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Herpes simplex virus type 1 and Alzheimer's disease: The autophagy connection

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The causes of Alzheimer's disease (AD) and of the characteristic pathological features—amyloid plaques and neurofibrillary tangles—of AD brain are unknown, despite the enormous resources provided over the years for their investigation. Indeed, the only generally accepted risk factors are age, Down syndrome, carriage of the type 4 allele of the apolipoprotein E gene (APOE-ε4), and possibly brain injury. Following the authors' previous studies implicating herpes simplex virus type 1 (HSV1) in brain of APOE-ε4 carriers as a major cause of AD, the authors propose here, on the basis of their and others' recent studies, that not only does HSV1 generate the main components of amyloid plaques and neurofibrillary tangles (NFTs)—β-amyloid (Aβ) and abnormally phosphorylated tau but also, by disrupting autophagy, it prevents degradation of these aberrant proteins, leading to their accumulation and deposition, and eventually to AD. *Journal of NeuroVirology* (2008) **14,** 1–4.

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Autophagy and Alzheimer's disease

The autophagy-lysosome pathway is the main intracellular process for clearance of large proteins, protein complexes, and cellular organelles (Cuervo *et al*, 2005; Martinez-Vicente *et al*, 2005). It is a mechanism that enables cells to survive when subjected to starvation, and is involved also in the degradation of microbial intruders. Autophagy (the term usually abbreviated from macroautophagy) takes place within double membrane-bounded structures called autophagosomes or initial autophagic vacuoles (AVs). These do not contain any enzymes and so the degradation of the unwanted material occurs only after fusion of autophagosome with lysosomes, which contain hydrolases.

It has recently been realised that autophagy is an essential process for maintaining homeostasis and that a decrease in efficiency of autophagy occurs during aging, resulting in the accumulation of undigested products inside lysosomes (Cuervo *et al*, 2005; Ward, 2002). A role for autophagy in several neurodegenerative diseases has been proposed also, mainly because a defect in autophagy might account for the continuous residence of aberrant proteins in the brain in these diseases and because mice in which basal autophagy in neural cells has been suppressed (by deletion of auto-phagyrelated gene [atg] 5 or 7) suffer neurodegenerative disease, with an accumulation of inclusion bodies and intracellular protein aggregates in brain (Hara *et al*, 2006; Komatsu *et al*, 2006). In the case of AD, the relevant proteins—β-amyloid (Aβ) and abnormally phosphorylated tau—are generally thought to be the major culprits in the neurodegenerative process of the disease, although their precise involvement is still unknown; in fact, they are considered by some to be protective, entombing possible damaging agents. The exact nature of the defect in autophagy or the cause of its disruption is unknown. But in Alzhemier's disease (AD) brain, autophagosomes and AVs accumulate in dystrophic neurites (Nixon *et al*, 2005) and amyloid precursor protein (APP), Aβ, and the enzymes responsible for Aβ formation are present in AVs (Yu *et al*, 2004).

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Autophagy and microbes

The autophagy system is a mechanism for dealing not only with unwanted cell proteins but also with combating microorganisms entering cells. In this case the term heterophagy, rather than autophagy, is used. Some infectious agents have devised ways of overcoming and even of using the system to their own advantage, e.g., several bacteria and viruses replicate within autophagic vacuoles (Colombo, 2005; Wileman, 2006). Herpes simplex virus type 1 (HSV1) encodes a protein—infected cell polypeptide 34.5 (ICP34.5)—that can disrupt autophagic processes (Talloczy *et al*, 2002; Talloczy *et al*, 2006), thus protecting itself against destruction. This protein is involved also in counteracting the protein kinase R (PKR) defense mechanism, which is activated by the presence of double-stranded RNA (dsRNA). Cells infected with HSV1 and with many other types of virus produce dsRNA, either because in the case of DNA viruses, including HSV1, transcription occurs from genes on both strands or in the case of RNA viruses a complementary strand is needed, or the genome is in fact double stranded. Activated PKR, which is found in AD brains, phosphorylates elongation initiation factor 2α (eIF2α), which in turn causes the cessation of protein synthesis (Cassady *et al*, 1998). Such cessation would preclude synthesis of viral proteins, initiate apoptosis (Chang *et al*, 2002), and enhance autophagy. HSV1 counteracts the PKR system by dephosphorylating eIF2α, and ICP34.5 was shown to be responsible for these effects: infection of cells with ICP34.5-deleted virus allowed activation of PKR, phosphorylation of eIF2α and protein synthesis cessation (Chou *et al*, 1995). The disruption by wild-type HSV1 of autophagy and the PKR system is yet another example of the way the virus takes over host cell processes. Other such viral effects include stopping the synthesis of most cell proteins but allowing production of cell and viral proteins required for virion formation, and causing cells to enter cycle but blocking them at the G2/M stage—which, interestingly, occurs also in AD.

