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Intracerebral propagation of Alzheimer's disease: strengthening evidence of a herpes simplex virus etiology

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Abstract

Background—A faulty human protein, abnormally phosphorylated tau, was recently publicized to spread "***like a virus***" from neuron to neuron in Alzheimer patients' brains. For several decades, we have been amassing arguments showing that herpes simplex virus type 1 (HSV-1), not p-tau, propagates this inter-neuronal, trans-synaptic pathological cascade.

Methods—We reiterate convincing data from our own (and other) laboratories, reviewing the first anatomic foothold neurofibrillary tangles gain in brainstem and/or entorhinal cortex; the chronic immunosurveillance cellularity of the trigeminal ganglia wherein HSV-1 awakens from latency to reactivate; the inabilities of p-tau protein's physical properties to promote it to jump synapses; the amino-acid homology between human p-tau and VP22, a key target for phosphorylation by HSV serine/threonine-protein kinase UL13; and the exosomic secretion of HSV-1-infected cells' L-particles, attesting to the cell-to-cell passage of microRNAs of herpes viruses.

Results—The now-maturing construct that reactivated HSV-1 best accounts for the intracerebral propagation of AD changes in the human brain should at last seem highly attractive. This hypothesis might even explain statins' apparent mechanism in some studies for lowering AD incidence.

Conclusion—Provided that funding agencies will quickly ignite a new realm of investigation, the rejuvenated enthusiasm for testing this optimistic construct holds incalculable potential for rapid, efficacious clinical application, through already available and relatively safe anti-viral therapeutics.

1. Introduction

The recent report and publication that Alzheimer's disease "… seems to spread ***like a viral infection*** from brain cell to brain cell" has prompted us to restate compelling evidence

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garnered for several years that in many patients, the agent triggering human Alzheimer's disease (AD) is herpes simplex virus type 1 (HSV-1).[1,2] While the mice studied in the reported experiment were assumed to be free of infectious agents, the potential confounding effects of endogenous betaherpesviruses and gammaherpesviruses seen in Mus musculus cannot be fully discounted.[3]

2. Neuropathological evidence

The earliest suggestion that HSV-1 was an appealing trigger in AD pathogenesis originated in Ball's published hypothesis that the limbic predilection for the neuropathological lesions in the human brain likely indicated a reactivation of latent HSV-1 as a cause of AD.[4] The concept that reactivation of latent HSV, travelling centripetally from trigeminal ganglia into neighboring mesial temporal cortex (rather than centrifugally to evoke “cold sores” in distal nerve endings) would best explain the earliest predilection of entorhinal and hippocampal neurons to neurofibrillary tangle formation,[5] was more forcefully underscored, when an in situ hybridization study by Deatly et al from Ashley Haase's laboratory was published.[6]

In this study Deatly et al.[6] used in situ hybridization and detected latency-associated transcripts (LAT) in the human trigeminal ganglia (TG). The Stevens lab had also conducted this sort of study.[7] Deatly et al.,[6] using in situ hybridization to detect LAT, reported they could not find LAT by this technique in their human CNS samples. However, since that time very sensitive quantitative RT-PCR has reported quantification of LAT in numerous human and animal tissues latent with HSV-1. For example, the laboratory of Straus has characterized the HSV LAT 2 in human sacral ganglia.[8] In unpublished studies, LAT has also been detected by Hill et al., from human TG and brain. Also, we have unpublished results where we have shown HSV-1 DNA in 67 out of 70 human brains, all from confirmatory autopsies of clinically diagnosed Alzheimer's patients. We are aware of no reports which were unable to detect LAT transcripts by sensitive RT-PCR in any neural tissue latent with HSV.

