

Herpes simplex virus type 1 and Alzheimer's disease: possible mechanisms and signposts

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ABSTRACT: Support for the concept that herpes simplex virus type 1 (HSV1), when present in the brains of apolipoprotein E- ϵ 4 carriers, is a major risk for Alzheimer's disease (AD) is increasing steadily, with over 120 publications providing direct or indirect evidence relevant to the hypothesis. No articles have contested the concept, apart from 3 published 13–18 yr ago. This review describes very recent studies on the role of HSV1 but refers also to older studies that provide background for some lesser-known related topics not covered in other recent reviews; these include the relevance of herpes simplex encephalitis and of epilepsy to AD, the action of IFN, and the possible relevance of the different types of DNA damage to AD—in particular, those caused by HSV1—and mechanisms of repair of damage. New epidemiologic data supporting previous studies on mild cognitive impairment and progression to AD are reviewed, as are those examining the relationship between total infectious burden (additive seropositivity to various microbes) and cognition/AD. The latter indicates the involvement of HSV1 and cytomegalovirus (and the necessity of taking into account any marked differences in sensitivity of antibody detection). Recent studies that provide further support for the occurrence of repeated reactivation of latent HSV1 in the brain in AD pathogenesis are also discussed.—Itzhaki, R. F. Herpes simplex virus type 1 and Alzheimer's disease: possible mechanisms and signposts. *FASEB J.* 31, 3216–3226 (2017). www.fasebj.org

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"Heresy is a cradle; orthodoxy, a coffin"

—Robert Green Ingersoll

Support for a major role of infection in Alzheimer's disease (AD) continues to grow, whereas scientific opposition, as opposed to knee-jerk hostility, is as invisible and inaudible as ever. This review aims to update certain aspects of the topic and to introduce new facts that will further examine the key role of 1 of the microbes implicated: herpes simplex virus type 1 (HSV1), although first, it seems essential to restate the evidence that has given rise to the concept of a viral role in AD pathogenesis.

Studies from the author's laboratory and subsequently, from other groups (1), indicate that HSV1 is present in a latent form (usually) in a high proportion of elderly brains

ABBREVIATIONS: A β , β amyloid; ACV, acyclovir; AD, Alzheimer's disease; aMCI, amnesic mild cognitive impairment; APOE, apolipoprotein E; BER, base excision repair; BRCA1, breast cancer factor 1; CMV, cytomegalovirus; DDR, DNA damage response; DSB, double-stranded break; DSBR, double-stranded break repair; HHV6, human herpes virus type 6; HSE, herpes simplex encephalitis; HSV1/2, herpes simplex virus type 1/2; IB, infectious burden; NHEJ, nonhomologous end-joining; OGG1, 8-oxoguanine glycosylase; P-tau, tau phosphorylation; PK, protein kinase; PKcs, protein kinase catalytic subunit; PNS, peripheral nervous system; ROS, reactive oxygen species; SIRT, sirtuin; SSB, single-stranded break; TG, trigeminal ganglia; TLE, temporal lobe epilepsy

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(detected by PCR in postmortem specimens), including those of patients with AD. It is absent in the brains of most younger people (2). Further work suggests that the virus in the brain of elderly apolipoprotein E (APOE)- ϵ 4 carriers confers a strong risk of AD (3). A concurrent related finding was that APOE- ϵ 4 is a strong risk for herpes labialis—cold sores that are usually caused by HSV1 infection in the peripheral nervous system (PNS) (2)—consistent with the joint role of the 2 factors in the CNS. The proportion of patients in the groups studied (admittedly small in number) who have HSV1 in the brain and carry an APOE- ϵ 4 allele is ~60%, as is the proportion of individuals with cold sores who carry the same allele. The virus can be activated (as in the PNS), leading to a productive infection, probably limited in extent but still damaging, through direct viral action and/or an inflammatory response (4). Reactivation is probably recurrent so that its effect may be cumulative, eventually leading to AD. Subsequent studies revealed that HSV1 caused β -amyloid (A β) deposition (5–8) and AD-like tau phosphorylation (P-tau) (9–12) in infected cell cultures and (13) in mice (also see refs. 1, 14). Recently, the closely related virus HSV2 (15) was found to cause similar AD-like effects. Amyloid accumulation occurs via HSV1-induced protein kinase (PK) RNA activation, followed by phosphorylation of eukaryotic translation initiation factor 2- α (16), which activates β -site cleavage enzyme 1 translation. *In situ* PCR showed that in AD

brains, HSV1 DNA was located specifically within amyloid plaques (17). Antiviral treatment of HSV1-infected cells greatly decreased the amounts of A β and P- τ (see refs. 1, 18).

Work from many laboratories, using a variety of techniques and presented in >70 publications, plus work from the author's laboratory, has strongly supported the concept that proposes that HSV1 enters the brain in older age as the immune system declines and establishes latent residence there. During events such as immunosuppression, peripheral infection, and stress, the virus reactivates, causing localized damage and inflammation (in effect, a mild type of encephalitis). Repeated reactivations lead to the accumulation of damage, the formation of amyloid plaques and neurofibrillary tangles, and eventually to AD. The roles of the main components of plaques and tangles—A β and abnormally P-tau, respectively—now seem less certain than previously (see Discussion in ref. 1); in the case of A β , its action initially is probably antiviral, but whether it becomes toxic (as apparently it does *in vitro*) at higher concentrations in the brain is unknown. However, the striking colocalization of HSV1 DNA and amyloid plaques (17) suggests that it might be entombing the virus, thereby preventing viral replication, a strategy first suggested by Robinson and Bishop (19). Likewise, the mode of interaction between HSV1 and APOE- ϵ 4 is unclear, although it might be direct between the virus and the apoE protein (20) competing for entry into cells. Whatever the mechanisms, there is strong evidence that HSV1, in combination with APOE- ϵ 4, has a major role in AD.

