

# Herpes Simplex Virus Type 1 in Alzheimer's Disease: The Enemy Within

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**Abstract.** Alzheimer's disease is a modern scourge and is likely to become increasingly so in the future, with increasing longevity. The disease has been investigated for over one hundred years yet its causes and that of the neuropathological characteristics seen in AD brain are still completely unknown. Evidence for a major causative role of a common virus, herpes simplex virus type 1 (HSV1), acting in combination with a genetic factor – the type 4 allele of the apolipoprotein gene, a known susceptibility factor – is presented here. The characteristics of the virus, some of which make it an especially likely candidate for this role, are described, as are the many precedents for the action of a genetic factor modulating outcome of infection. Various possible ways in which HSV1 might lead to development of AD, such as its up-regulation of various enzymes and in particular certain kinases, its effect on the cell cycle, on autophagy, and its inflammatory and oxidative effects are also discussed. It is concluded that there is strong evidence that the virus is indeed a major factor in AD and therefore there is a strong case for appropriate treatment, and possibly for prevention in the future.

**Keywords:** Abnormal tau phosphorylation, Alzheimer's disease, amyloid- $\beta$ , apolipoprotein E, autophagy, cell cycle, cholesterol, herpes simplex virus type 1, inflammation, oxidation

Alzheimer's disease (AD), like cardiovascular disease, is generally an affliction of the elderly. However, unlike the latter – in which several major risk factors have been discovered, such as smoking, hypertension, and obesity – few risk factors and certainly no causes of AD are known at present, despite the vast amount of research and vast sums expended, especially on the main neuropathological features that are thought to be central to disease pathogenesis.

Several chronic disorders are known to be caused by pathogens, including viruses in various cancers and the bacterium *Helicobacter pylori* in stomach ulcers [56]. There is a good rationale for considering that AD too has an infectious aetiology: in particular, herpes simplex virus type 1 (HSV1), a common neurotropic virus that infects most humans, is implicated for several rea-

sons. Firstly, during acute brain infection with this virus, the brain regions that are afflicted are the same as those that exhibit the neuropathological features of AD. Also, survivors of this HSV1 acute brain disease exhibit long-term effects that include memory loss [58]. A further reason why HSV1 might be involved in AD is that the virus is ubiquitous – infecting about 90% of the adult population – a necessary characteristic, in view of the relatively high prevalence of AD. Finally, the ability of the virus to remain in a latent form throughout life – and to reactivate – in the peripheral nervous system (PNS) after infection early in life could explain why AD symptoms manifest in older people. This review discusses the involvement of HSV1 in AD in more detail, stressing the similarity between the effects of HSV1 infection and those seen in AD.

## THE LIFECYCLE OF HERPES SIMPLEX VIRUS TYPE 1

Herpes simplex virus type 1 (HSV1), a member of the herpes virus family, causes several diseases includ-

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ing cold sores, genital herpes, keratitis, and herpes simplex encephalitis (HSE). Most of the population is infected with this virus, usually at an early age, although the rise in socio-economic level has led to an increase in the age at which primary infection occurs. Once infected, the virus persists in the peripheral nervous system, usually in the trigeminal ganglia (TG), for the remainder of the infected person's life.

HSV1 has a double-stranded DNA genome, which is encased in an icosahedral protein capsid. The capsid is surrounded by a complex protein structure known as a tegument, which is further enclosed by a lipid membrane into which several glycoproteins are embedded [71].

The key "aim" of HSV1 (like other viruses) is to further its own replication, which it does by subverting cell mechanisms to provide all the components needed. Also, during the course of infection, the virus has to preserve itself from destruction by the host, and to preserve the host cells until they have served their purpose. Ultimately, the virus leaves the confines of the host cell, fatally wounding its temporary home in the process.

