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HSV1 IN ALZHEIMER'S DISEASE: MYTH OR REALITY?

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Abstract

Alzheimer's disease (AD) is the most frequent cause of dementia in the elderly, characterized by the presence of cerebral amyloid plaques and neurofibrillary tangles. The causes of the disease are not well understood, especially considering that more than 95% of AD patients are non-familial. Due to the similarity of brain regions affected in herpes simplex encephalitis to those mainly affected in AD, and owing to the very high prevalence of latent herpes simplex virus type 1 (HSV1) infection, reactivation of HSV1 was proposed as one of the possible causes of AD. The trigeminal ganglion, located only a few millimeters from the entorhinal cortex, is the primary site of HSV1 latency, although other sites including the sensory neurons, the nodose ganglion of the vagus nerve and other regions of the brain may be involved, possibly in relation to very early neurofibrillary AD changes in the dorsal raphe, locus coeruleus and other brainstem nuclei. Novel data obtained upon infection of cultured neuronal cells and mouse brain with HSV1 further show that HSV1 infection causes intracellular amyloid-beta protein accumulation, as well as abnormal phosphorylation of tau protein, the major component of tangles. Another interesting fact is the existence of a significant degree of homology between HSV1 components and AD susceptibility genes. In this review we summarize findings that reveal connections between the two conditions, as well as different suggestions for the mechanisms of HSV1-induced AD. As most of the available results support a connection of AD and HSV1 infection, antiviral therapy should be taken into consideration for AD treatment following early diagnosis.

Keywords

• Alzheimer's disease • Herpes Simplex Virus Type 1 • Apolipoprotein E • Antiviral Therapy

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1. Introduction

Alzheimer's disease (AD, OMIM #104300) is a chronic progressive neurodegenerative disease and by far the most common irreversible cause of dementia syndrome in elderly people. It accounts for over 60% of all dementia cases, and it presently affects over 24 million people worldwide. It is expected to increase as a greater proportion of the population ages [1].

The disease is divided into two subtypes based on the age of onset: early-onset AD (EOAD) and late-onset AD (LOAD). Early-onset AD accounts for approximately 1% to 6% of all cases and ranges roughly from 30 years to 65 years. Both EOAD and LOAD may occur in people with a positive family history of AD (familial AD, FAD). Approximately 60% of EOAD cases have multiple cases of AD within their families, and of these familial EOAD cases, 13% are inherited in an autosomal dominant manner with at least three generations affected [2].

Early-onset disease can also occur in families with late-onset disease [3]. With the exception of a few autosomal dominant families that seem to be single-gene disorders (and accounting for less than 1% of cases), most AD cases appear to be a complex disorder that is likely to involve multiple susceptibility genes and environmental factors [2]. Although the first-degree relatives of patients with LOAD have approximately twice the expected lifetime risk of the disease, the pattern of transmission is rarely consistent with Mendelian inheritance [4].

Clinical symptoms of both EOAD and LOAD include gradual decline in memory (typical initial presentation is an inability to retain recently acquired information), language, abstract reasoning and decision making. Changes in mood and affect often accompany or precede the memory decline. Death commonly occurs from general wasting, malnutrition, and pneumonia [3].

Clinical diagnosis of AD is currently based on core diagnostic criteria that include objective evidence of gradual and progressive decline in episodic memory, which may be associated with other cognitive changes concerning executive function, language, complex visual processing and gnosis. These criteria can be accompanied by supportive features that include atrophy of medial temporal structures (e.g., entorhinal cortex, hippocampal formation, parahippocampal gyrus) on MRI, abnormal cerebrospinal fluid biomarkers (low amyloid beta (Aß1-42), or increased total-tau or phospho-tau concentrations), specific metabolic patterns evident with molecular neuroimaging methods, in particular reduced glucose metabolism in bilateral temporo-parietal regions and in the posterior cingulate cortex on positron emission tomography, and familial genetic mutations in amyloid precursor protein gene (*APP*), presenilin 1 (*PS1*) and presenilin 2 (*PS2*) genes [5]. The European

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Federation of Neurological Societies guidelines suggest several additional criteria for AD diagnosis: defining the impact of cognitive decline on activities of daily living, inclusion of information on past medical history, assessing comorbidity (e.g., depression, cardiovascular and pulmonary diseases, infections, sleep disturbances), and electroencephalography measurements [6].