Earlier, with multivariate analysis and statistical rank ordering of morphometric data from 1,328,743 microscopic fields in brains of 45 demented patients and 12 age-matched controls, Fewster et al in Ball's laboratory clearly suspected a “…gradual spread of lesions favoring a neuron-to-neuron dissemination of an etiological trigger, such as a reactivated latent viral agent” more than 20 years ago.[9]

In their exciting new review of AD pathogenesis, Braak and Del Tredici profferevidence that the earliest “pre-tangle” abnormality (AT8-immunoreactive abnormal tau aggregates) may actually appear earliest in locus coeruleus nerve cells of the brain stem, passing from there to the trans-entorhinal region before more widespread dissemination.[10] Regrettably, however, they then posit some form of trans-synaptic transport of tau protein aggregates, a hypothetical mechanism which we avidly dispute below. Even before afflicting entorhinal/hippocampal nerve cells, why might HSV-1 grab a very first foothold in locus coeruleus and other pontine tegmental projection neurons?[11] Braak's group does not consider viral agents, speculating merely about some “… ***tauopathy, possibly beginning in childhood.***” But anatomic knowledge of the trigeminal nerve's root entry zone recalls that its very short centripetal ganglionic branches first reach the sensory, motor, and mesencephalic nuclei of the Vth cranial nerve, the neighboring locus coeruleus, and the pontine raphe nuclei. Since virtually from birth more than 90% of all North American adults harbor latent HSV-1 in our trigeminal ganglia, some bizarre “childhood tauopathy” is not as appealing as a viral reactivation and propagation of this latent HSV-1.

The approximately 80,000 nerve cells in each Gasserian ganglion are surrounded from birth by vast numbers of chronic inflammatory, immunosurveillance cells, especially lymphocytes.[12] Should such immunoprotection fail later in life, perhaps the reactivating HSV-1 can then creep inexorably along those trigeminal branches, entering the lower brainstem, and thence into entorhinal and eventually many more distant cortical nerve cells.

Nay-sayers to the "herpes reactivation" hypothesis have been misled by earlier observations of lack of difference in anti-HSV antibodies in AD patients versus age-matched controls. At last, however, Letenneur et al. have clarified this conundrum.[13] In the sera of 512 elderly people initially free of dementia and followed for 14 years, they measured both anti-HSV immunoglobulin G (IgG), an indicator of any lifelong infection to HSV, but most importantly also the anti-HSV immunoglobulin M (IgM), an indicator of primary infection or of ***reactivation of the virus***. Cox proportional hazard models showed that, when controlled for age, gender, educational level, and ApoE e4 status, although there was no significantly increased risk found in the 424 IgG-positive subjects, the 43 individuals who were IgM-positive had a much higher risk of developing Alzheimer's disease (HR=2.55; 95% CI). This heightened risk seems specific for AD, since the hazard ratios for other dementias were not significant. Since IgM antibodies are present in the blood for only a limited time period (the IgM response tends to decline within about 1 to 2 months, although in HSV encephalitis it may persist for 56 to 328 days), they conclude that the HSV reactivation in this group was recent. They believe they identified subjects involved in a "***chronic infection to regularly reactivating HSV***" (a mechanism well accepted in another manifestation, cold sores or fever blisters). Their graph of cumulative AD rate plotted against antibodies' status reveals that the dynamics of the clinical AD incidence according to IgM status demonstrate an increase ***more than 7 years after blood sampling.*** They thus posit that these reactivations do not lead to instantaneous cognitive impairment, but perhaps weaken cerebral tissue, leading to AD only several years later. Letenneur and colleagues speculate that, as the AD pathology begins many years before frank dementia, recurrent reactivation of HSV could act as a potent stimulus to brain microglia, increasing cytokine levels, and triggering a positive feedback cycle leading to an increasing accumulation histopathologic changes.[13]

Should viral reactivation evoke intraneuronal tangles? The neurofibrillary tangles in substantia nigra nerve cells typical of **postencephalitic Parkinson disease**, clinically appearing long after the original encephalitis lethargica disorder, are generally agreed to reflect an initial viral trigger. Additionally, we have not only the well-documented co-localization of measles virus genome in the tangle-laden nerve cells of **subacute sclerosing panencephalitis**,[14] but in a serendipitous display of how a smoldering viral inflammation might evoke tangle formation, Ball has published photomicrographs from the brain of an 87-year-old man after 14 years of progressive dementia, demonstrating one microglial shrub or nodule engulfing a dying locus coeruleus nerve cell, and a second, neighboring coerulean neuron containing a typical tangle while simultaneously undergoing identical microglial "neuronophagia."[15] These microglial nodules and neuronophagia are strongly accepted as neuropathologic markers of cerebral viral infection, such as by herpes simplex.

