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Herpes Simplex Virus Infections and Alzheimer's Disease

Implications for Drug Treatment and Immunotherapy

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Abstract

Interest in the possible role of herpes simplex virus type 1 (HSV1) as a cofactor in the pathogenesis of Alzheimer's disease (AD) has re-emerged following the detection of viral DNA sequences in the central nervous system (CNS). Evidence from 2 independent laboratories indicates that HSV1 may interact with a host-specific factor, the apolipoprotein Eε4 allele, to further augment the risk for AD. In this review, we consider the arguments implicating HSV1 in the pathogenesis of AD. Although further studies are required to confirm a role for HSV1 in AD and to elucidate its underlying molecular basis, implicating a virus in the pathogenesis of this insidious disease clearly offers novel potential treatments.

Alzheimer's disease (AD) is a chronic, progressive neurodegenerative disorder characterised histopathologically by intracellular accumulation of neurofibrillary tangles, abnormal extracellular deposition of the β-amyloid (Aβ) protein into focal plaques and extensive neuronal cell death. These changes predominantly occur in limbic brain structures such as the medial temporal and frontal cortex and hippocampus,[1] while other regions such as the cerebellum and occipital cortex are mostly spared. A small proportion of AD cases have a clear genetic basis, with most familial forms attributed to autosomal-dominant mutations in 3 genes: β-amyloid precursor protein and presenilin-1 and -2.[2] A further genetic risk is associated with an increased susceptibility for AD among individuals carrying the type ε4 allele of the apolipoprotein E gene (ApoE4).[3] However, the aetiology of AD is multifactorial and the vast majority of cases cannot be attributed to these genetic factors alone, indicating that environmental factors may modulate the onset and/or progression of the disease. Although many factors have been proposed (head injury, heavy metal, infectious agents), there has been renewed interest in the neurotropic herpes simplex virus type 1 (HSV1).[4] This article examines the evidence implicating HSV1 as a potential cofactor in the pathogenesis of some AD cases and considers the prospects for relevant drug therapy and immunotherapies.

1. An Historical Perspective

The hypothesis that HSV1 might play a role in AD was formally proposed by Melvyn Ball[5] in the early 1980s, based primarily upon several observations. Firstly, there is precedence for viral involvement in other complex neurological disorders (e.g. subacute sclerosing panencephalitis, caused by the measles virus and, more recently, AIDS-related dementia, caused by the human immunodeficiency virus). Secondly, because of the high incidence of AD, any candidate infectious agent would likely have to be ubiquitous; HSV1 clearly meets

this criterion since most persons have been infected with the virus by the time they reach adulthood.[6] Thirdly, and provocatively, the brain regions most severely affected in cases of HSV encephalitis (HSE) are principally the same as those affected in AD, notably limbic structures,[7] and some cases of acute HSE have resulted in memory deficits.[8] Finally, HSV1 has the capacity to remain latent in infected neurons and can be spontaneously reactivated in response to various noxious stimuli, which could lead to its dissemination into previously uninfected areas. These characteristics of HSV1 are consistent with the chronic, progressive nature of AD.

Ball[5] outlined plausible anatomical routes of viral entry and dissemination into the central nervous system (CNS). In the peripheral nervous system (PNS), HSV1 commonly resides in sensory ganglia, particularly the trigeminal ganglion (TGG). Upon reactivation, the virus can spread to the periphery, where it is known to cause herpes labialis (cold sores) in some individuals. Ball postulated that under certain conditions, such as immunosuppression (a natural occurrence of aging), the virus in the TGG might reactivate and spread 'centripetally' via retrograde trans-synaptic and -neuronal transport into limbic brain regions: medial temporal cortex, basal forebrain, amygdala and hippocampus.[5,9] Another documented route of HSV1 entry into the CNS is via the olfactory ciliary nerve epithelium.[10-12] The latter route is consistent with the observation that the olfactory bulb and olfactory cortices frequently exhibit the pathological lesions of AD.[13,14] Notably, these structures project to both the amygdala (via pyriform cortex) and hippocampus (via entorhinal cortex), principle sites of AD neurodegeneration. Also consistent with this possibility is the interesting finding that hyposmia and other perturbations in olfaction are early clinical manifestations of AD.[15]

2. Detecting Herpes Simplex Virus Type 1 (HSV1) in the Brain

Establishing HSV1 as a cofactor for AD requires the demonstration of the virus or its genome in cells of the CNS. Direct evidence for the presence of HSV1 in postmortem brain tissue was, until recently, marked by conflicting results and controversy. The earliest attempts to detect viral DNA in the brain used hybridisation techniques such as dot and Southern blotting and *in situ* hybridisation that require approximately 1 viral genome per 1 to 200 cell genomes for reliable detection. Although several hybridisation studies successfully demonstrated HSV1 DNA in the human brain,[16-19] other studies failed to detect the viral genome.[20-23] The sensitivity of these hybridisation techniques may account for the inconsistent detection of HSV1 DNA in postmortem tissue, particularly if active HSV1 infection is not a prominent feature of late-stage AD. Along these same lines, HSV1 antigens have occasionally been detected in brains of individuals who had AD,[24] in some but not in all studies.[25]

