wild-type mice in an HSV1 encephalitis model [19].

It is important to recognize that even unequivocal evidence supporting biological plausible mechanisms by which HSV (or another virus) could lead to AD does not provide direct evidence for causality. Population-based epidemiological studies help establish the case for association and causality in ways that "biological plausibility" studies cannot. One of the most-cited such efforts was by Tzeng et al. [20], published in this journal. They utilized a retrospective cohort study design to examine the risk of dementia in Taiwanese patients over age 50 during the ten-year period after diagnosis of HSV infection, compared to dementia incidence in non-HSV infected age- and sex-matched controls. They estimated the adjusted hazard ratio (aHR) for development of dementia at 2.6 (95% CI 2.4–2.8, $p < 0.001$) in the HSV-infected group compared to uninfected controls. The aHR was similar when looking specifically at the diagnosis of AD (aHR 2.7, 95% CI 2.5–3, $p < 0.001$). HSV-positive patients taking antiviral medications (typically but not exclusively acyclovir and famciclovir) had a remarkably reduced (91%) aHR for developing dementia compared to those who had never received anti-herpetic medications (aHR 0.09, 95% CI 0.08–0.11, $p < 0.001$). It should be pointed out that this treated population likely had "severe" HSV infection as only patients who had made three or more outpatient visits related to treatment during the index calendar year were included in this analysis. Lindman et al. [21] used a similar retrospective matched cohort design to examine dementia risk in Swedish patients over the age of 50 in the presence or absence of "herpevirus" (HSV or VZV) infection, and with or without documented antiviral (typically valacyclovir or acyclovir) treatment. Even though the aHRs were significantly smaller than those in the Taiwanese study, the adjusted hazard ratio (aHR) for risk of subsequent dementia in herpesvirus-infected subjects was 1.5 (95% CI 1.3–1.7, $p < 0.001$). As in the previous study, the use of antiviral drugs reduced the risk of dementia, although the magnitude of this effect was significantly smaller. Herpesvirus-positive subjects who received antiviral treatment had an aHR for dementia of 0.9 (95% CI 0.82–0.98, $p = 0.015$) compared to age- and sex-matched controls, and this aHR was even lower (0.75, 95% CI 0.68–0.83, $p < 0.01$) when the comparison was limited to treated and untreated subjects within those who were herpesvirus-positive.

This issue of *Neurotherapeutics* contains the latest entry into the epidemiological debate [22]. Young-Xu and colleagues performed another retrospective matched cohort

study using US Veterans Health Administration data and, in contrast to prior studies (see above), found a lower aHR (0.8, 95% CI 0.78–0.83) for dementia among those with symptomatic HSV1/2 infection relative to those without. Among the HSV-positive group those who received antiviral therapy, the aHR for dementia was 0.75 (95% CI 0.72–0.78) compared to those not receiving antiviral therapy, a risk reduction almost identical to that reported in the Swedish cohort [21] but quite a bit different from the 91% reduction reported in the Taiwan cohort [20].

So where does all this leave us? In 1965, Sir Bradford Hill developed a set of criteria for evaluating when observed associations allowed for "a verdict of causation" [23]. Critically weighing evidence in each of these areas is beyond the scope of this viewpoint, but it is reasonable to assume that the link between HSV infection and AD is biologically plausible, with credible experimental evidence that provides conceivable mechanisms by which this infection could contribute to known pathways implicated in AD pathogenesis. Much of this evidence is not unique or specific to HSV, and in some cases the evidence may be as strong or stronger for other herpesviruses. Importantly, as emphasized earlier, it is critical to recognize that no amount of biological plausibility establishes causation. Attempts to identify direct footprints of HSV infection including detection of intrathecal antibody synthesis, or of HSV antigen or nucleic acid in brain tissue have not shown convincing or consistent differences between AD patients and controls. The consistently high "background signal" in control patients who do not have AD makes this approach unlikely to produce a "smoking gun" for causality. An added complexity comes from the fact that a relatively ubiquitous event such as HSV infection or reactivation could lead to AD only in the context of specific host co-factors. Studies suggesting differences in distribution of HSV DNA in amyloid plaques or potential differences in distribution of known AD risk factors such as APOE-E4 alleles between HSV-positive and HSV-negative AD patients, even if not consistently confirmed, leave these "multi-hit" models intellectually and scientifically viable. The fact that initial HSV CNS infection is likely decades removed from the development of AD further complicates analysis, as the indicators of such remote infection may be indistinct or absent, although presumably ongoing viral CNS reactivation from latency, a key component in most proposed pathogenesis models, would be less occult.