REACTIVATION

One of the main features of the concept that HSV1 plays a major role in AD is that stressful or traumatic events periodically reactivate HSV1 in the CNS, causing direct cytotoxicity and inflammatory damage in the brain; these events include immunosuppression or hyperthermia (in murine studies), neurosurgery, radiotherapy, or chemotherapy (summarized in refs. 1, 21). In particular, the studies by Peter and Sevall (22) and Ramakrishna *et al.* (23) strongly support the occurrence of frequent reactivation events in brains harboring HSV1. A study by DeBiasi *et al.* (24), using PCR for the diagnosis of herpes virus infection and including data from 2214 cerebrospinal fluid specimens, showed that 3.6% were positive for HSV1, a much greater percentage than accounted for by clinical herpes simplex encephalitis (HSE), the annual incidence of which is ~2 cases per million of the population. Interestingly, the researchers comment on mild or atypical forms of HSE, which, they suggest, might account for 17% of total cases, consistent with the results of Klapper *et al.* (25) and the concept of recurrent activation of HSV1 in the AD brain.

If reactivation of HSV1 does indeed occur in AD (although unfortunately, no current technique can provide direct evidence for reactivation in the human brain), the extent of damage related to any episode must be limited; otherwise, patients would display symptoms of acute HSV1 infection of the CNS, namely, encephalitis. HSE is the most common type of sporadic viral encephalitis in

humans, although fortunately, it is extremely rare. Therefore, it is not surprising that diagnostic detection of reactivation of the virus is also very rare. Nonetheless, there are a number of case reports of people who developed HSE, and many experienced a relapse months or years later. For example, neurosurgery can trigger the reactivation of HSV, with a long inactive interval of as much as several years, after an initial diagnosed episode of HSE (26–28). However, whether the neurosurgery itself or simply the stress of the surgical procedure causes reactivation of HSV1 is not known. It is likely that the frequency of relapses is underestimated, as the initial symptoms of the illness are nonspecific. A main diagnostic criterion of HSE is the appearance of HSV1 DNA in cerebrospinal fluid, but as the DNA persists there for only ~1 wk (29), any delay in performing a lumbar puncture, if HSE is suspected, might result in the analysis being too late to detect HSV1 DNA. Because of the serious consequences of HSE, which can include permanent impairment of cognition and memory, clinicians strongly advocate starting antiviral treatment as soon as possible if there is even a slight possibility of the illness being HSE. Usually such treatment is effective, but an occasional patient does not respond. In some cases, this might be because the infection is caused by a drug-resistant strain of virus, but clinicians might wrongly attribute the lack of response to an incorrect diagnosis, again contributing to underestimation of the frequency of occurrence of reactivation. Fortunately, drug resistance is usually rare: the prevalence of acyclovir (ACV) resistance in HSV isolates from immunocompetent hosts is ~0.1–0.6%, but in immunocompromised patients, who are at much greater risk, its prevalence is 3.5–10% (30).

Recent studies on reactivation include that of Yao *et al.* (31), showing that in HSV1-infected mice, the virus could be reactivated in explant cultures of brain tissue. The result has recently been confirmed in tree shrews by Li *et al.* (32). These animals are more closely related to primates at the genomic and transcriptome level, so the effects of HSV1 in the shrews may be more relevant to humans than are those in rodents. Ocular inoculation of the virus followed the usual pattern: acute infection in the brain, 5 d postinoculation, with the most highly infected animals exhibiting symptoms similar to those of human viral encephalitis. Approximately 2 wk later, the infection became latent and the viral DNA undetectable. With the use of the modified explant cocultivation technique of Chen *et al.* (33), the researchers were able to recover reactivated virus from the CNS, indicating that in these animals, as in mice, HSV1 can be reactivated from latency in the CNS. These data suggest that experiments in these animals (because of their relationship to primates) might be preferable to mice for future viral studies.

One objection to the proposal, which is implicit in the HSV1–AD concept that HSV1 reactivation occurs in human brain, is based on the assertion that reactivation occurs only in the periphery—in the trigeminal ganglia (TG)—where it has long been recognized. This objection is based on early studies that showed that in HSV1-infected mice harboring latent HSV1 in the brain, massive doses of radiation and immunosuppression were required to effect

reactivation *in vivo* and that the latent virus could not be reactivated from brains of infected mice by explant culture (34). In humans, although there are substantial neuropathological data from fatal cases of HSE, there are necessarily none from those who survive, merely limited information about subjects who experienced HSE relapse and recovered after treatment. Despite these misgivings, there are now sufficient data to provide convincing, if indirect, evidence of HSV1 reactivation, with consequent damage, in the human brain. [Even during periods of latent HSV1 infection, significant damage might occur (35)].