The primary lesions of AD pathology are senile plaques (SP) composed of 39-43 amino acid fragments of the Aß protein that accumulate in the extracellular space and neurofibrillary tangles (NFT) made of hyperphosphorylated microtubule-associated protein tau (MAPT) in the neurons. The causes of FAD are known and include mutations in the APP gene, as well as in PS1 and PS2 genes, which code for subunits of the APP cleaving enzyme γ-secretase. The dominant view on AD pathogenesis for the last 20 years has been the amyloid cascade hypothesis [7] that is based on the discovery that a mutation in *APP* gene was able to induce AD in familial cases of the disease [8]. This led to a (somewhat premature) conclusion that Aß is the main trigger of the disease in both FAD and sporadic AD, and that it causes all subsequent changes, including tangle formation. The finding that mutations of the *MAPT* gene cause a pure tauopathy without Aß accumulation [9,10] further supported this framework of interpretation.

However, neuropathological data actually did not fit well with the amyloid hypothesis [11]. For example, Braak and colleagues have shown that tau pathology in the entorhinal and hippocampal regions precedes Aß accumulation by as much as 27 years [12] and recent data suggest that this delay may even be much longer. Tau-positive materials labeled by the AT8 monoclonal antibody (that shows early neurofibrillary degeneration), were observed in high proportion (38/42) of children and young adults in the absence of Aß accumulation [13]. As AD is traditionally viewed as a disorder associated with aging (more specifically, with old age) early tau pathology seen in children and young adults, if confirmed, could completely alter our view on brain aging [14]. Taking all of these findings into account it is not surprising that over twenty recent clinical trials of potential disease-modifying drugs based on manipulation of Aß (including active immunization) have failed [15]. Moreover, in the recently abandoned semagacestat trial, some patients on the drug got worse - the drug that was designed to inhibit formation of Aß sped up cognitive decline. One plausible explanation is that the formation of Aß might be in fact the brain's protective mechanism against the disease process, whereas the progression of the pathological changes of the neuronal cytoskeleton, rather that Aß burden, is crucial in determining the severity of the dementia syndrome in AD [16-19].

Under the assumption that the pathological lesions developing during the course of AD are directly or indirectly attributable to the aging process, a central position in the pathogenesis of AD has been variously assigned to all factors that are known to be capable of damaging postmitotic cells (a risk that increases during aging and old age), such as greater oxidative stress, chronic inflammation, mitochondrial dysfunction and failure of the ubiquitin-proteasome system [20].

One of the underestimated potential causes of all of these changes is reactivation of a latent infection with herpes simplex virus type 1 (HSV1). As inflammatory responses and oxidative changes have been amply documented in AD brains [21,22] and most people are latently infected by HSV1 (worldwide rates are between 65-90%, [23], several authors recently suggested that both aggregation of Aß and formation of NFT could represent inflammatory responses to infection and oxidative stress caused by HSV [for review see 24]. During latent HSV1 infection of neurons in mice there is evidence of ongoing inflammation and oxidative damage not only to infected neurons but also, by measuring the amount of oxidative damage, in nearby uninfected cells [24]. Itzhaki and Wozniak further suggest that aggregation of Aß and tau could be responses to oxidative stress.

1.1 Herpes Simplex Virus Type 1

HSV1 is a dsDNA virus which causes several diseases in humans, including herpes labialis and herpes simplex encephalitis (HSE). It is composed of four concentric compartments. The central DNA core is coding for at least 74 genes.