A recent landmark publication from Minneapolis offers remarkably cogent observations of anatomic and functional deficits induced in mice followed through the acute into the chronic phase of herpes simplex encephalitis (HSE).[16] As Cheeran and colleagues remind us, HSV-1 viral brain infection occurs either as a primary infection, commonly in neonates, or due to reactivation of latent virus, in immunocompetent adults. The long-term sequelae in adults are manifested as anterograde memory loss, anosmia (loss of smell), and dysphasia (language loss). But little is known about the precise pathogenesis of the long-term neurological outcomes after such HSE. The long-term damage, both functional and

anatomic, in humans is confined to the limbic system, including particularly the hippocampus. Based on the lesions' locations, it is postulated that the virus enters the CNS either through the olfactory bulb and/or the trigeminal nerves, possibly after a reactivation event in the trigeminal ganglion. Murine models have shown more demonstrable evidence that both olfactory and trigeminal nerve routes are likely conduits into the CNS. However, until their publication, it was not known whether experimental infection would eventuate in "chronic neuropathology."

In BALB/c mice intranasally administered the HSV-1 strain 17Syn+, inflammatory cells were identified as lymphocytes, neutrophils, macrophages, and plasma cells. Immune cells' identities were confirmed immunohistochemically with antibodies to CD3 (T lymphocytes), myeloperoxidase (neutrophils), and Mac2 (macrophages). The distribution of HSV-1 antigen was assessed throughout the brain sections, identifying unequivocally immunopositive cells. The sequential spread of acute to then chronic histopathologic changes was documented at 2 days post-inoculation, 5, 14, 30, and 60 days p.i. The Morris water maze test designed to measure spatial memory function showed poor performance in all animals which survived to 30 days p.i., indicating a severe deficit in spatial memory. This behavioral observation accords very well with the often first-noticed spatial disorientation of the early-stage Alzheimer patient, who notices trouble finding his/her way home, or the car's location in the parking lot.

From the focal chronic lesions detected as the virus progresses through different associated routes, Amrien et al. posit they are "localized along neural circuits that can be traced back to their source at the olfactory…and trigeminal nerve endings."[16] The presence of ***activated microglia*** appears sustained in damaged areas during the chronic phase. These cells immunostain intensely with Mac2, a marker known to be upregulated in macrophages involved in phagocytosis. Amrien and colleagues conclude that both in their animal models and in human patients surviving HSE, the neuropathologic changes correspond to the mesial temporal cortex and hippocampus, and are associated with long-term ("smoldering") inflammation.[16]

3. Propagation of abnormal tau highly unlikely

It is most difficult for us to conceive of how the tau protein could possibly spread in a paracrine or cell-to-cell fashion. We envision any efficient propagating factor passing trans-synaptically between nerve cells as a molecule shaped much more like a bullet or other highly compacted particle (e.g., such as the HSV-1), rather than as some long, flexible bundle resembling cooked spaghetti (see partial 3-dimensional structure of a tau protein interaction region with PIN1 (PDB 118H) at http://www.rcsb.org/pdb/explore/explore.do?pdbId=1I8H).