HSV1's assault on the cell begins with attachment to heparan sulfate proteoglycan (HSPG) molecules on the cell surface [100], which involves two viral glycoproteins – gB and gC [30]. Subsequently, the virus binds to specific cell surface receptors, including a member of the tumour necrosis factor receptor family [60], an HSPG molecule with specific structural features [80], and nectin-1 [29]. Interestingly, nectin-1, a member of the immunoglobulin superfamily that is related to the poliovirus receptor, and which is involved in the formation of synapses, is processed by a  $\gamma$ -secretase-like enzyme [46]. Binding to these specific receptors is mediated by glycoprotein D [50]. Once binding has occurred, the virus enters the cell via fusion with the cell membrane, although recent studies have revealed that in some cell types HSV1 enters by endocytosis [64]. Once inside the cell, the tegument components and the capsid are available to cause havoc to the cell's machinery. The function of many of the tegument proteins remains unclear but one of the best described is the virion host shutoff (vhs) protein, which shuts off host protein synthesis by degrading mRNA molecules [51]. The capsid, meanwhile, travels to the nucleus where it is disassembled, releasing the viral DNA. Subsequently, the viral genome circularises and viral gene expression is initiated by a protein complex consisting of two host proteins and an HSV1 tegument protein.

There are three temporal classes of HSV1 genes: immediate early, early and late. The immediate ear-

ly genes encode proteins that interfere with antigen presentation, disrupt protein synthesis and control the expression of the other two classes of HSV1 genes. The early genes are primarily involved in DNA synthesis whereas many of the late genes encode structural proteins. Once sufficient viral proteins are available, capsids assemble and viral DNA is packaged into them. The tegument proteins then bind to the capsid and the viral particles acquire an envelope by budding into the inner nuclear membrane. Subsequent egress from the cell involves fusion of the enveloped viral particles within the perinuclear space with the outer nuclear membrane, and the naked capsids produced obtain their lipid envelope either from the plasma membrane or from organelles such as the Golgi apparatus and the endoplasmic reticulum (ER) before being transported to the cell surface [75]. HSV1 infection causes severe structural and biochemical changes that ultimately lead to cell death. Such changes include alterations in the nucleolus, non-random, site-specific chromosomal damage, cytoskeletal and matrix abnormalities, and modifications to the plasma and organelle membranes.

In addition to the productive lifecycle described above, HSV1 can undergo latency. In latency, the viral genome is present but no viral particles are produced; although exposure to certain stimuli can cause the virus to awaken from its latent state and enter a productive lifecycle. Viral gene expression during latency is limited to one locus – the gene encoding the latency associated transcript (LAT). LATs are abundantly expressed mRNA molecules and they appear not to be translated. The precise function of the LATs is unknown. It might be involved in reactivation of the virus since HSV1 mutants that do not express LAT exhibit reduced or delayed reactivation [12]. However, LAT might also function in the establishment phase of latency: strains of HSV1 that are mutant in LAT establish latency in fewer cells than wild type strains [87].

Also, recent studies have demonstrated that LAT has anti-apoptotic properties, including evidence that LAT encodes a microRNA that interferes with apoptosis [33]; although other parts of LAT might have anti-apoptotic activity [19].

## HERPES SIMPLEX VIRUS TYPE 1 IN BRAIN

Herpes simplex encephalitis (HSE) – the severe brain disorder caused by HSV1 – is extremely rare, affecting 1-3 people in every million. The brain regions that are primarily affected in this disorder are the frontal and

temporal cortices, which are the brain regions that exhibit the most damage in AD [2]. Several early studies attempted to determine whether the virus was in human brain under normal, non-HSE circumstances but the results obtained were equivocal, mainly because of the insensitivity of the techniques used [77,85]. However, the advent of the ultra-sensitive polymerase chain reaction (PCR) provided a means of overcoming this sensitivity problem. Indeed, using PCR, Jamieson et al. [42] discovered that HSV1 DNA is present in a high proportion of brains of elderly people, including AD patients. The viral DNA was found in the temporal and frontal cortices but it was absent from the occipital cortex, which is much less affected in AD [43]. The virus was also absent from the brains of most young people tested, suggesting that the virus reaches the brain in older age as the immune system declines [43].