The HSV1 life cycle follows two paths: lytic and latent. During the lytic cycle, new viral particles are produced. The virus promotes expression of immediate early or α proteins, and subsequent expression of intermediate or ß, and late or γ proteins, which all leads to the assembly of novel viral particles. During the latent cycle, viral DNA remains circularized inside the nuclear envelope and expresses two known isoforms of a single-gene product, named the latency-associated transcript [25]. The trigeminal ganglion, located only a few millimeters from the entorhinal cortex, is the primary site of HSV1 latency [26], although other sites including the sensory neurons [27], the nodose ganglion of the vagus nerve [28] and other regions of the brain may be involved [29], possibly in relation to very early neurofibrillary AD changes in the dorsal raphe [19], locus coeruleus and other brainstem nuclei [13].

In the case of herpes labialis, after the initial infection of the mucosal membranes of the mouth and eye, the virus travels along nerve cells and becomes latent in the peripheral nervous system. Stimuli such as neuronal injury, steroids, hypothermia, ultraviolet light exposure and fever cause HSV1 to break out of latency and to reactivate. The activated virus re-infects the mucosal epithelium, causing the well-known recurrent herpetic sore. Both symptomatic and asymptomatic infection enables virus spread through saliva. The infection with HSV1 occurs usually in infancy, residing in about 90% of the adult population. It is interesting to note that although most of the population is infected with this virus only 25–40% develop cold sores [30].

Herpes simplex encephalitis is a rare, very serious acute neurological condition. Interestingly, infection afflicts regions most prominently affected in AD, such as the hippocampus and fronto-temporal cortices [31]. Further, those who survive HSE usually suffer from memory loss and cognitive impairment.

The entry of the virus is dependent on interactions of the envelope glycoproteins

B, C and D (gB, gC and gD respectively) with surface molecules of host cells. Following the initial interaction between surface heparan sulfate proteoglycans and viral gB and gC [32], virus facilitates entry via binding of the cells herpesvirus entry (Hve) proteins and gD and fusing with the host cell membrane [33]. Since it has been shown that during entry, HSV1 interacts with lipid rafts, it has been proposed that gB associates with as yet an unidentified receptor localized inside lipid rafts [34].

1.2 HSV1 and APOE

It is widely accepted that sporadic AD is a multifactorial disease, encompassing several genetic and environmental factors. Many risk factors for AD have been explored, including smoking, elevated blood pressure, brain nerve growth factor, gender, race, head trauma and intelligence, but so far the only certain risk factors are increasing age and carriage of the isoform 4 of the apolipoprotein E gene (APOE) [35-40]. The disease usually occurs after the age of 65 and the risk doubles every additional 5 years.

APOE is a 299 amino acid protein involved in the mobilization and redistribution of lipids and cholesterol during neuronal growth and repair. Protein apoE exists in three major isoforms, types ε2, ε3, and ε4, which differ only in residues 112 and 158, placed in the N-terminal, receptor-binding domain. In the early 1990s isoform 4 of the APOE gene was revealed as a susceptibility factor for AD [40]. APOE4 has been associated with enhanced depolymerization of microtubules [41], lower density of cholinergic neurons in AD [42], reduced neuronal remodeling [43], but also with a worse prognosis after HIV infection [44] and traumatic brain injury [45].

APOE-ε4 plays different roles during infection by certain pathogens - while associated with a worse prognosis after HIV infection, it also acts protectively during hepatitis C virus-induced liver damage [46]. APOE genotype has been found to determine the outcome of infection by HSV1 – people who develop cold sores after HSV1 infection are more likely to carry APOE-ε4 [47], while there is preliminary evidence on a relatively small number of cases that APOE-ε2 might be a risk factor for HSE [48]. Furthermore, the APOE-ε4ε4 variant has a protective effect in female postherpetic neuralgia, while giving susceptibility to shingles in women. APOE-ε2ε2 causes earlier infection with malaria, while APOE-ε2 determines the outcome of pulmonary tuberculosis in women [24].