The microtubule-associated tau protein (MAPT; constituting a family of six major isoforms from 352 to 441 amino acids in size; the monomer has a molecular weight of 43–67 KDa, with 79 potential serine and threonine phosphorylation sites on the longest tau isoform), which accumulates as intensively hyper-phosphorylated isoforms in AD brain, is almost certainly too large, too extended ("non-compact") in conformation, too highly charged, and too biologically reactive to exist as a single "spreading" or synapse-crossing entity, either inside or outside of the neuron.[17] Indeed the list of tau's high affinity interactions, and aggregate-ability with itself and with other cytoskeletal- and neuronal-associated components such as α-synuclein and S100β protein is extensively documented.[18,19]

4. Viral kinase best explains tau phosphorylation

We dispute the assumption that the only cause of hyperphosphorylated tau observed in AD is a human kinase, and contend that an HSV kinase is implicated in the creation of p-tau. We hold that the phosphorylation of tau by a human kinase does not preclude the occurrence of a similar phosphorylation by a viral kinase. Our contention involving an HSV kinase is based upon evidence of other human proteins that are observed to be phosphorylated by HSV kinases, and data that show human kinases can phosphorylate HSV proteins, illustrating a cross-species interchange of enzyme/substrate interactions which should be considered in pathobiology. Indeed, we provide specific evidence that a HSV kinase is implicated in the creation of p-tau. Recent data from Itzhaki's laboratory show that anti-HSV-1 agents in vitro reduce the p-tau accumulation induced by HSV-1, and that p-tau accumulation is found to depend on HSV-1 DNA replication.[20] A second group also has shown that HSV-1 infection gives rise to an increase in tau phosphorylation, and that hyperphosphorylated tau accumulates in the nerve cell nucleus, forming defined structures in HSV-1-infected neuronal cells reminiscent of the common sites of viral DNA replication.[21] While the presence of p-tau had earlier been attributed to human kinase GSK3,[22] we contend that other evidence indicates at least some of the p-tau characteristic of AD is in fact produced by a ***viral kinase***, likely HSV kinase UL13, a serine/threonine kinase similar to the human kinases identified as agents of tau phosphorylation. Our hypothesis of a viral kinase giving rise to p-tau is based on published data demonstrating cross-species kinase “promiscuity,” with human and viral kinases each shown to phosphorylate both human and viral proteins. For example, there is evidence that human casein kinase (CK2) phosphorylates both human tau and HSV VP22.[23–29] In addition, HSV kinase UL13 is the primary HSV kinase responsible for phosphorylation of the critical HSV tegument protein VP22, and this viral kinase has been observed to phosphorylate human EF1-delta, again illustrating a cross-species kinase/target interaction.[28,30]

The HSV kinase UL-13 has been observed to mimic the cellular human kinase cdc2,[31] prompting us to wonder: if viral kinase UL13 can phosphorylate a human protein such as EF1-delta, why would it not also be capable of phosphorylating human tau? In fact, a very similar phenomenon has been reported whereby the HSV kinase Us3 is observed to mimic human cellular kinase Akt and phosphorylates human tuberous sclerosis complex 2 (TSC2) on the same sites as Akt.[32]

Lastly in support of our contention that a viral kinase contributes to the occurrence of p-tau, we point to our novel observation that there is a notable amino acid homology between human tau and HSV VP22, the target of kinase UL-13. For tau and VP22, we note a 35% identity in a sequence of 63 residues and a 37% identity in a sequence of 32 residues, supporting the likelihood that a kinase which phosphorylates one will also phosphorylate the other. In fact, this cross-species phosphorylation is observed to occur when human kinase CK2 phosphorylates both human tau and HSV VP22, illustrating the functional implications of this striking homology. Likewise, a viral kinase such as UL-13, which phosphorylates HSV VP22, may also be observed to phosphorylate human tau. Hence, the above reports on cross-species phosphorylation by kinases, in combination with the sequence homology between tau and VP22, strongly suggest that HSV kinase UL13 phosphorylates human tau.