EPIDEMIOLOGY

In an investigation of potential differences between subjects with amnesic mild cognitive impairment (aMCI), who, over a 24-mo period, convert to AD and those who do not, Agostini *et al.* (36) found that at baseline, serum HSV1-specific IgG antibody titers were higher and the avidity of the antibodies significantly greater in aMCI nonconverters than in aMCI-converters. HSV1 antibody titers correlated with MRI-evaluated cortical volumes of the left hippocampus and amygdala. As might be expected, the percentage of APOE- ϵ 4 was higher in the converters, although not significantly so. The researchers concluded that stronger HSV1-specific humoral responses are associated with protection against AD conversion and better-preserved cortical volumes and that the data supported the concept of a role for HSV1 in the pathogenesis of AD.

In contrast, in a longitudinal study in a Korean population, Shim *et al.* (37) found that levels of plasma IgG against HSV1 were not higher for converters, although converters' values for IgG against Epstein-Barr virus, another herpes virus, were higher, and these IgG values correlated with cognitive decline. The researchers suggested that the differences between their results implicating Epstein-Barr virus but not HSV1, and those implicating HSV1 (36, 38) might be attributable to differences in epidemiologic methods, to the assays (using plasma rather than serum or different antibody kits), or to ethnic differences between the populations.

Olsson *et al.* (39) examined brains from a large population over a range of ages for the presence of HSV1 by PCR and for A β using formalin-fixed sections. They found an unusually small percentage (~2%) with HSV1 DNA in the brain—an incidence much lower than found in other studies, which range from 35% by Baringer and Pisani (40) for subjects with a range of ages to almost 100% [for a very small group (41)] and 70% (3, 42) for elderly subjects. Olsson *et al.* (39) found that most of the HSV1-positive subjects showed A β staining in the brain, but many of those apparently not harboring HSV1 were also positive for the peptide. The difference between these results and previous studies was attributed to the use of fixed brain specimens in the latter, as several studies had shown that formalin storage, if lengthy, greatly increased the likelihood of loss of viral DNA (43, 44).

The first study on total infectious burden (IB), defined by Strandberg *et al.* (45) as systemic microbial burden (as

measured by serum antibodies), was conducted on 383 home-dwelling individuals, mean age 80 yr, of whom 82 and 37%, respectively, had a history of coronary heart disease or stroke, and 18% had type 2 diabetes. Viral burden, measured as seropositivity for HSV1, HSV2, or cytomegalovirus (CMV), and bacterial burden (*Chlamydia pneumoniae* and *Mycoplasma pneumoniae*) were determined. The data showed that greater viral burden, namely, HSVs and CMV, was associated with cognitive impairment, whereas there were no significant associations between bacterial burden and cognition. Aiello *et al.* (46) studied a subset (1204/1789) of participants in the Sacramento Area Latino Study on Aging, aged 60–100, and reported that the rate of cognitive decline varied significantly over a 4-yr period (even allowing for age, education, sex, and chronic health conditions) with the level of anti-CMV antibody but not with that of HSV1. They raised the possibility that the quantification of the antibodies might be more appropriate than just determining their presence. However, assay sensitivity is another important factor in such studies. The latter was highlighted in a response (47) to the study of Barnes *et al.* (48), implicating CMV but not HSV1 in AD; for HSV1, their assay detected merely a single protein of the virus, whereas the assay for CMV detected all of its many viral proteins.

Subsequent investigations of IB were described in the author's previous reviews. A more recent study (49) investigated IB in a subsample of 588 stroke-free participants, mean age 72 ± 8 yr, in the Northern Manhattan Study, assaying antibody titers for *C. pneumoniae*, *Helicobacter pylori*, CMV, and HSV1 and HSV2. The researchers found that quantitative stroke risk-weighted IB index was inversely associated with executive function at baseline and cognitive decline in the memory domain. They suggested that these findings might indicate that common infections (and the consequent inflammatory response) interact with different processes, such as aging, vascular, and neurodegenerative, which were static or progressive depending on the individual. They concluded that past exposure to common infections might contribute to vascular cognitive impairment and commented that 2 prior studies (45, 50) suggested that it is the viral component of overall IB that might be primarily responsible for the relationship. The latter study (50), an exploratory analysis in Northern Manhattan Study on IB index and global measures of cognitive performance, showed that association with cognition was essentially unchanged on restricting IB to viral serologies, consistent with the notion that it is viral exposure that is more likely to be implicated in cognitive impairment.

The only study investigating IB in relation to AD, rather than cognition *per se*, is that of Bu *et al.* (51). They measured antibody titres to HSV1, *Borrelia burgdorferi*, *C. pneumoniae*, and *H. pylori* in 128 patients with AD and 135 healthy age-matched controls. Participants were stratified into 3 categories, defined by the number of antibody types detectable in an individual's serum: those seropositive for 0–2 antibodies, those for 3, and those for 4–5, representing, respectively, lower, middle, and higher IB values. More antibody types to viruses and bacteria were found in patients with AD than in the controls, the patients usually

being in the category 4–5 (36 vs. 16%) and less often in the category 0–2 (20 vs. 44%) than healthy controls ($P < 0.001$); the category of 3 types (44 vs. 40%) did not differ significantly between the 2 groups. Division into separate categories for viruses and bacteria showed that in each case, more antibody types were found in patients with AD than in controls. IB, bacterial burden, and viral burden were independently associated with AD after adjusting for age, gender, education, APOE genotype, and various comorbidities. Levels of serum A β and of inflammatory cytokines were significantly higher in individuals exposed to 4–5 pathogens than in those exposed to 0–2 or 3 pathogens. The researchers concluded that their data support the role of infection/inflammation in the aetiopathogenesis of AD.