After infection, the viral DNA load in mice depends on the presence of APOE gene, and is even higher when the host carries an APOE4 allele (13-fold more HSV1 DNA in brain compared to APOE3 carriers) [49]. Furthermore, HSV1 is vertically transmitted, and the virus colonizes brains of all offspring of infected mothers [50]. Vertical transmission does not depend on APOE genetic status, but the amount of virus in the brain is dependent on APOE allele type, dosage and gender, with female offspring carrying APOE4/4 genotype having the highest viral loads [51].

To study the impact of different isoforms of APOE on HSV1 gene expression, transgenic mice that express human isoforms ε2, ε3, ε4 and no APOE were constructed [52]. The HSV1-infection of primary neuronal cortical cultures of these animals revealed higher expression of immediate early genes of the virus in APOE-ε4 and knockout strains. Regarding infection of the APOE-ε4 strain, a delay in expression of the latency associated transcripts was observed.

Together these data suggest that APOE-ε4 allele provides a more conducive environment for HSV replication and spread than the other APOE alleles, perhaps retaining the virus in the lytic phase of its life cycle for a longer period.

1.3 Summary of selected research and hypotheses in support of the connection between HSV1 infection and AD

It was noted that a combination of APOE-ε4 carriage and latent HSV1 infection confers greater risk of developing AD [47,53]. The supposed link between APOE and HSV1 could be their competition for binding to heparin sulfate proteoglycans on the cell surface. The isoform ε4 could posses weaker binding properties compared to isoforms ε2 and ε3. Another explanation is that ε4 is less efficient in repairing neuronal cells after damage, i.e. in removing excess lipid or supplying necessary lipid to the site of injury.

Binding to transporter associated with antigen processing (TAP) protein is thought to be one of the main ways in which HSV1 evades the immune response of the host. The viral protein ICP47 blocks the antigen-binding site of TAP, preventing the antigen presentation to the host immune system. TAP2687C variant of the TAP allele seems to give susceptibility to infection and if associated with APOE4 carriers, yields a high risk for AD development [54].

HSV1 was found to cause an accumulation of cholesterol in cells by inhibiting cholesterol esterase and the efflux of cholesterol out of cells [55]. Hill *et al.* [56] elaborated their hypothesis on the connection between cholesterol levels, HSV infection and the risk of developing AD. As cholesterol is an important component of lipid rafts, and lipid rafts have been associated with HSV1 gB-mediated virus entry, they suggested that lowering cholesterol levels, and in that way decreasing the activity of lipid rafts, should reduce neuronal spread of HSV1. They find support for their theory in the fact that statin use has been attributed to reduced risk of developing AD [57].

Latent HSV1 is very rarely present in brains [58] or cerebrospinal fluid (CSF) [59] of younger people (actually, even in HSE sufferers HSV1 is present in CSF for only about one week after infection), but resides latently in a high proportion of aged brains [60]. It was proposed that HSV1 infects the brain following decline in the immune system with increasing age. Systemic infection, stress and further immunosupression could lead to brain inflammation and subsequent reactivation of the virus. The reactivation could cause upregulation of enzymes involved in APP processing and Aß formation, and as the infection progresses, cells would die causing the release of amyloid aggregates which develop into classic amyloid plaques. Cheon *et al.* [61] suggested that latently infected cells express at least one antigen which causes the production of IL-6 and a local inflammatory response which damages infected and neighboring neurons.