Epidemiological studies of the HSV-AD association have understandably relied on retrospective cohort studies. These studies have major inherent methodological issues including the types of dementia and their diagnostic criteria considered, the viral infections studied and the basis for their diagnosis, and the time window following viral infection interrogated. The published studies vary in the populations surveyed with variations in geographic, racial, ethnic, and other demographic factors. Perhaps the one consistency has been an association of "antiviral therapy" with reduced risk of dementia generally and AD specifically and predominantly in those with prior herpesvirus infections. Unfortunately, the nature of the available studies encompasses a wide variety of drugs (typically but not exclusively acyclovir and valacyclovir) and often little useful information on therapeutic doses and treatment duration. It is also worth remembering that although the available antiviral drugs most commonly used in treatment in these studies were designed to preferentially target the herpesvirus DNA polymerase, with variably inhibitory concentrations across the individual human herpesvirus family members, that like all drugs they also have known and as yet unidentified "off target" actions on host cell proteins and functions. The efficacy if any of antiviral drugs in reducing risk of AD specifically or dementia more generally is again supportive but not conclusive of a role for drug-susceptible viruses in AD pathogenesis. Retrospective analyses of the type available to date are among the weakest types of epidemiological evidence for effects and need to be confirmed in prospective trials.

From a purely pragmatic perspective, the idea of a randomized double-blind placebo-controlled trial of valacyclovir in a population of patients with mild AD and evidence of HSV infection as documented by serology seems an eminently reasonable approach based on the preponderance of evidence currently available in published studies (see [24] and ClinicalTrials.gov NCT03282916 for one proposed protocol). The evidence for a causal linkage between HSV and AD is neither "overwhelming" [25] nor dismissible as "alternative fact" [26], but remains a testable hypothesis with practical therapeutic implications. In a world where a treatment with substantial potential safety concerns, extensive and expensive monitoring requirements, and post-approval healthcare costs estimated to extend into the billions of dollars can be approved based on limited and inconsistent evidence of efficacy, and despite overwhelming opposition from experts, supporting a trial of a relatively safe, inexpensive intervention like valacyclovir in AD seems like a "no brainer"!

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References

1. Noguichi H, Moore JW. A demonstration of Treponema pallidum in the brain of cases of general paralysis. J Exp Med 1913; 17:232-8.