From these data, it appears that at least with respect to impaired cognition, the main IB is probably that of CMV and/or HSV1, although the bacteria *Borrelia* and *C. pneumoniae* and *H. pylori* are also implicated. Possibly the 2 herpes viruses act in concert. An investigation of the level of serum antibodies to CMV and to HSV1 (52) suggested that CMV is the trigger for the immune dysregulation seen in a number of age-related diseases, leading to infected subjects experiencing poorer health. Thus, control of HSV1 infection in CMV-seropositive subjects might be impaired, leading to reactivation of latent HSV1. This suggestion would account for the epidemiologic results and would be consistent with the concept of the role of HSV1 in AD and other studies implicating CMV.

HSE, HSV1, HHV6, EPILEPSY, AND AD

HSE is a major cause of seizures and subsequent temporal lobe epilepsy (TLE) (53, 54). Michael and Solomon (54) have pointed out that the proportion of subjects who become epileptic is probably underestimated, as seizures are often subtle, and diagnosis may be difficult. HSV1 has been implicated in TLE, in that viral DNA was detected in the brains of some patients with TLE by Gannicliffe *et al.* (55), Jay *et al.* (56)—3 of whose subjects had a previous history of HSE—and Yamada *et al.* (57). However, the total number of cases investigated was small, and the data need confirmation. Another herpes virus, human herpes virus type 6 (HHV6) DNA (see details below) was found in brains of patients with TLE (58, 59). In the latter study, the presence of virus was associated with a history of encephalitis with viral involvement.

In independent studies, a relationship has been found between certain aspects of TLE and the presence of APOE- ϵ 4: these include an increased frequency of APOE- ϵ 4 in epilepsy with senile plaques (60), earlier onset of TLE in APOE- ϵ 4 carriers (61), and an association of APOE- ϵ 4 with seizure development after severe traumatic brain injury (62). Carriage of APOE- ϵ 3,3 in patients with epilepsy conferred greater neuronal resilience (as indicated by lower levels of markers for RNA and DNA damage and lower susceptibility to inappropriate cell cycling and death pathways) than in those carrying other APOE alleles (63). The obvious corollary of these data is that both the presence of HSV1 and APOE genotype should be investigated in the same samples from patients with TLE. Such a study

has been performed, examining HHV6 and APOE. HHV6 comprises 2 types: HHV6A and -B. These are lymphotropic herpes viruses, which infect most of the population at a young age and, like other herpes viruses, can enter a latent state. Huang *et al.* (64) sought HHV6B in brain samples from patients with mesial TLE and also determined their APOE genotype. HHV6B DNA was present more often in patients than in controls, but APOE genotypes did not differ significantly between patients and controls nor between virus-positive or -negative patients. However, patients who were HHV6B positive and APOE- ϵ 4 positive had a higher seizure frequency, and their brain samples had a higher viral load and greater viral protein expression than did virus-positive but APOE- ϵ 4-negative patients. The researchers suggested that APOE- ϵ 4 might affect virus reactivation, replication, and protein expression, as well as seizure frequency.

Recently, more emphasis has been placed on the relationship between seizure activity and AD, with the number of occurrences noted to be greater in those with early-onset AD and greater still in those with autosomal-dominant, early-onset disease. Ten to 22% of patients with sporadic AD have at least one unprovoked seizure during their illness (65). In a retrospective study, Mucke and colleagues (66) found that most seizures in AD are non-convulsive and could easily go unrecognized, although such activity can acutely impair cognition. With the use of neurophysiological recordings, epileptiform activity was detected even in patients without a history of clinical seizures (67): 42% of patients with AD showed subclinical epileptiform activity compared with 11% of age-matched, cognitively normal controls, and although the patients with AD with seizures did not differ clinically from those without such activity, the subsequent decline of their global cognition and their executive function was greater.

Hence, there is a striking parallelism between the risk factors HSV1 and APOE- ϵ 4 for both AD and TLE seizure frequency, suggesting a connection between TLE and AD. In fact, the diseases share several characteristics (68), namely, changes in cognition and behavior, including impairment of episodic memory, executive function, language, and visuospatial ability, and both display mood disorders. There are also defects in regulation of excitability, although the defects responsible might not be the same in the 2 diseases (68). Furthermore, lobectomy specimens from patients with TLE (but not dementia) showed the presence of senile plaques in 10% of cases, correlating with the patients' age (69). The numbers and density of the plaques were the same as in subjects without epilepsy or dementia, but their age-related incidence was significantly greater in the patients with epilepsy.