Several groups have since confirmed that HSV1 DNA is present in human brain tissue, some of them also demonstrating that the viral DNA is present in AD patients [4,9,32,61,72]. PCR is not the only technique to show HSV1 presence in human brain: enzyme linked immunosorbent assay was used to detect antibodies to the virus in the cerebrospinal fluid (CSF) of both AD sufferers and aged normals [98]. The rationale for seeking these antibodies was that an intrathecal antibody response is seen after HSE where antibodies in the CSF can persist for several years. Our demonstration of this intrathecal immune response in AD and elderly normals subjects not only confirms that HSV1 is present in human brain but it also reveals that the virus has replicated there, causing an acute, perhaps recurrent, infection. Also, consistent with the PCR findings, younger people were found to lack this HSV1-specific intrathecal immune response [98]. More recently, we have used the technique of *in situ* PCR to confirm that the brains of AD patients and age-matched controls contain HSV1 DNA and to show that the virus is present in neurons (Wozniak, Mee, Itzhaki, in preparation).

### **HERPES SIMPLEX VIRUS TYPE 1, APOLIPOPROTEIN E AND ALZHEIMER'S DISEASE**

The fact that HSV1 is present in the brains of elderly controls as well as AD patients does not weaken the argument that HSV1 is involved in the disease. A major concept pertinent to micro-organisms is that susceptibility to, and outcome of, infection is modulated by genetic factors, which means that an "infected" person

may not necessarily be "affected" and consequently, that not only patients but also "controls" may be infected. For example, cold sores, which are caused by HSV1, afflict only 20–40% of the population, despite the high prevalence of HSV1 and its reactivation in everybody infected [45]. Thus a host factor might well determine whether an infected person develops cold sores and similarly, whether an individual with HSV1 in brain develops AD. In fact this was found to be so: HSV1 confers a high risk of AD when in brain of carriers of a specific allele of the gene called APOE – the APOE- $\epsilon$ 4 allele [41,53]. APOE- $\epsilon$ 4, although a known susceptibility factor for AD [23,74], is neither necessary nor sufficient for the disease, i.e., it must act in conjunction with another factor(s). The joint genetic-environmental risk factor that we have discovered accounts for 60% of our cases. Presumably, other factors account for the remaining 40%.

Our results showed that the AD brain is not predisposed to HSV1 infection, as the proportion of elderly controls harbouring HSV1 in brain is similar to that of AD patients [41,42,53]. Similarly, APOE- $\epsilon$ 4 carriers are not predisposed to HSV1 infection, as very few of the brain-infected elderly controls carry an  $\epsilon$ 4 allele. Intriguingly – and consistently, we also found that APOE- $\epsilon$ 4 is a risk for cold sores.

Mechanistically, there are several possible ways in which HSV1 and the protein, apoE, might interact. It could be at the level of the immune system, if the immune system of APOE- $\epsilon$ 4 carriers were less able to combat HSV1 infection. Alternatively, the damage caused by HSV1 in brain might be repaired less well in APOE- $\epsilon$ 4 possessors. ApoE and HSV1 might also interact at the level of the cell surface: both the virus and the protein bind to HSPG in the cell surface before attaching to specific receptors for entry into the cell [44, 100]. Thus, HSV1 and apoE might compete for HSPG binding and cell entry, and if apoE4 were to compete less well than the other isoforms, more cells would be infected, causing greater damage and leading to AD. This hypothesis is strongly (even if indirectly) supported by our studies showing that APOE genotype governs outcome of infection by several other infectious agents that use HSPG and/or specific apoE receptors for binding and entry (Table 1). These include HSV1 not only in cold sores [41,53] but also in HSE [54], hepatitis C virus (HCV)-induced damage in liver [96], and varicella zoster virus (VZV) in post-herpetic neuralgia (PHN) [99]. Another group found that in HIV-infected people, APOE influences damage in brain and PNS pre-AIDS [22] and two recent studies of ours sug-