More recent attempts at the detection of HSV1 in autopsy brain specimens, however, have employed the polymerase chain reaction (PCR), which can be several orders of magnitude more sensitive than hybridisation-based techniques. Using PCR, along with a number of cautionary controls to guard against crosscontamination, Itzhaki et al.[26] demonstrated the presence of HSV1 DNA in the temporal and frontal cortex of the majority of tested AD patients (14 of 21) and age-matched controls (9 of 15). In contrast, analysis of the occipital cortex – a region that is relatively spared in AD – failed to reveal the presence of the viral genome in AD (9 of 9) and control (5 of 5) cases; likewise, viral DNA was not detected in any tested brain region obtained from middle-aged individuals (5 of 5) or infants (5 of 5). Other investigators have also documented the presence of HSV1 genomic sequences in the human brain using PCR.[27]

In summary, these findings indicate that the presence of HSV1 in the CNS is more prevalent

than previously documented and, moreover, that it is brain region-specific and age-related.

3. Apolipoprotein E Gene (ApoE) as a Host-Factor

How could HSV1 play any role in the pathogenesis of AD if it occurs as frequently in the brains of both affected and non-affected individuals? If HSV1 does play a role, then there must be some additional host-specific factors which differ between the 2 groups. One host factor for AD that has been established as playing a role in sporadic (non-familial) cases is the apolipoprotein E gene (ApoE) gene.[3] Individuals, homozygous or heterozygous for the ApoEε4 allele, are at an increased risk for AD compared with individuals with other ApoE genotypes. Itzhaki et al.[28] examined the relationship between ApoE genotype and the presence of HSV1 DNA in postmortem brain specimens from 46 patients with AD and 44 age-matched control patients. Their results were intriguing: they reported that the frequency of the ApoEε4 allele was considerably higher in HSV-positive AD patients. In other words, a diagnosis of AD was more frequent among individuals carrying one or more ApoEε4 alleles and whose brains simultaneously harboured HSV1 than those possessing only one or none of these risk factors (table I). Thus, although HSV1 by itself was not found to be a risk factor for AD, these data suggest that infection of the CNS with this neurotropic virus, in combination with the ApoEε4 allele, increases the risk for AD.

An inherent difficulty associated with studies requiring nondemented elderly individuals is that ApoEε4 is itself a risk factor for AD; consequently, the older the population, the smaller the proportion of ApoEε4 carriers who remain that have not developed AD. As a result, some of the critical comparison groups in the study of Itzhaki et al.[28] contained as few as 2 individuals [e.g. ApoEε4 carriers without AD who were HSV1-negative (table I)]. Clearly, additional samples need to be analysed to properly evaluate the magnitude and veracity of the role of HSV in AD.

In a similar study carried out by Itabashi et al.,[29] PCR was again used to detect the presence of HSV (types 1 and 2) DNA in postmortem temporal and frontal cortex brain samples from 46 AD cases and 23 age-matched controls. Their findings were broadly similar to those of Itzhaki et al.,[28] albeit with some important distinctions. They also reported that a diagnosis of AD was more likely among individuals who were positive for both the ApoEε4 allele and HSV in brain compared with those possessing only one or none of these cofactors (table I). However, among ApoEε4 carriers, there was no significant difference in the incidence of AD between the HSV-positive and HSV-negative groups (table I). One possible explanation for the discrepancy between these 2 studies may lie in the fact that a lower proportion of brains were found to be HSV-positive in the study of Itabashi et al.[29] (19 of 69 or 27.5%) than in the study of Itzhaki et al.[28] (64 of 90 or 71.1%). Whether this disparity reflects methodological differences between the 2 studies and/or epidemiological differences between the patient populations is presently unclear. Nonetheless, these differences serve to underscore

Table I. Number (and percentage) of patients with and without Alzheimer's disease (AD) having various combinations of risk factors in 2 independent studies

ApoEε4	HSV	Itzhaki et al.[28]		Itabashi et al.[29]	
		AD	non-AD	AD	non-AD
+	+	29 (93.5)	2 (6.5)	12 (92.3)	1 (7.7)
+	–	2 (50.0)	2 (50.0)	13 (81.3)	3 (8.7)
–	+	7 (21.8)	26 (78.8)	2 (33.3)	4 (66.7)
–	–	8 (36.4)	14 (63.6)	19 (55.9)	15 (44.1)
Overall		46 (51.1)	44 (48.9)	46 (66.7)	23 (33.3)

ApoEε4 = ε4 allele apolipoprotein E gene; **HSV** = herpes simplex virus; **+** = risk factor present; **–** = risk factor absent.

the need for analysis of larger numbers of brains in independent studies.