HSV1-induced reduction in autophagic processes would be in keeping with the reduction in such processes occurring at least at later stages of AD. Similarly, PKR activation in AD may well be at a late stage of the disease, in reaction to stress (Peel, 2004)—stress due perhaps to infection. However, it is worth emphasising that although certain other microorganisms might cause some similar changes in the cells that they infect, HSV1 alone is known to be present—and therefore capable of action—in the normal elderly brain.

Herpes simplex virus type 1 and Alzheimer's disease

As to the way HSV1 relates to AD and to autophagy, the virus was originally proposed many years ago as a possible candidate agent in AD. This was mainly because in herpes simplex encephalitis (HSE), the virus affects the same brain regions as those most affected in AD (Ball, 1982), but also because of the high prevalence of HSV1, and its propensity for infecting neurons and ability to remain within them for the life time of the host. Much later, the first study experimentally linking virus and AD showed that HSV1 DNA is present in a latent state in brain of a high proportion of AD patients and normal elderly people (Jamieson *et al*, 1991), although in only a very low proportion of younger people (Jamieson *et al*, 1992). Several studies by others have since substantiated this in detecting HSV1 DNA in an appreciable proportion of human brains (Baringer and Pisani, 1994; Bertrand *et al*, 1993; Gordon *et al*, 1996; Itabashi *et al*, 1997; Mori *et al*, 2004; Rodriguez *et al*, 2005). In some of these cases, the proportion detected was lower than in ours, but this was due probably to a difference in prevalence of HSV1 infection in some countries (e.g., Japan [Itabashi *et al*, 1997]), or to age of subject not being taken into account. Two other studies (Hemling *et al*, 2003; Marques *et al*, 2001), however, detected HSV1 DNA in only a very low proportion of brains; the reason for this is not known.

We subsequently showed that the virus, when in brain of carriers of the apolipoprotein E gene (APOE-ε4), confers a strong risk of AD (Itzhaki *et al*, 1997; Lin *et al*, 1998). Since then, the modulatory effect of APOE on outcome of infection has been strongly substantiated by our studies showing that in several diseases of known microbial cause, including herpes labialis and hepatitis C virus–induced liver damage, APOE determines the occurrence or the severity of disease (Itzhaki *et al*, 1997; Lin *et al*, 1998; Wozniak *et al*, 2002). Subsequently we found evidence that HSV1 had replicated in the elderly brain, possibly recurrently, i.e., that it was not merely a passive passenger (Wozniak *et al*, 2005). Recently, we have linked the virus with the pathological features of AD brain: HSV1 infection causes deposition of Aβ and AD-like phosphorylation of tau and increases the enzymes responsible for their formation (Wozniak, Itzhaki, Shipley, and Dobson, submitted; Wozniak and Itzhaki, manuscript in preparation).

Several other studies strongly even if indirectly support our data: APOE influences HSV1 infection (Burgos *et al*, 2003; Burgos *et al*, 2006; Miller and Federoff, 2006); links exists between HSV1 and Aβ (Cribbs *et al*, 2000; Satpute-Krishnan *et al*, 2003); peripheral infection causes cognitive decline in elderly people and AD patients, presumably via induction of inflammation in brain (Holmes *et al*, 2003; Konsman *et al*, 2002; Strandberg *et al*, 2003)—which could reactivate latent HSV1.

Hypothesis

We propose the following scenario: HSV1 enters the brain in older age, as the immune system declines,

either from the peripheral nervous system (PNS) where, after infection earlier in life, it has resided latently, or as a new infection via the olfactory route. The virus becomes latent in the brain but reactivates periodically under conditions of immunosuppression or stress—e.g., during systemic infection. The reactivated virus then causes limited local damage, i.e., an atypical type of HSE (a number of cases of "mild" and recurrent HSE have been reported (Fodor *et al*, 1998; Klapper *et al*, 1984)). The damage occurs probably through direct viral action and also indirectly, via inflammatory/oxidative effects, thereby augmenting any such processes if already occurring, and these processes lead to deposition of Aβ and to AD-like phosphorylation of tau. Defective autophagy—a consequence of aging—is exacerbated by viral action via the ICP34.5 gene and this prevents not only degradation of the HSV1 but also degradation of the aberrant cell proteins, so that they remain as permanent residents in the brain. Subsequent deposition of other materials on the Aβ and abnormal tau leads to formation respectively of amyloid plaques and neurofibrillary tangles.

References

Ball MJ (1982). Limbic predilection in Alzheimer dementia: is reactivated herpesvirus involved? *Can J Neurol Sci* **9:** 303–306.