5. HSV in the CNS: an association with increased risk of AD

In addition to the numerous publications from Professor Itzhaki's laboratory showing HSV-1 DNA in human brains from England and Scotland,[33–41] numerous other investigators[42–48] that have reported HSV-1 DNA in human brains. These studies have been from Japan, Canada, Finland, Germany, and Austria. All of these have documented the presence of

HSV-1 DNA in human brains. A number of investigations have tried to link various genetic factors, age, and gender to HSV-1 DNA in humans. These reports are somewhat controversial, and in some cases are contradictory. However, there is general agreement among those who have investigated this area that a high percentage of the human population has HSV-1 DNA in the CNS.

Evidence from human and animal studies suggests a strong correlation between HSV-1 in the brain and the likelihood of AD. Human apolipoprotein allele e4, especially the homozygote e4/e4, is an accepted risk factor for the development of AD.[49–51] As noted above, greater than 90% of the adults in the USA have HSV-1 DNA in the neurons of their trigeminal ganglia.[53] Many studies have shown HSV-1 DNAwithin the human brain.[33–48,50,53,54]

One report found HSV DNA located in AD amyloid plaques.[55] The ability of HSV-1 to reactivate and cause diseases is well established.[56,57] One explication we are considering is that patients who develop AD are latent with a “high phenotypic `reactivator strain” of HSV.[58] Not all people with HSV DNA in their brain develop AD.[33] Thus, these “non-AD” subjects could have no HSV DNA in their CNS or a very low percentage of a “low phenotypic reactivator strain” of HSV. Since AD is a complex condition for which there is no perfect animal model, we have posited that risk factors for AD may well act in concert.[59] For example, one subject could be latent with a “high phenotypic reactivator” of HSV and yet despite his APOE allele status of e3/e3 (the most common allele), he could still develop AD. Numerous reviews certainly concur that the strong suggestive evidence of HSV in the human brain is a risk factor for AD.[60–62]

We have hypothesized that statins passing through the blood-brain barrier might lower the risk of AD by limiting lipid raft endocytosis and thus neuron-to-neuron spread of HSV.[63] Some clinical studies proffered evidence that statins decrease the risk of AD.[64–66] Tong et al have reported age-dependent rescue by simvastatin of Alzheimer's disease cerebrovascular and memory defect.[67,68] We are aware that many AD studies in humans, animal models, and in vitro remain controversial. It is true that one review has reported that statins do not affect cognitive decline in Alzheimer's patients.[69] However, we would point to other studies that are positive, showing statins causing a decrease in the memory loss.[67,68,70–72] The experimental designs of these negative studies could be incorrect. The model, the design, the length of time etc., all play major roles in clinical studies. We recall the mystery of the ApoE e4 allele. A few decades ago, there were publications that concluded, “the e4 allele of ApoE has no role in Alzheimer's.” With more extensive research, including meta-analysis, the majority of Alzheimer's workers agree that the e4/e4 allele can be a significantly high risk factor for AD. We forecast that future publications including large numbers of patients over a significant period of time will show specific statins do reduce memory defects.[64]

Wolozin et al. report that statins, specifically simvastatin, can be associated with reduced dementia in AD.[70–72] It is accepted that statins act by pleotropic mechanism involving two or more pathways, one of which certainly involves cholesterol. A relationship between reduced cerebrovascular accidents and memory improvement in AD is hinted at, yet the exact mechanism of how statins might act is not known. However, it is generally becoming accepted that there is a reduction in the memory loss in patients with Alzheimer's disease treated with statins. This diminished risk remains uncertain because other reports claim statins have no protective effect.[69]

In animal models, it is known that HSV DNA can progress from the initial site of ocular infection (original inoculation site) to those ganglia innervating the site of infection, and from there the virus can proceed into the CNS. We therefore hypothesize that frequent

neuronal ganglionic reactivation of HSV-1 results in transport of the virus sometimes toward the human CNS, as well as sometimes toward the periphery. Within the brain, the entorhinal cortex is considered one of the key starting points for AD histopathology ("***locus minoris resistentiae***") and is also an area in which there is an especially significant amount of HSV-1 DNA. Our related belief is that viral strains which are high phenotypic reactivators, either alone or in combination with other risk factors, exhibit viral reactivation changes in the neurons, leading to an increased spread, oxidative damage and stress, and major AD histopathology.

