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Perspectives on herpes-APP interactions

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We recently reported a physical association between the herpes simplex virus type 1 (HSV) that causes the common cold sore and the amyloid precursor protein (APP) whose proteolytic fragment, Ab, is the major component of senile plaques in Alzheimer's disease (Satpute-Krishnan, DeGiorgis and Bearer 2003). This physical association raises many new questions concerning a role for HSV infection in central nervous system function, including whether infection affects the risk of Alzheimer's disease or promotes other types of dementias.

Herpes simplex virus type 1 (HSV) infects the epithelial cells of the mucous membranes of the face where it secondarily infects sensory nerve terminals. Once within the neuron, HSV travels back to the neuronal cell body located in the trigeminal ganglion, which lies at the base of the skull adjacent to the brain stem. HSV remains in the ganglion in a latent form, until reactivation when newly synthesized virus is transported back to the mucous membrane. Until our study (Satpute-Krishnan *et al.*, 2003), the molecular mechanisms of this transport were unknown. In fact, the mechanism by which any intracellular particle, be it endogenous organelle or exogenous microbe, obtains motors for transport is a current major area of investigation in cell biology, as the large number of recent reviews and reports on this topic demonstrates.

We began our investigation with the goal of identifying the general mechanism by which a particle obtains a motor, using HSV as a tool to uncover universal paradigms governing this process. The identification of amyloid precursor protein (APP) as the putative motor receptor for HSV, and the implications of this for Alzheimer's disease (AD), was a surprise – an unexpected result, a 'discovery'. Our experiments began by demonstrating that HSV undergoes fast axonal transport when injected into the giant axon of the squid (Satpute-Krishnan *et al.*, 2003). The squid axon allowed us to develop a novel functional assay that tests any exogenous particle, in this case HSV, for its ability to obtain motors and be transported. We had previously used this assay to demonstrate that removal of the viral envelope mimicked infection and produced HSV capable of being transported in the retrograde direction towards the neuronal cell body upon injection into the squid axon (Bearer *et al.*, 2000). These initial results revealed the highly conserved nature of the transport process, which supported our idea that experiments using a human virus in a squid axon would produce universally applicable paradigms.

To discover the molecular basis of this activity, we undertook a 'candidate gene' approach. APP was selected based on work from the Goldstein laboratory that was published just after our first experiments had successfully reconstituted HSV motility in the giant axon. It had long been known that APP, a transmembrane glycoprotein, was transported to the cell surface and, in neurons, to synaptic terminals (Koo *et al.*, 1990). Goldstein's work showed

that APP (1) bound the microtubule motor, kinesin, through its light chains (Kamal *et al.*, 2000), (2) was associated with transport vesicles (Kamal *et al.*, 2000, 2001) and (3) produced similar phenotypes to kinesin when disrupted in *Drosophila* (Gunawardena & Goldstein, 2001).

Our biochemical and morphological analyses of HSV revealed that motile virus was physically associated with large amounts of cellular APP. Thus we now believe that APP plays a role in the anterograde transport of HSV from its site of synthesis in the cell body to distant neuronal termini. Whether this role is direct or indirect remains to be tested in a functional assay, and we are in process of doing this (P. Satpute-Krishnan *et al.*, in preparation).

That HSV associates to such a degree with APP has raised numerous and disturbing questions. APP is a known risk factor for AD as the parent protein of the toxic A β fragments thought by some to be the primary factor causing synaptic dysfunction and neurodegeneration. Once we discovered this association, we were excited to find reports on epidemiological correlates between the presence of HSV DNA in the central nervous system (CNS) and AD, particularly those by Itzhaki and co-workers (Itzhaki *et al.*, 1997, 2001; Dobson & Itzhaki, 1999; Grant *et al.*, 2002). However, there is a wide gap between these epidemiological correlations and the molecular interactions between the virus and APP that we discovered. The accompanying correspondence by Itzhaki *et al.* raises a number of intriguing questions that should prompt many new and significant studies.

One key question is whether HSV enters the CNS via transport within neurons from the trigeminal pathway. This is certainly possible. The trigeminal ganglion contains bipolar neurons, each of which extends processes both to the periphery and into the CNS to synapse in the midbrain (the principal sensory trigeminal nucleus and spinal trigeminal nucleus) or in the amygdala (Fig. 1) (Jasmin *et al.*, 1997).

Other mechanisms of viral entry have also been proposed, including spread through the meninges or haematological dissemination during the viraemia of the initial infection (Burgos *et al.*, 2002). For the latter, a role for one of the lipoproteins that transports lipid in serum, ApoE, has been proposed. The ApoE4 allele is known to be associated with AD (Holtzman *et al.*, 2000; Mahley & Rall, 2000). Itzhaki and coworkers have shown an increased risk in patients with both the ApoE4 allele and HSV infection in a small cohort of cases (Itzhaki *et al.*, 1997; Dobson & Itzhaki, 1999).

These correlations raise another key question: what role does ApoE play in HSV susceptibility and consequent pathogenicity? In addition to its potential role in haematogenous dissemination of HSV, ApoE might be involved in the transmission of virus within the nervous system by promoting viral passage across the synapse. ApoE is a component of senile plaques and localizes at synapses in the CNS. It is endocytosed by LPR1, a lipoprotein receptor that also binds the APP-secreted fragment (Kounnas *et al.*, 1995; Pietrzik *et al.*, 2002). One unexplored possibility is that this endocytotic event might mediate HSV transmission between neurons, possibly at the synapse. There are no current data on whether HSV crosses synapses, and if so what receptors receive the virus and allow entry, nor do we know if the virus has any direct effect on synaptic transmission.