What is uncertain is the nature of the association between AD and TLE: whether seizures contribute to the development of AD or whether they are a consequence of late-stage AD. However, it is now clear that seizures can occur early in AD and might be a basic part of the disease. Another possibility, if seizures and dementia start at about the same time, is that the dementia seen in TLE does not result from AD but instead, from the therapy used to treat the epilepsy. Furthermore,

TLE induced in triple-transgenic familial AD model mice has been found to cause enhanced AD-like neuropathology (70). In humans, infection with HSV1 early in life appears to be associated with a greater risk of seizures later in life, a finding substantiated by studies in mice (71). Younger age is also a risk factor for seizures in AD (72). This might be particularly relevant in the developed world, where the age of primary infection with HSV1 has risen appreciably with increasing socioeconomic level, so that far fewer individuals are infected in infancy. Based on animal models of AD and on the observations cited above, Palop and Mucke (73) have suggested that aberrant excitatory activity caused by A β , together with other age-related changes, might contribute to the cognitive deficits observed in the animals and that similar changes might explain the impaired cognition of patients with AD.

A small number of studies have found that HHV6, like HSV1, is associated with AD, as well as with TLE (as mentioned above), although as yet, there are no data suggesting a causal role for HHV6 in either disease. HHV6 was shown to be present in a high proportion of patients with AD (74), but in this case, unlike that of HSV1, the proportion in age-matched normals was significantly lower (70 vs. 40%, $P = 0.003$). In the AD brains, the 2 types of viral DNA overlapped extensively, although HHV6, unlike HSV1, was not directly associated in AD with APOE- $\epsilon 4$. It was suggested that HHV6 might enhance the damage caused by HSV1 and APOE- $\epsilon 4$ in AD, as in AIDS; in progressive multifocal leukoencephalopathy, caused by John Cunningham (JC) virus, HHV6 is associated with characteristic demyelinating brain lesions; and in both cases, it has been suggested that HHV6 activation might accelerate the disease, although alternatively, it might act opportunistically. Furthermore, HHV6 augments the damage caused by certain viruses in cell culture and in animals. In contrast to these data, tentatively supporting a role for HHV6 in AD, Agostini *et al.* (36) found that HHV6 seroprevalence, antibody titres, and avidity index were similar in patients with AD, subjects with aMCI and age-matched controls. However, antibody levels in serum have been related to viral presence or absence in the PNS, not necessarily in the CNS, and this study did not seek viral DNA presence in the brain, which would presumably be a prerequisite for a role in AD. Furthermore, as the researchers themselves point out, their data did not preclude activation of latent HHV6 in the brain by the reactivation of another virus.

DNA DAMAGE AND REPAIR IN AD AND HSV1

This section will describe studies comparing the main types of damage and repair of DNA in the brain or cells from patients with AD and age-matched normal individuals (age-matching is important, as DNA repair efficiency declines with age) and also studies on the effects of HSV1 on these processes. HSV1 is well known to cause oxidative stress *via* reactive oxygen species (ROS) and also reactive nitrogen species (75). Oxidative damage to DNA by ROS is a significant cause of lesions that might promote AD

progression; this would be consistent with the brain's particularly high consumption of oxygen, probably one-fifth of the total oxygen consumed by the human body. As Wang *et al.* (76) commented, "Chronic oxidative damage together with genome repair deficiency in the neurons is a double whammy for neurodegeneration."

Early work investigated mainly single-stranded breaks (SSBs) or double-stranded breaks (DSBs) in DNA or chromosomal aberrations in cultured lymphocytes, lymphoblastoid cells, or fibroblasts. AD and Parkinson's disease lymphoblastoid cells were found to be hypersensitive to X-rays (77). Gamma-irradiation of AD lymphocytes caused a significantly higher number of chromosome dicentric (78) than in controls, but numbers of acentrics and DNA SSBs and their rate of repair (79) did not differ significantly. Furthermore, the number and rate of DSB repairs (DSBRs) did not differ significantly, although mis- or nonrepair of a small fraction of DSBs, undetectable by the techniques then used, might have accounted for the greater radiosensitivity of the AD cells (80). Studies on DNA damage and repair in lymphocytes after treatment with alkylating agents, such as *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, yielded conflicting results, some indicating defective repair in AD but others finding no differences when compared with controls (81–85).

Subsequent studies using more sensitive techniques have shown that DSBs and also SSBs, base mismatches, insertions, and deletions are more frequent in DNA from patients with AD than from age-matched controls, presumably because of a greater rate of formation and/or less-efficient repair processes in AD. DSBR usually occurs *via* nonhomologous end joining (NHEJ); it operates throughout interphase and is inhibited during the process of mitosis. DSBs occurring during the S and G₂ phases of the cell cycle are repaired by homologous recombination.

Unrepaired or misrepaired DNA DSBs have been described (86) as the most significant forms of DNA damage. DSBs can cause loss of genetic material and genomic rearrangements and can lead eventually to genomic (or mitochondrial) instability, events especially deleterious in neurons because of their lower DNA repair ability and slower rate of repair compared with proliferating cells.

Weissman *et al.* (87) found significant base excision repair (BER) dysfunction, caused by reduced processing of damaged bases by DNA glycosylases and reduced DNA synthesis capacity by DNA polymerase in AD brains (taken at a short postmortem interval) and in aMCI brains compared with age-matched controls. In the aMCI brains, the dysfunction correlated with neurofibrillary tangle pathology. The total BER capacity inversely correlated with age of controls but not with the age of patients with AD. Therefore, the researchers suggested that the low BER capacity in AD, regardless of age, indicated premature aging—the dysfunction occurring at the disease's earliest stages—and so defective BER might play an important role in AD progression.