Baringer JR, Pisani P (1994). Herpes simplex virus genomes in human nervous system tissue analyzed by polymerase chain reaction. *Ann Neurol* **36:** 823–829.

Bertrand P, Guillaume D, Hellauer L, Dea D, Lindsay J, Kogan S, Gauthier S, Poirier J (1993). Distribution of herpes simplex virus type 1 DNA in selected areas of normal and Alzheimer's disease brains: a PCR study. *Neurodegeneration* **2:** 201–208.

Burgos JS, Ramirez C, Sastre I, Bullido MJ, Valdivieso F (2003). ApoE4 is more efficient than E3 in brain access by herpes simplex virus type 1. *Neuroreport* **14:** 1825–1827.

Burgos JS, Ramirez C, Sastre I, Valdivieso F (2006). Effect of apolipoprotein E on the cerebral load of latent herpes simplex virus type 1 DNA. *J Virol* **80:** 5383–5387.

Cassady KA, Gross M, Roizman B (1998). The second-site mutation in the herpes simplex virus recombinants lacking the gamma134.5 genes precludes shutoff of protein synthesis by blocking the phosphorylation of eIF-2alpha. *J Virol* **72:** 7005–7011.

Chang RC, Suen KC, Ma CH, Elyaman W, Ng HK, Hugon J (2002). Involvement of double-stranded RNA-dependent protein kinase and phosphorylation of eukaryotic initiation factor-2alpha in neuronal degeneration. *J Neurochem* **83:** 1215–1225.

Chou J, Chen JJ, Gross M, Roizman B (1995). Association of a M(r) 90,000 phosphoprotein with protein kinase PKR in cells exhibiting enhanced phosphorylation of translation initiation factor eIF-2 alpha and premature shutoff of protein synthesis after infection with gamma 134.5-mutants of herpes simplex virus 1. *Proc Natl Acad Sci U S A* **92:** 10516–10520.

Colombo MI (2005). Pathogens and autophagy: subverting to survive. *Cell Death Differ* **12(Suppl 2):** 1481–1483.

Cribbs DH, Azizeh BY, Cotman CW, LaFerla FM (2000). Fibril formation and neurotoxicity by a herpes simplex virus glycoprotein B fragment with homology to the Alzheimer's A beta peptide. *Biochemistry* **39:** 5988–5994.

Cuervo AM, Bergamini E, Brunk UT, Droge W, French M, Terman A (2005). Autophagy and aging: the importance of maintaining "clean" cells. *Autophagy* **1:** 131–140.

Fodor PA, Levin MJ, Weinberg A, Sandberg E, Sylman J, Tyler KL (1998). Atypical herpes simplex virus encephalitis diagnosed by PCR amplification of viral DNA from CSF. *Neurology* **51:** 554–559.

Gordon L, McQuaid S, Cosby SL (1996). Detection of herpes simplex virus types 1 and 2 and human herpes virus 6 DNA in human brain tissue by polymerase chain reaction. *Clin Diagnost Virol* **6:** 33–40.

Hara T, Nakamura K, Matsui M, Yamamoto A, Nakahara Y, Suzuki-Migishima R, Yokoyama M, Mishima K, Saito I, Okano H, Mizushima N (2006). Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice. *Nature* **441:** 885–889.

Hemling N, Roytta M, Rinne J, Pollanen P, Broberg E, Tapio V, Vahlberg T, Hukkanen V (2003). Herpesviruses in brains in Alzheimer's and Parkinson's diseases. *Ann Neurol* **54:** 267–271.

Holmes C, El-Okl M, Williams AL, Cunningham C, Wilcockson D, Perry VH (2003). Systemic infection, interleukin 1beta, and cognitive decline in Alzheimer's disease. *J Neurol Neurosurg Psychiatry* **74:** 788–789.

Itabashi S, Arai H, Matsui T, Higuchi S, Sasaki H (1997). Herpes simplex virus and risk of Alzheimer's disease. *Lancet* **349:** 1102.

Itzhaki RF, Lin WR, Shang D, Wilcock GK, Faragher B, Jamieson GA (1997). Herpes simplex virus type 1 in brain and risk of Alzheimer's disease. *Lancet* **349:** 241–244.

Jamieson GA, Maitland NJ, Wilcock GK, Craske J, Itzhaki RF (1991). Latent herpes simplex virus type 1 in normal and Alzheimer's disease brains. *J Med Virol* **33:** 224–227.

Jamieson GA, Maitland NJ, Wilcock GK, Yates CM, Itzhaki RF (1992). Herpes simplex virus type 1 DNA is present in specific regions of brain from aged people with and without senile dementia of the Alzheimer type. *J Pathol* **167:** 365–368.