Table 1  
Summary of infectious diseases with an APOE association

| Pathogen                    | Disease                          | Allele/genotype conferring risk           | Allele/genotype conferring protection     | Ref.           |
|-----------------------------|----------------------------------|---|---|----------------|
| Herpes simplex virus type 1 | Alzheimer's disease              | APOE- $\epsilon$ 4                        |   | [40,52]        |
| Herpes simplex virus type 1 | Cold sores                       | APOE- $\epsilon$ 4                        |   | [40,52]        |
| Herpes simplex virus type 1 | Encephalitis                     | APOE- $\epsilon$ 2                        |   | [53]           |
| HIV                         | Dementia                         | APOE- $\epsilon$ 4                        |   | [22]           |
| HIV                         | Peripheral neuropathy            | APOE- $\epsilon$ 4                        |   | [22]           |
| Hepatitis C virus           | Severe liver disease             |   | APOE- $\epsilon$ 4                        | [95]           |
| Varicella zoster virus      | Shingles                         | APOE- $\epsilon$ 4 $\epsilon$ 4 (females) |   | [98]           |
| Varicella zoster virus      | Post herpetic neuralgia.         |   | APOE- $\epsilon$ 4 $\epsilon$ 4 (females) | [98]           |
| Plasmodium falciparum       | Malaria (earlier susceptibility) | APOE- $\epsilon$ 2 $\epsilon$ 2           |   | [94]           |
| Mycobacterium tuberculosis  | Pulmonary tuberculosis           | APOE- $\epsilon$ 2 (females)              |   | in preparation |

gest that APOE determines age of first infection by the malaria protozoan [95] and outcome of infection with *Mycobacterium tuberculosis* (Wozniak, Maude, Innes, Hawkey, Itzhaki, in preparation).

Further evidence supporting the importance of APOE in HSV1 infection is provided by studies on APOE-transgenic mice. These demonstrate that the ability of HSV1 to enter the brain is dependent on APOE gene dose and that during acute and latent HSV1 infection of mice, the viral load in brain is greater in animals expressing the human APOE- $\epsilon$ 4 allele than in those expressing the APOE- $\epsilon$ 3 allele [15–17]. These data suggest that the APOE- $\epsilon$ 4 allele either facilitates entry of the virus into human brain or allows the virus to spread and replicate more efficiently in brain. These effects might well be explained by a competition effect with apoE4, as described above.

Another potential mechanism of interaction between APOE and HSV1 is at the level of viral gene expression. Miller and Federoff investigated the effect of APOE on the expression of specific HSV1 genes during acute and latent infection [59]. They used primary neuronal cultures from APOE-transgenic and APOE-knockout (KO) mice to assess viral IE gene expression, and found the highest level in cells from KO and APOE- $\epsilon$ 4 mice. Examination of the animals' trigeminal ganglia showed that LAT expression was least in KO and APOE- $\epsilon$ 4 animals. The authors conclude that in the latter two groups, the virus remains longer than the usual five or so days in the replicative phase prior to latency, thus prolonging lytic infection and cell death, and that the data support the concept that HSV1 in brain and APOE- $\epsilon$ 4 synergistically promote neuronal death – death occurring in AD. Interestingly, a study by Bhattacharjee et al. similarly showed the importance of APOE in the response to HSV1, latency being less efficiently established in the trigeminal ganglia of APOE KO mice than in control animals [10]. The Miller-Federoff data

are also consistent, as the authors point out, with those of Perng et al. indicating that LAT expression *in vitro* blocks apoptotic death induced by various insults [68].

Miller and Federoff suggest that the critical damage relevant to AD caused by HSV1 and APOE- $\epsilon$ 4 is a greater vulnerability of infected neurons in APOE- $\epsilon$ 4 carriers, leading to A $\beta$ -mediated synaptic and cellular dysfunction [59].