Lovell *et al.* (88) investigated levels of oxidative damage in the brain from subjects with preclinical AD, defined as those whose neuropsychological test scores antemortem were normal but who had significant AD

pathology at autopsy. Many previous studies had found that levels of oxidative DNA and RNA damage were greater and levels of 8-oxoguanine glycosylase (OGG1)-mediated BER lower in vulnerable brain regions (hippocampus/parahippocampal gyri) of subjects with MCI or late-stage AD compared with age-matched controls. Lovell *et al.* (88) noted also that lipid peroxidation and protein oxidation levels were greater. In the preclinical AD study, the researchers showed that in the vulnerable brain regions, levels of DNA- and RNA-associated 8-hydroxyguanine were higher than in control hippocampus/parahippocampal gyri, as was that of OGG1, although the latter difference was not statistically significant. They concluded that oxidative damage to nucleic acids and a compensatory increase in OGG1 expression are early events in the pathogenesis of AD.

Levels of expression of various BER components were found to be greater in postmortem brain tissue than in blood cells from live patients with AD or MCI and controls (89). The data indicated that changes in expression of BER genes precede AD development and might be useful as predictive biomarkers. However, Suberbielle *et al.* (90) found reduced levels of breast cancer factor 1 (BRCA1) but not of other DNA repair factors in AD brains and in human amyloid precursor protein-transgenic mice. BRCA1 is a tumor-suppressor gene encoding a protein essential for DSBR by homologous recombination, which binds directly to DSBs associated with the phosphorylated histone variant γ H2A histone, member X (γ H2AX). The latter allows DSB signaling and retention of repair proteins at the break site. A β oligomers reduced BRCA1 levels in primary neuronal cultures. From investigation of wild-type mice in which neuronal BRCA1 was knocked down, the researchers concluded that BRCA1 is regulated by neuronal activity, protects the neuronal genome, and supports neuronal integrity and cognitive functions.

Despite the quantity of detailed information about DNA repair mechanisms in the brain, it is still uncertain whether the greater DNA damage in neurodegenerative diseases is a consequence rather than the cause of neurodegeneration (86). Hou *et al.* (91) maintain that no genome-wide association studies have yet found an association between genes encoding DNA repair factors and AD. It is unclear whether loss of DNA repair is a risk factor for AD onset; it might instead more directly affect the rate of AD progression.

As for the effects of HSV1 infection on damage and repair of host cell DNA, studies have more often been concerned with the reverse—the use of the cell's DNA damage response (DDR) for repairing the viral DNA (92). Among those who have examined viral-induced damage in the host DNA, Aranda-Anzaldo (93) showed that HSV1 infection of cultured cells induced SSBs in the host cell DNA in a time-dependent fashion and that the early DNA damage observed in virus-infected cells was related to modifications in the higher-order structure of host-cell chromatin (94). Fortunato and Spector (95) found that at early times postinfection, HSV induces breaks and specific uncoiling of the centromeres of chromosomes 1, 9, and 16. Later, a more complete breakage of all other

chromosomes, termed pulverization, occurred. Viral entry and synthesis of certain viral proteins were required to produce the damage.

A more general study found that HSV infection of cultured cells affects nuclear structure, compressing and marginalizing host chromatin; furthermore, it disrupts the nuclear lamina by forming replication compartments in which various viral DNA processes, such as viral DNA replication and exit of nucleocapsids from the nucleus, can occur (96). Interestingly, Frost (97) proposed that in tauopathies, such as AD, disruption of the lamin nucleoskeleton causes heterochromatin relaxation, DNA damage, and eventually, neuronal death.

Deng *et al.* (98) discovered that telomeres were another target of HSV1, accumulating dysfunction-induced DNA damage foci. At the molecular level, the virus induced transcription of telomere repeat-containing RNA, followed by the proteolytic degradation of the telomere protein tripeptidyl-peptidase 1 (TPP1), *via* the HSV1-encoded E3 ubiquitin ligase infected cell protein 0 (ICP0), and loss of the telomere repeat DNA signal. The researchers suggested that HSV1 reorganizes telomeres to overcome barriers to viral infection of the nucleus and uses telomere structures or molecules to aid virus replication. They commented also that nuclear envelope components, including lamin B, have been implicated in telomere regulation and stability. In relation to AD, a meta-analysis of telomere length studies by Forero *et al.* (99) found consistent evidence of shorter telomeres in patients with AD.

The effect of HSV1 on DNA repair is complex. Smith *et al.* (100) showed that virion DNA activates the cellular DDR kinase, DNA-PK, which comprises the 470-kDa catalytic subunit DNA-PK (PKcs) and the Ku70/80 heterodimer and that this effect—in stimulating the NHEJ repair pathway—is intrinsically antiviral and leads to a less-efficient HSV1 lytic infection. However, the HSV1 ubiquitin ligase protein, ICP0, which has multifunctional properties, can combat the inhibitory effects of DNA-PK activation so that productive infection occurs.