4. Is There A Role for ApoE in Cold Sores?

Since it appears that HSV1 might be involved in mediating CNS damage, Itzhaki et al.[28] also examined the possibility that the susceptibility to disease in the CNS rendered by the ApoEε4 allele might be paralleled in the PNS. They investigated the relationship between ApoE genotype and HSV1-induced damage in the PNS (i.e. cold sores). Analysis of the ApoE genotypes of 40 people with recurrent cold sores and 33 others who did not experience cold sores demonstrated that the ApoEε4 allele frequency was significantly higher among those with cold sores (36%) than those without (9%). These results strengthen the hypothesis that ApoEε4 and HSV1 interact to cause disease. Nevertheless, it is still possible that ApoEε4 carriers are more readily infected by HSV1, but that the virus is innocuous. Further experiments that address the underlying molecular mechanism by which HSV1 causes disease are required.

5. Viral Factors

Supporting evidence from our own laboratory further implicates a role for HSV1 in AD pathogenesis. Whereas Itzhaki et al.[26,28] investigated host-specific factors that influence susceptibility to AD, our laboratory has focused its attention on virus-specific factors that could play a role in the pathophysiology of this insidious disease. In this regard, we have found that a portion of the glycoprotein B (gB) of HSV1 exhibits striking similarities to Aβ (unpublished observations). These proteins share extensive primary sequence homology and exhibit strong similarities in tertiary structure, including the ability to form β-pleated sheets as determined by circular dichroism measurements. At the ultrastructural level a viral glycoprotein, like Aβ, forms amyloid fibrils spontaneously *in vitro*. Moreover, we have demonstrated that the portion homologous to Aβ is neurotoxic to primary cortical neuronal cultures. Intriguingly, gB has been shown to interact with lipoproteins, particularly apoE,[30] which may represent an important molecular interaction that leads to further neurodegeneration.

6. Therapeutic Implications

If confirmed, the evidence reviewed here suggests that a selective subpopulation of AD patients might profit from antiviral drug therapy – the challenge lies in identifying the susceptible subgroup. Although ApoE genotyping is relatively straightforward, detecting viral DNA in the CNS of living patients is clearly more problematic, especially since seropositivity does not necessarily have to correlate with HSV1 infection of the CNS. Brain biopsy is presently the most accurate method for diagnosing HSE, but such a radical diagnostic procedure presents obvious risks and is not feasible for screening a large population base. An alternative approach might be PCR analysis of cerebrospinal fluid (CSF), since recent evidence suggests that this technique approaches the reliability of brain biopsy in the diagnosis of HSE.[31] However, it remains to be determined whether markers indicative of subacute HSV1 brain infection will likewise be detectable in the CSF.

Trying to minimise the effects of HSV1 in AD is a complex issue, especially since herpes infection can occur at any time from birth throughout life. The best possible anti-herpetic strategy would clearly be one that prevents the primary infection of the organism, i.e. vaccination. However, along with other problems intrinsic to such forms of immunotherapy, any vaccine against HSV1 would have to be administered very early in life to prove effective for the majority of the population.

For those individuals who already harbour HSV1, the next realistic goal would be to prevent reactivation and subsequent dissemination of the virus within the CNS. Presumably, recurrent reactivation of latent HSV1 infection in the CNS leads to progressive neuronal damage that facilitates the the neurodegeneration associated with AD, and thus the therapeutic goal is to intervene to minimise

cellular damage. Effective antiviral compounds presently exist for the treatment of acute HSV infection of the CNS (i.e. HSE), principally, nucleoside analogues such as aciclovir. However, in contrast to HSE which has a relatively clear clinical presentation, the milder, recurrent reactivation of HSV1 that is hypothesised to be a cofactor in AD is not likely to be accompanied by overt, external indicators. Thus, unless or until markers of reactivation are defined, acute treatment of reactivated HSV1 might not be indicated. Consequently, long term prophylactic use of these drugs may represent a viable treatment strategy. On the other hand, if an at-risk patient were to experience circumstances that are known to cause reactivation of HSV1 (such as immunosuppression or head injury), then more acute therapeutic or stronger prophylactic treatment might be indicated.

The most promising drug therapies will most likely emerge from research aimed at elucidating the interaction between HSV1 and hosts carrying ApoEε4. In this regard, there is evidence that suggests a direct interaction between viral glycoproteins and apolipoproteins.[30] Disrupting this interaction may yet represent a novel therapeutic target. If such a direct link can be found, then such drugs might not be a long way off.

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