## HERPES SIMPLEX VIRUS TYPE 1 AND THE NEUROPATHOLOGICAL FEATURES OF ALZHEIMER'S DISEASE

The AD brain is characterized by two abnormal features: amyloid plaques and neurofibrillary tangles (NFT). Plaques are composed of amyloid- $\beta$  (A $\beta$ ), which is derived from amyloid- $\beta$  protein precursor (A $\beta$ PP) via proteolytic cleavage by the enzymes  $\beta$ - and  $\gamma$ -secretase (reviewed in [11]). NFT consist mainly of tau, a microtubule-associated protein which, in AD, is abnormally and hyper-phosphorylated by several enzymes including protein kinase A (PKA) and glycogen synthase kinase 3 $\beta$ . Interestingly, HSV1 encodes a protein that activates, and is functionally homologous to, PKA [8].

Despite the amount of research being carried on amyloid plaques and NFT, their cause is still unknown; however, several lines of evidence have linked these neuropathological features with HSV1. Firstly, Cribbs et al. discovered that an HSV1 glycoprotein (gB) has a sequence highly homologous to a segment of A $\beta$  and that synthetic peptides derived from this homologous region accelerate the formation of A $\beta$  fibrils *in vitro* and are neurotoxic at similar doses to A $\beta$  [24]. These peptides also self-assemble into fibrils that are ultra structurally indistinguishable from A $\beta$  [24]. The authors therefore suggested that HSV1 might act as a "seed" for

plaque formation [24]. Secondly, Satpute-Krishnan et al. showed that A $\beta$ PP is associated with HSV1 during anterograde transport of the virus [76], a process which might affect A $\beta$ PP degradation and synaptic function. Consistently, our findings reveal that HSV1 indeed affects breakdown of A $\beta$ PP – in human neuroblastoma cells: Western blotting, and more recently immunocytochemistry, shows that A $\beta$ PP levels decrease on infection (an expected effect of the virus, as it causes a general decrease in host cell protein synthesis), and there is a large increase in a 55 kD C-terminal fragment, which includes the A $\beta$  sequence [79]. Moreover, we have since found that infection with HSV1 leads to A $\beta$  accumulation in cells and consistently that the enzymes responsible for A $\beta$  production are increased in HSV1-infected cells too [97]. Interestingly, it is now thought that intracellular formation of A $\beta_{1-42}$  peptide, rather than extracellular deposition of A $\beta$ , is an early event in AD. We have linked HSV1 to tau too by showing that HSV1 infection of human neuroblastoma and glioblastoma cells in culture causes abnormal, AD-like phosphorylation of tau. Also, the enzymes responsible for this abnormal phosphorylation increased in infected cells (Wozniak, Frost and Itzhaki, in preparation). Taken together these findings strongly support a causal role for HSV1 in plaque and NFT formation (Fig. 1). The possibility that deposition of A $\beta$  peptide and abnormal tau phosphorylation are a consequence of cell death caused by the virus rather than of direct action by the virus can be discounted, as tau phosphorylation is maximal by 6 hours post-infection; this is well before the virus replication cycle has been completed and presumably, the cell machinery providing the necessary components for viral replication could not function once the cell has died.

Interestingly, brain injury and other viruses (e.g., HIV [27,69] and measles viruses [3]) cause some of the neuropathological features of AD but it should be stressed that HSV1, unlike these other viruses or brain injury, is uniquely able to cause such damage, as it is present in a high proportion of elderly brains.

### **HERPES SIMPLEX VIRUS TYPE 1, ALZHEIMER'S DISEASE AND THE CELL CYCLE**

Accumulating evidence suggests that the cell cycle is activated in AD and that it contributes to disease pathogenesis. In AD neurons, aberrant expression of many cycling enzymes occurs but the cells do not en-

ter mitosis; instead there is evidence of arrest – at the G2/M phase – and this leads to cell death [63,93]. In addition, phosphorylation of certain tau residues has been shown to occur during mitosis (in SHSY5Y cells) suggesting that inappropriate stimulation of cells into cycle contributes to the abnormal phosphorylation of tau in AD [25]. Interestingly, the tau phosphorylation we have observed in infected cells is identical to that occurring in the mitotic cells, signifying that many infected cells have entered the cell cycle. In fact HSV1 infection subverts various cell processes, including the host cell cycle machinery, to benefit viral survival and replication. In particular, despite its decreasing most host cell protein synthesis, it activates many kinases, including cdc2 kinase, an enzyme not normally expressed in resting neurons [70], and interestingly, in AD this kinase is upregulated [92]. Also, although the virus shuts down host DNA synthesis fairly soon after entry, later in infection it causes cells, including neurons – cells that do not normally go into cycle – to re-enter cycle; however, in a situation that parallels that in AD, HSV1 stops the progression at the G2/M stage as mitosis would presumably greatly perturb viral replication [102].