Lilley *et al.* (101) found that activation of the DDR is beneficial for viral replication, but this cellular response is abrogated in neuronal cells; therefore, they suggested that cellular DNA damage proteins may be involved in controlling viral latency. However, De Chiara *et al.* (102) recently showed that HSV1 infection of rat embryo cortical neurons leads to DNA lesions, including SSBs and DSBs, and causes γ H2AX formation and accumulation, revealing that DNA lesions and DDR are, in fact, produced in neurons, not just in undifferentiated cells. The researchers propose that DDR occurs during HSV1 replication, whatever the cell type, but not during HSV1 latency in neurons. Brown (103) has suggested that failure of neurons to mount an adequate DDR to HSV1 may contribute to the establishment of latency. Perhaps relevantly to HSV1 reactivation from latency in neurons, Cliffe *et al.* (104) showed that a neuronal pathway involving activation of JNK, common to many stress responses, is essential for initial HSV1 gene expression during reactivation. Van Meter *et al.* (105) found that JNK activity (in response to oxidative stress) leads to the stimulation of DSBR *via* phosphorylation of sirtuin 6 (SIRT6), a member of the SIRT

family, on serine 10. SIRT6, which is sited in the nucleus, is needed for maintaining genomic stability.

The SIRT1 gene, like SIRT6, is linked to longevity maintenance in mammals. Phosphorylation by JNK stimulates its activity (105). The stress sensors AMPK and Sirt1 are involved in neuronal survival and neuroprotection. Otth *et al.* (106) proposed that HSV1 could activate the AMPK/Sirt1 axis as a strategy for establishing latency through inhibition of apoptosis and restoration of energy status. They demonstrated that HSV1 modulated the AMPK/Sirt1 axis differentially during infection, interfering with proapoptotic signaling and regulating mitochondrial biogenesis, both of which are essential processes in the lifetime of CNS neurons. They viewed their findings as supporting a role of HSV1 in neurodegeneration and, specifically, in AD.

Lees-Miller *et al.* (107) and Parkinson *et al.* (108) found that HSV1 targets the DNA-PKcs component of DNA-PK for proteasomal degradation in epithelial cells. De Chiara *et al.* (101) detected SSBs and DSBs in HSV1-infected neurons and further found that HSV1 decreases the expression of the Ku80 component involved in NHEJ, the usual pathway for DSBR. The researchers suggest from their data that HSV1 promotes the proteasomal degradation of Ku80, which, by impairing NHEJ activity, causes DSB accumulation. The data support the concept that repeated reactivation of HSV1 can cause cumulative neurotoxicity and neurodegeneration, possibly *via* the virus-induced damage and misrepair of DNA.

Interestingly, Kanungo (109) has pointed out that in AD brains, NHEJ activity and levels of DNA-PKs and the Ku protein are lower than in age-matched controls. However, normal aging brains also show reduced DNA-PKs and Ku levels, weakening the hypothesis of a direct link between NHEJ activity and AD and suggesting that there must be additional factors in AD pathogenesis. The researcher also proposed that the deficiency of Ku80, as a somatostatin receptor, can disrupt somatostatin signaling, thereby inducing A β generation, which can then potentiate DNA-PKcs degradation with consequent loss of NHEJ activity and prevention of DSBR; activation of these two different pathways, culminating in genome instability, might explain the differences in outcome between AD and normal aging.

To summarize, despite extensive studies on DNA damage and repair in general and some analyses of the effects of HSV1 in general, only a single study (102) has attempted to link these aspects. Clearly, much more work needs to be done to determine if HSV1-induced damage in DNA does indeed play a role in the development of AD.

IFN: IMMUNE ANTIVIRAL ACTIVITY AND RELATION TO HSV1 AND AD

IFNs are cytokines that inhibit viral infections, including those caused by HSV1, and stimulate the immune system, activating, in particular, T cells modulating neuroinflammatory pathways. There is a precedent for linking IFN antiviral action to AD. Grimaldi *et al.* (110) treated 23 mild-to-moderate patients with AD with 22 μ g subcutaneous

injection of IFN- β 1a, 3 times per week for 28 wk, followed by 24 wk of observation, and compared the effects with those of 19 patients who received placebo. IFN- β 1a was well tolerated, with only rare and mainly mild adverse events. A nonstatistically significant reduction in progression of cognition during follow-up occurred in the treated patients. However, there were significant improvements in the Instrumental Activities of Daily Living and Physical Self-Maintenance Scale. IFN is known to cause a transient mood decline during the first months of treatment. Surprisingly, the placebo was found to have an antidepressant effect, which might have counterbalanced an IFN-induced reduction in rate of cognitive decline. The researchers considered an antiviral action of IFN, but attributed its beneficial effect on their patients with AD to its immunomodulatory and anti-proliferative properties. They suggested that as IFN- β 1a appeared to be safe and well tolerated in patients with early AD, its possible beneficial role should be further investigated in larger studies.