One possibility concerning Aß accumulation suggests that Golgi transmembrane APP could transport HSV-containing vesicles anterogradely, which would protect APP from

proteolysis. After the release of the virus, the protection would cease, leading to hydrolytic proteolysis of APP by γ-secretase. The APP has been identified as a putative motor receptor. In vitro, 47-amino acid terminal tail of APP binds the kinesin light chain 1, which leads to the formation of a ternary complex with the kinesin heavy chain, which contains the motor domain [62]. During the lytic cycle of HSV1, membrane glycoproteins are synthesized and incorporated into the Golgi membrane, where they promote the budding of the virus into the Golgi network. The possibility that HSV1 is being transported to the cell surface via anterograde transport inside Golgi vesicles was tested through observation of movements of the virus inside the squid giant axon [63]. The tegument protein, VP16, was tagged with GFP and injected into the axon after which movements were observed by confocal microscopy. The velocity of anterogradely transported particles was 5-fold higher than the retrograde and anterograde movements of rhodamine-labeled mitochondria. Immunofluorescence staining confirmed that viral particles associated directly with the Golgi cisterns and APP.

Effects of HSV infection on APP and its degradation were studied in a neuronal SHSY5Y cell line [64]. Western blot analysis of cell lysates 3 hours postinfection revealed a significant decline in all bands of full-length APP corresponding to the three main isoforms in multiple glycosylation states. The decline could simply be the result of rapid shut-off of protein synthesis prior to viral replication, but an interesting finding was an increase in band intensity of a C-terminal 55 kDa fragment. The phenomenon was not restricted to HSV1 infection, since HSV2 infection provided same results. Furthermore, it has been shown that infection of cultured neuronal and glial cells with HSV1 leads to a dramatic increase in the intracellular levels of Aβ1-40 and Aβ1-42, whilst levels of APP in cells decreased [65]. Similarly, Aβ1-42 deposits were found in mouse brain after HSV1 infection [65].

The HSV1 causes changes in neuronal excitability and intracellular Ca^{2+} signaling, which results in abnormal processing of APP, causing an increase in intracellular Aß. The neuronal excitability changes occur because HSV1 binds to potassium channel, reducing efflux of K^+ and causing depolarization. The major pathway of increasing neuronal excitability, however, is the enhanced activity of the persistent sodium channels by HSV1. These changes in turn cause the activation of inositol triphosphate receptor, which enables the release of Ca^{2+} from the endoplasmatic reticulum. Activation of voltage-gated calcium channels by the increased activity of the persistent sodium channels, however, plays a more important role in changed intracellular Ca^{2+} levels, by the influx of Ca^{2+} from the extracellular space. This enhances the activity and calcium-dependent phosphorylation of APP and the intracellular accumulation of Aß and Aß generation, which in turn causes more Ca^{2+} dysregulation, generating a self-sustaining vicious cycle [66].

In situ PCR of HSV1 DNA and thioflavin S staining of amyloid plaques in postmortem brain tissue revealed their colocalization [67]. While the amount of plaques that associated with HSV1 DNA was similarly high in AD and normal brains, being 90% and 80% respectively, the amount of HSV1 DNA that colocalized with plaques was significantly higher in AD brains, being 74%, compared to 24% in normal temporal and frontal cortices. It is interesting to note that all but one AD case carried at least one APOE ε4 allele, and none of the aged controls.

Kammerman *et al.* [68] suggested that Aß aggregates form as a result of interaction between Aß42 and L particles. According to these authors Aß42 is a product of the innate immune system which creates pores in membranes. The L particles are synthesized by HSV1-infected cells to neutralize Aß42 and complement 3b components and to create a safer environment for virus migration among cells. When Aß42 perforates L particles via pore formation, the L particle releases the tegument proteins it carries, which then bind with Aß42 creating insoluble amyloid plaques. The tangles of AD could be attributable to the release of VP22 from the L particle, which is a microtubule organizing protein and an organizer of viral assembly in HSV1 infected cells. The VP22 has homologous regions to the tau protein, and VP22 could have been mistakenly identified as tau, because immunochemistry is not able to differentiate between VP22 and tau.