Finally, most of what we know about HSV comes from its behaviour in the peripheral nervous system. We currently assume that HSV behaviour in the CNS is similar, but this remains unproven. Variables that will be important to consider when attempting to link HSV infection with age of onset, rate of progression, and type and severity of cognitive dysfunction include amount and location of HSV in the CNS, viral strain, age of infection and numbers of latency-eruption cycles. A recent report from Finland did not find

correlation between HSV infection and AD (Hemling *et al.*, 2003), suggesting that other variables in the affected population may also contribute to Alzheimer's risk. The correspondence by Itzhaki *et al.* raises the intriguing idea that HSV synthesis may continue at low levels even during latency periods. In fact, several studies show that HSV is shed in saliva in a small number of infected people (1–5%) without causing symptoms, i.e. an ulcer (Hatherley *et al.*, 1980; Okinaga, 2000). If a constant low level of viral production occurs asymptotically in the brain, chronic neuronal damage could ensue.

One question is the source of the APP in our study. We used HSV synthesized in infected Vero cells, a monkey kidney epithelial cell line. Vero cells express all three of the APP homologues, APP, APLP1 and APLP2 (Lo *et al.*, 1995), each of which contains the first seven amino acids of the 15 amino acid epitope from the C-terminus of APP recognized by our antibody, GTENPTYKFFEQMQN. Thus, our study may not distinguish between these homologues and it is possible that in Vero cells HSV associates with all three isoforms. APLP1 and APLP2 are also expressed in neurons and localize with APP in senile plaques (Crain *et al.*, 1996; Galvan *et al.*, 2002). Although cleavage of APLP1 and APLP2 does not produce the toxic A β fragment, the proteolysis of these two isoforms is neurotoxic (Galvan *et al.*, 2002). Thus HSV would be predicted to damage neurons via APP regardless of which isoform it binds. Because we do not yet know the specific chemical basis of the interaction between APP and HSV, we cannot determine how specific this interaction is for one or the other isoform.

Could the virus itself encode an APP-like protein that is recognized by our anti-APP peptide antibody? Indeed, the viral envelope glycoprotein, gB, has some homology to the N-terminus of AP: 33% identity, 64% similarity in a 42-amino-acid sequence from aa 1 to 42 in APP (Cribbs *et al.*, 2000). However, a Blast search of the NCBI database for viruses [ORGN] with the C-terminal peptide sequence of APP yielded 106 hits but no matches to HSV proteins even though all are expected in the database (search performed January 2004 by M. Conley and E. L. Bearer, unpublished data). Furthermore, our data show that the size of the C-APP antigen detected in Western blots is ~120 kDa, consistent with the size of cellular APP and not with the smaller viral gB (55 kDa) (Satpute-Krishnan *et al.*, 2003). These results demonstrate that the APP antigen associated with HSV harvested from Vero cells derives from the host cell.

HSV is among the largest human viruses known, with a genome of ~ 152 kb encoding 87 proteins in 89 reading frames. These proteins interact and interfere with host cell machinery in numerous ways – transcriptional regulation, phosphorylation and replication have been the most studied. Recent data suggest interactions of the viral protein, VP22, with cellular proteins involved in RNA trafficking and processing (Sciortino *et al.*, 2002). Our data indicate interactions of HSV with host cell membrane trafficking and transport factors including motors and their receptors (Satpute-Krishnan *et al.*, 2003). These many activities of viral proteins combine to produce a cytotoxic effect on epithelial cells. In neurons, HSV infection seems to be less immediately toxic, possibly in part because of the HSV LAT gene product associated with viral latency and, selectively expressed in neurons, which inhibits apoptosis (Bloom, 2004). Should HSV cause defects in transport within neurons, which our data suggest, this alone could account for synaptic dysfunction (Bayer *et al.*, 2001; Higuchi *et al.*, 2002; Koo, 2002; Morfini *et al.*, 2002). The additional toxic effect of a viral infection together with repeated rounds of viral synthesis and transport could cause multiple functional defects in an infected neuron that cumulatively result in synaptic failure and consequent cognitive impairment. To determine how these many variables might induce or exacerbate AD is the next challenge.

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Fig. 1.

Hypothetical pathway of HSV transport to the brain via bipolar neurons in the trigeminal ganglion. HSV type 1 enters the sensory nerve terminals in the mucous membranes of the face by fusing viral envelope with the neuronal surface membrane. The viral nucleocapsid and tegument is then transported within the neuron back to its cell body in the trigeminal ganglion adjacent to the brain stem where it enters latency or is replicated. After replication in the neuronal nucleus (N), viral particles recruit cellular membranes containing APP, probably during packaging in the Golgi. The sensory neurons of the trigeminal nerve are bipolar, extending a single process that reaches both outwards to the face and inwards to the brain. Thus, newly synthesized virus emerging from the bipolar cell body could be transported either outwards to the face or inwards to enter the brain. In the brain, infective virus may bud from the neuron at the synapse where it has access across the cleft to the post-synaptic neuron. APP, released from virus, could then be hydrolysed and deposit in the synaptic cleft as amyloid.