Viral infection, IFN, and AD also have been linked in a review that presented strong evidence for a role of neuroinflammation in both the onset and progression of AD (*inter alia*), although the underlying initiating mechanisms are unclear (111). The researchers proposed that environmental factors are probably involved, possibly *via* dysregulation of the antiviral response. They point out that many of the mutations linked to neurologic conditions occur in genes related to the antiviral response, such as sterile α motif/histidine-aspartate-domain-containing protein 1 (SAMHD1), which regulates cellular deoxyribonucleotide triphosphate levels and has been shown also to restrict reverse transcription of HIV. The mutations can cause gain or loss of function, and although the gene products may have potent antiviral activity, they might be harmful if not appropriately regulated, and so, their expression is tightly controlled. The type I IFN response is the primary pathway stimulated on sensing viral pathogen-associated molecular patterns. The IFNs subsequently stimulate production of cytokines, chemokines, and antiviral factors, which, although protective in early immunologic responses, might cause extensive damage if unregulated. Microglia, although normally protective, can cause excessive neuroinflammation if overactivated, perhaps contributing to the development of neurodegenerative diseases. The researchers conclude that as only a subset of type I interferonopathies is associated with neurologic symptoms, neuroinflammation alone might be insufficient to trigger disease and that environmental triggers/risk factors might be required for initiation. They urge a more thorough investigation into the role of viral infection as triggers or amplifiers of disease, attributing direct viral effects to a variety of possible mechanisms (*e.g.*, protein misfolding, ROS induction, stress granule formation, or generating pathologic products), or indirectly, by triggering or exacerbating disease-promoting inflammation.

IFN action has been studied in HSV1-infected TG cells in cultures treated with enriched IFN supernatant from IFN-transfected cells (112). IFN- β was found to be more

effective in inhibiting HSV1 lytic gene expression than IFN- α . The antiviral efficacy of IFN- β was dose-dependent and correlated with induction of the IFN-inducible, antiviral genes, 2'-5' oligoadenylate synthetase and double-stranded RNA-dependent PK. A later study (113) investigated the potential efficacy of IFNs in blocking reactivation of latent HSV1. On transducing HSV1-infected TG cells in explant culture under conditions stimulating viral reactivation with IFN- β or IFN- γ , transgene expression in both cases delayed and suppressed HSV1 reactivation. In HSV1-infected animals, in which reactivation was induced in the eye by UV irradiation, and the corneas were transduced with both types of IFN, only IFN- γ delayed and reduced HSV1 reactivation. However, a number of other studies (114–116) have shown that IFN- β and IFN- γ act synergistically in combating HSV1 replication in cell culture and in immunocompetent mice.

The clinical trial described above (110) used IFN to treat AD, with intriguing results possibly implicating its antiviral action. Therefore, there is a case for its use in future trials, perhaps in combination with the obvious choice of drug, ACV, or its biodrug, valacyclovir (which is much better absorbed in the body), as ACV is highly effective in treating HSV1 infections, including HSE (117, 118). By suppressing HSV1 replication, ACV would reduce the extent of direct viral damage and the accompanying inflammation during its putative reactivation in the brain. However, ACV needs to be activated by the HSV1 thymidine kinase, so if ACV-resistant thymidine kinase mutants occur, then these can cause serious illness in immunocompromised subjects. Fortunately, the combined use of IFN- β with IFN- γ was shown to be highly effective in reducing mutant virus replication in cell cultures and *in vivo* in mice [Huang *et al.* (119)]. The researchers suggested that for therapy, frequent IFN treatment or IFN together with anti-HSV drugs, such as foscarnet, which target viral DNA polymerase, could be used.

CONCLUSIONS

The proposal that a microbe is a cause of a chronic disease has long been regarded with suspicion, even when supported by overwhelming evidence. Perhaps this is partly because microbes were thought to cause only acute diseases, but also because a few decades ago, PCR was used for microbe detection in certain chronic diseases, and the results were considered unreliable because of the technique's proneness to artifacts; furthermore, control subjects were also sometimes found to harbor the microbe (as is the case with HSV1 and AD). Yet, even Koch (120) realized eventually that his postulates did not apply to everyone who was infected and that some people could be infected, but were asymptomatic and so were seen as controls. That highlights the importance of understanding that microbial presence in controls does not preclude a causal role for the microbe in the disease in question.

The topics discussed above and in previous reviews by the author (and in the ~30 reviews by other researchers on

microbes and AD) should help to elucidate the role of HSV1 in the development of AD. However, many questions remain: what damage, if any, occurs in human brain during latency? How can the virus or any of its components be detected in the brain in life, during latency, as well as during reactivation, and during any future antiviral therapy? Are several types of microbe (including not just HSV1 but also other herpes viruses) involved in AD, and do those cases of AD that are caused by microbes result from a single agent or from more than one? And with the view of the importance of the gut microbiota, is the gut involved in AD, specifically, viruses in the gut that are so under-represented in the current plethora of studies on gut microbes? A role for gut microbes seems especially intriguing now because of recent work (121) that supports early studies, suggesting that a gut bacterium is involved in Parkinson's disease. And how does another recent study (122) that suggests that site-specific P-tau inhibits A β toxicity relate to the antiviral action of A β (123–125)? What are the roles of A β and P-tau, especially considering the proposal by Braak and Del Tredici (126), from anatomic considerations, that extracellular and aggregated A β might be produced by abnormal P-tau, the latter caused by a pathogen that, they suggest, is HSV1? One can only hope that funding will, at last, become available for research to answer these and other questions and to set up clinical trials of antimicrobial agents.

NOTE ADDED IN PROOFS

The relevance of HSV1 effects in brain to the development of AD is supported further by current studies on APOE- ϵ 4 transgenic mice, which show marked behavioral and pathological changes in HSV1-infected animals (R. L. Thompson, University of Cincinnati College of Medicine, Cincinnati, OH, USA, and M. T. Williams and N. M. Sawtell, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA; personal communication, May 13, 2017). F

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