It has been hypothesized that Aß42 may act to downregulate pathologic effects of HSV1 either by adsorption and sequestration or formation of pores in the membrane. Although Aß42 is central to the pathology of AD, it also seems that it is a product of the innate immune system designed to adsorb a wide variety of intra- and extracellular ligands. Therefore, the response of the immune system to viral infection could be the reason for the accumulation of Aß42 [69].

The infection with HSV1 can cause abnormal folding and phosphorylation of tau, confirmed by immunocytochemical analysis [70]. The phosphorylation occurs at multiple residues, including the ones specific for AD, T212 and S214. Further research revealed increased levels of GSK3ß and PKA, kinases known to phosphorylate tau. It is interesting to note that lithium, which inhibits the activity of GSK3*β*, also has an inhibitory effect on HSV1 [71]. Wozniak *et al.* [70] postulate that HSV1-induced tau phosphorylation could be a consequence of inappropriate cell cycle entry stimulation. In support of their theory is the fact that HSV1 infection increases the activity of cdc2 kinase [72], a cell cycle enzyme which was also found upregulated in AD [73]. Neurons do not actually go through with division, but instead are arrested at G_2/M stage after which abortive apoptosis ("abortosis") usually follows. The possible reason for the virus-induced loss of tau function could be making the microtubules available for their interaction with viral proteins.

Letenneur *et al.* [74] examined 14-year old serum samples of 512 individuals aged 65 and over for the presence of anti HSV1 and HSV2 IgG and IgM antibodies, to confirm whether lifelong infection (IgG) or primary infection/ reactivation (IgM) correlated with risk of AD development. A significant association was found between the IgM-positive status and AD. Given that seropositivity was analyzed only once, the authors could only hypothesize that HSV chronic infection may therefore contribute to the progressive brain damage characteristic of AD.

Bullido *et al.* [75] studied the association of AD with polymorphisms of *EIF2AK2*, its translational product acting by subsequently shutting-off host-driven protein synthesis induced by many viruses. They found that an SNP inside an exonic splicing enhancer (rs2254958), which terminates its function, is more frequent in AD patients, and is correlated with a 3.3-years earlier age of onset.

Infection of a human neural cell culture with a highly reactivating strain of HSV1 (17 syn +) resulted in an upregulation of proinflammatory markers cytosolic phospholipase A_2 ($cPLA_2$), cyclooxygenase-2 (COX-2) and interleukin-1ß, all found to be elevated in AD brains [76]. The infection also caused a 5-fold increase in miRNA-146a levels, implicated in the regulation of the host immune and inflammatory response, which could have been the primary cause of the observed downregulation of an immune system repressor complement factor H.

The degree of homology between human and HSV1 virus was analyzed using the NCBI BLAST server [77]. It appears that intraprotein sequences of AD susceptibility gene products, such as APOE4, clusterin, complement receptor 1, and PICALM are identical to those of the HSV proteins. These genes share this homology with a number of infectious agents, including *Chlamydia pneumoniae*, the influenza virus, HHV-6 and some phages that affect commensal bacteria. It is interesting to note that a VGGVV sequence, expressed in different viruses (including fragments of the HSV1 glycoprotein B peptide), is identical to the C-terminus of Aß, and this epitope has been used to label Aß of AD patients. Carter suggested that viral proteins with homology properties could interfere with normal human protein/protein interactions, especially by mimicking tau-interacting proteins [77]. The other possible outcome is the development of an autoimmune response, initiated by viral protein expression.

Santana *et al.* [78] examined the effects of HSV1 infection in a human neuroblastoma cell line, SK-N-MC, overexpressing *APP*. Immnuofluorescence staining revealed that the infection caused accumulation of intracellular Aß40 and Aß42 as soon as 2 hours postinfection, even before the replication of the virus. The infection with HSV1 also resulted in decreased activity of α-secretase and the processing of APP by the non-amyloidogenic pathway. Aß accumulated in autophagic vesicles, but additional tests showed that these vesicles did not fuse with lysosomes, pointing to the impairment of the autophagic pathway caused by the infection. The group also confirmed that Aß accumulation did not depend on the formation of autophagosomes, since its inhibition resulted in the accumulation of Aß inside late endosomes.