6. Endocytosis, microvesicles (L-particles), exosomes, and endosomes

We have previously put forth three specific interactions between HSV L-particles and beta amyloid.[73] These three hypotheses were supported by reported biomolecular changes and neuropathological changes simultaneously observed in both AD- and HSV-infected brains. Recent studies have shown a relationship between microvesicles and viral infection.[74] Microvesicles (L-particles) are secreted from HSV-infected cells and this phenomenon offers a highly plausible mechanism for microvesicular-mediated intracellular communication. A recent study with another herpes virus, Epstein-Barr (EBV) suggested that this tumorigenic virus also utilizes exosomes as a mechanism of cell-to-cell communication, through the transfer of signaling component proteins and functional microRNA.[74] Lukiw, Hill et al. in our group have shown that specific host microRNAs, for example miRNA146a, are significantly upregulated during HSV-1 infection of primary neuronal and retinal cells.[75] This in turn drives the down-regulation of complement factor H (CFH) expression, and implicates HSV-1 induced miRNA-146a in the evasion of HSV-1 from the complement system and in the induction of Alzheimer-type pro-inflammatory signaling.[75,76] Other studies with EBV have shown that miRNAs can be transported to recipient cells via small nano-sized vesicles (known as exosomes) that are of endosomal origin.[77] Unquestionably, much more study is needed on this mode of transport. However, the overwhelming evidence points extremely firmly toward a relationship between viral particles in the CNS and the spread of proteins as well as the neuron-to-neuron propagation of the characteristic neuropathological lesions in the Alzheimer's victim's CNS.

7. Conclusion and reason for optimism

Both the gross (naked-eye) and the microscopic appearance of a typical peptic ulcer of duodenum or stomach was for many years believed incorrectly to reflect a slow, indolent erosion of the gastrointestinal lining, such as would logically be evoked by a chronicity of excess digestive acids. Only after several decades of less than ideal therapeutic response to antacid treatments was it finally proven that the initial trigger for many such ulcerations is actually a bacterium (*Helicobacter pylori*), a family of micro-organisms far more commonly associated with rapid, short-term, "acute" diseases.[78]

The history of AD research efforts, since the earliest publications first describing this entity, has likewise been replete with a wide variety of unsatisfying causative hypotheses, ranging from environmental toxins, to genetic triggers such as abnormalities of chromosome 21 in autosomal dominant-inheritance families, and ApoE e4 influence on beta-amyloid pathologies. That some ubiquitous microbiological agent, instead, might play a seminal role would actually be in accord with recent evidence that beta amyloid protein has antimicrobial peptide activity, a phenomenon which we and others hypothesized in 2006 and 2010.[73,79]

Our present postulate therefore optimistically pleads here for yet another etiological construct, that of the **reactivated HSV-1**, a hypothesis which to date still cries out for a serious thrust of new, well-funded exploration. Should this pathogenetic concept then prove

robust, how quickly astute clinicians will move to trials of anti-viral agents such as acyclovir, especially in aging patient populations exhibiting or at risk for mild cognitive impairment.

All investigators in medical bioscience contribute very critical building-blocks to the frustratingly slow but requisite progress which inevitably predates any final disease break-through. We therefore are always indebted to every participant in this struggle. At the same time, however, since just as in other realms of competitive human endeavor the research of bioscience too has its fashionable “band-wagons,” we now implore the federal and other funding agencies to immediately allocate at least half of all new AD budgets to younger, smaller laboratories burning the midnight oil to find the “missing link” responsible for this enormously tragic and costly Alzheimer burden.

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A man walking through the forest sees blackened branches, fallen tree-trunks, and still-smoldering leaves. Did some “unknown mechanism” spread such havoc, or must this have been an actual forest fire?