2. Gajdusek DC, Gibbs CJ, Alpers M. Experimental transmission of a kuru-like syndrome to chimpanzees. Nature 1966; 209:794-6.
3. Chen TT, Watanabe I, Zeman W, Mealey J Jr. Subacute sclerosing panencephalitis: propagation of measles virus from brain biopsy in tissue culture. Science 1969;163:1193-4.
4. Payne FE, Baublis JV, Itabashi HH. Isolation of measles virus from cell cultures of brain from a patient with subacute sclerosing panencephalitis. N Engl J Med 1969; 281:585-9.
5. Snider WD, Simpson DM, Nielsen S et al. Neurological complications of the acquired immunodeficiency syndrome: Analysis of 50 patients. Ann Neurol 1983;14:403-18.
6. Ball MJ. Limbic predilection in Alzheimer's dementia: is reactivated herpesvirus involved? Canad J Neurol Sci 1982; 9:303-6.
7. Esiri MM. Viruses and Alzheimer's disease. J Neurol Neurosurg Psychiat 1982; 45:759-60.
8. Deatly AM, Haase AT, Fewster PH, Lewis E, Ball LMJ. Human herpes virus infections and Alzheimer's disease. Neuropathol Appl Neurobiol 1990; 16:213-23.
9. Jamieson GA, Maitland NJ, Wilcock, Craske J, Itzhaki RF. Latent herpes simplex virus type 1 in normal and Alzheimer's disease brains. J Med Virol 1991; 33:224–7.
10. Baringer JR, Pisani P. Herpes simplex virus genomes in human nervous system tissue analyzed by polymerase chain reaction. Ann Neurol 1994; 36:823-9.
11. Wozniak MA, Shipley SJ, Combrinck M, Wilcock GK, Itzhaki RF. Productive herpes simplex virus in brain of elderly normal subjects and Alzheimer's disease patients. J Med Virol 2005; 75:300-6.
12. Wozniak MA, Mee AP, Itzhaki RF. Herpes simplex virus type 1 DNA is located within Alzheimer's disease amyloid plaques. J Pathol 2009; 217: 131-8.
13. Itzhaki RF, Lin W-R, Shang D, Wilcock GK, Faragher B, Jamieson GA. Herpes simplex virus type 1 in the brain and risk of Alzheimer's disease. Lancet 31997; 49:241–244.
14. Burgos JS, Ramirez C, Sastre I, Valdivieso E. Effect of apolipoprotein E on the cerebral load of latent herpes simplex virus type 1 DNA. J Virol 2006; 80:5383-7.
15. Beffert U, Bertrand P, Champagne D, Gauthier S, Poirier J. HSV-1 in brain and the risk of Alzheimer's disease. Lancet 1998; 351: 1330-1.
16. Marques AR, Straus SE, Fahle G, Weir S, Csako G, Fischer SH. Lack of association between HSV-1 DNA in the brain, Alzheimer's disease and apolipoprotein E4. J Neurovirol 2001; 7:82-3.
17. Readhead B, Haure-Mirande J-V, Funk CC et al. Multiscale analysis of independent Alzheimer's cohorts finds disruptions of molecular, genetic, and clinical networks by human herpesviruses. Neuron 2018; 99:64-82.
18. Salt A, Angeli C, Doig AJ, Day PJR. Viral involvement in Alzheimer's disease. ACS Chem Neurosci 12:1049-60, 2021.
19. Eimer WA, Kumar DKV, Shanmugam NKN et al. Alzheimer's disease associated beta-amyloid is rapidly seeded by Herpesviridae to protect against brain infection. Neuron 2018; 99:56-63.
20. Tzeng N-S, Chung C-H, Lin F-H, et al. Anti-herpetic medications and reduced risk of dementia in patients with herpes simplex virus infections- a nationwide, population-based cohort study in Taiwan. Neurotherapeutics 2018; 15:417-29.
21. Lindman KL, Hemmingsson E-S, Weidung B, et al. Herpesvirus infections, antiviral treatment, and the risk of dementia-a registry-based cohort study in Sweden. Alz Dement 2021; 7:e12119. (https://doi.org/10.1002/trc2.12119)
22. Young-Xu Y, Powell EI, Zwain GM, Yazdit MT, Gui J, Shinert B. Symptomatic herpes simplex virus infection and risk of dementia in US veterans: a cohort study. Neurother 2021; (in press).
23. Hill AB. The environment and disease: association or causation? Proc R Soc Med 1965; 58:295-300, 1965.
24. Devanand DP, Andrews H, Kreisl WC, et al. Antiviral therapy: Valacyclovir treatment of Alzheimer's disease (VALAD) trial: protocol for a randomised, double-blind, placebo-controlled, treatment trial. BMJ Open 2020; 10:e032112. (https://doi.org/10.1136/bmjopen-2019-032112)
25. Itzhaki RF. Overwhelming evidence for a major role for herpes simplex virus type 1 (HSV1) in Alzheimer's disease (AD); underwhelming evidence against. Vaccines 2021; 9:679, 2021. (https://doi.org/10.3390/vaccines9060679),
26. Nath A. Association of herpes virus infections, antiherpetic therapy, and dementia; real or alternative fact? Neurother 2018; 15:415-8, 2018.