2. Conclusion

Accumulation of Aß could be a general response to infection, as other infectious agents, such as HIV [79], West Nile virus [80] and *C. pneumoniae* [81] can cause Aß deposition. On the other hand, Renvoize *et al.* [82] measured serum antibody titers to several pathogens implicated in AD, and found no differences in AD patients vs. "controls". Here it should be mentioned that, as, for example, with HSV1, not all infectious agents establish a detectable long-term infection and that in any case, serum analysis may not provide adequate information on the state of one's brain.

Wozniak *et al.* [83] measured HSV (1 and 2) and HHV6 IgG antibody levels in serum and CSF using ELISA, and found no differences between normal subjects and AD patients. All subjects were seropositive for HSV, but approximately half of normal subjects (69%) and AD patients (52%) were positive for intrathecal antibodies. There was no antibody leakage from blood to CSF since analysis by signal radial immunodiffusion confirmed that the blood brain barrier was not damaged. There was also no association between APOE-ε4 genotype and presence of intrathecal antibodies. Itzhaki and Wozniak found that younger subjects did not have intrathecal antibodies against HSV while older ones did have it suggesting that the intrathecal IgG is formed when the virus replicates thus causing a local, acute immune response to the replication [24].

By using Bradford Hill's criteria on several suspected pathogens, Honjo *et al.* [84] imply that no specific infection causes sporadic AD directly, but infection in general could contribute to AD onset and progression. They assign the greatest contribution to disease development to the bacterium *C. pneumoniae*. This bacterial species was described as late as 1990, infects the lung and nasal mucosa, but can systemically disseminate through monocyte employment. *C. pneumoniae* DNA was found in 80% of AD brain tissue samples, but in only 11,1% of controls [85]. The detected organism was viable and associated with plaques and tangles. Furthermore, the bacteria infected astrocytes, microglia, as well as neurons. It is suggested that this bacteria could gain access to the brain via the olfactory pathway, but it could as well cross the blood brain barrier following the uptake by monocytes. *C. pneumoniae* can induce strong inflammatory responses, which could in turn lead to AD-related neurodegeneration [86].

Co-infection of spirochetes, such as *Treponema pallidum* and *Borrelia burgdorferi*, with *C. pneumonias* and Herpes viruses is also frequent, and *T. pallidum* is known to cause slowly progressive dementia, cortical atrophy, microgliosis, and amyloid deposition in the atrophic form of general paresis [87].

Nevertheless, Itzhaki and Wozniak [88], in their reply to Honjo *et al.* [84], stress the importance of accumulating evidence on the connection between HSV1 infection and AD.

Despite some inconsistent findings on the involvement of HSV1 infection in AD, research in support of the theory encourages exploration of the effect of antiviral treatment in early stages of AD. HSV1 DNA polymerase activity is significantly decreased in the presence of Aß42 and acyclovir [69]. miRNA 146 seems to be upregulated by many viruses like EBV, HHV8, as well as HSV1, and its main function is to downregulate factor H, which is a repressor of the complement signaling cascade. In the presence of acyclovir and Ab42, there is no upregulation of miRNA 146 in HSV1-infected cells [69].

Antiherpetics, such as acyclovir and valacyclovir, act by inhibiting viral DNA replication. Intravenous immunoglobulin neutralizes extracellular virus and destroys infected cells, and both immunoglobulin and

antiherpetic drugs cross the blood-brain barrier, and so could prevent further brain damage caused by viral replication [89]. Other options would include anti-inflammatory therapy and vaccination against HSV1 in infancy. Vaccination with mixed HSV1 glycoproteins was shown to prevent establishment of a latent infection in mice [90]. Therefore, as most of the available results support a connection of AD and HSV1 infection, antiviral therapy should be taken into consideration for AD treatment following early diagnosis.

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