Klapper PE, Cleator GM, Longson M (1984). Mild forms of herpes encephalitis. *J Neurol Neurosurg Psychiatry* **47:** 1247–1250.

Komatsu M, Waguri S, Chiba T, Murata S, Iwata J, Tanida I, Ueno T, Koike M, Uchiyama Y, Kominami E, Tanaka K (2006). Loss of autophagy in the central nervous system causes neurodegeneration in mice. *Nature* **441:** 880–884.

Konsman JP, Parnet P, Dantzer R (2002). Cytokine-induced sickness behaviour: mechanisms and implications. *Trends Neurosci* **25:** 154–159.

Lin WR, Graham J, MacGowan SM, Wilcock GK, Itzhaki RF (1998). Alzheimer's disease, herpes virus in brain, apolipoprotein E4 and herpes labialis. *Alzheimer's Rep* **1:** 173–178.

Marques AR, Straus SE, Fahle G, Weir S, Csako G, Fischer SH (2001). Lack of association between HSV-1 DNA in the brain, Alzheimer's disease and apolipoprotein E4. *J NeuroVirol* **7:** 82–83.

Martinez-Vicente M, Sovak G, Cuervo AM (2005). Protein degradation and aging. *Exp Gerontol* **40:** 622–633.

Miller RM, Federoff HJ (2008). Isoform-specific effects of ApoE on HSV immediate early gene expression and establishment of latency. *Neurobiol Aging* **29:** 71–77.

Mori I, Kimura Y, Naiki H, Matsubara R, Takeuchi T, Yokochi T, Nishiyama Y (2004). Reactivation of HSV-1 in the brain of patients with familial Alzheimer's disease. *J Med Virol* **73:** 605–611.

Nixon RA, Wegiel J, Kumar A, Yu WH, Peterhoff C, Cataldo A, Cuervo AM (2005). Extensive involvement of autophagy in Alzheimer disease: an immuno-electron microscopy study. *J Neuropathol Exp Neurol* **64:** 113–122.

Peel AL (2004). PKR activation in neurodegenerative disease. *J Neuropathol Exp Neurol* **63:** 97–105.

Rodriguez JD, Royall D, Daum LT, Kagan-Hallet K, Chambers JP (2005). Amplification of herpes simplex type 1 and human herpes type 5 viral DNA from formalin-fixed Alzheimer brain tissue. *Neurosci Lett* **390:** 37–41.

Satpute-Krishnan P, DeGiorgis JA, Bearer EL (2003). Fast anterograde transport of herpes simplex virus: role for the amyloid precursor protein of Alzheimer's disease. *Aging Cell* **2:** 305–318

Strandberg TE, Pitkala KH, Linnavuori KH, Tilvis RS (2003). Impact of viral and bacterial burden on cognitive impairment in elderly persons with cardiovascular diseases. *Stroke* **34:** 2126–2131.

Talloczy Z, Jiang W, Virgin HW, Leib DA, Scheuner D, Kaufman RJ, Eskelinen EL, Levine B (2002). Regulation of starvation- and virus-induced autophagy by the eIF2alpha kinase signaling pathway. *Proc Natl Acad Sci U S A* **99:** 190–195.

Talloczy Z, Virgin HW, Levine B (2006). PKR-dependent autophagic degradation of herpes simplex virus type 1. *Autophagy* **2:** 24–29.

Ward WF (2002). Protein degradation in the aging organism. *Prog Mol Subcell Biol* **29:** 35–42.

Wileman T (2006). Aggresomes and autophagy generate sites for virus replication. *Science* **312:** 875–878.

Wozniak MA, Itzhaki RF, Faragher EB, James MW, Ryder SD, Irving WL (2002). Apolipoprotein Eepsilon 4 protects against severe liver disease caused by hepatitis C virus. *Hepatology* **36:** 456–463.

Wozniak, Itzhaki, Shipley, Dobson (2007). Herpes simplex virus infection causes cellular beta amyloid accumulation and secretase upregulation, is now published: *Neurosci Lett* **429:** 95–100.

Wozniak MA, Shipley SJ, Combrinck M, Wilcock GK, Itzhaki RF (2005). Productive herpes simplex virus in brain of elderly normal subjects and Alzheimer's disease patients. *J Med Virol* **75:** 300–306.

Yu WH, Kumar A, Peterhoff C, Shapiro Kulnane L, Uchiyama Y, Lamb BT, Cuervo AM, Nixon RA (2004). Autophagic vacuoles are enriched in amyloid precursor protein-secretase activities: implications for beta-amyloid peptide over-production and localization in Alzheimer's disease. *Int J Biochem Cell Biol* **36:** 2531–2540.