### **ALZHEIMER'S DISEASE AND HSV1'S CIRCUMVENTION OF HOST DEFENCE MECHANISMS**

HSV1 has developed several strategies to circumvent the cellular processes that protect the host from infection (Fig. 2). Firstly, one of the envelope glycoproteins present on the surface of the virus – gC – is able to bind to complement component C3b [28]. This interaction protects the virus from complement-mediated neutralisation. The virus is protected also from clearance by antibodies as two of its glycoproteins (gE and gI) form a heterodimer that binds to the Fc region of host IgG molecules [81]. Once inside the cell, HSV1 has additional evasion techniques. One method is by interfering with antigen presentation. MHC class I molecules present peptides to CD8+ T lymphocytes at the cell surface. Presentation of viral peptides would result in the infected cell being targeted for destruction; thus, it is advantageous for HSV1 to inhibit this process [88]. The virus does this by encoding an immediate early protein – infected cell polypeptide 47 (ICP47) – that binds to and inhibits the transporter associated with antigen processing (TAP), an essential component of MHC class I antigen presentation [88]. Interestingly, a

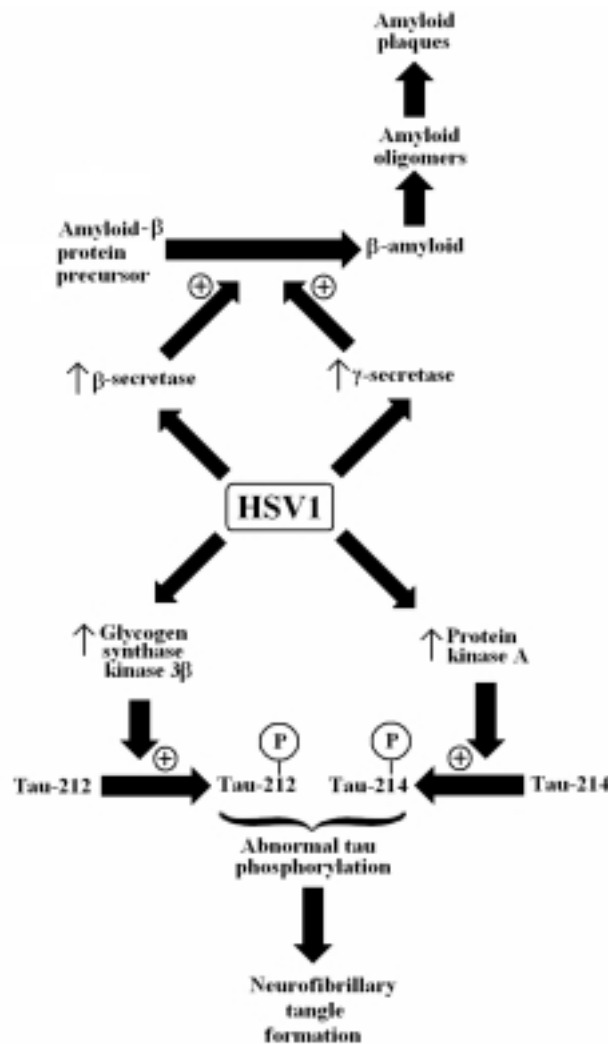


Fig. 1. Putative action of HSV1 in the formation of amyloid plaques and neurofibrillary tangles (see text for explanation).

recent study has shown that polymorphisms in the TAP gene are associated with AD, especially in carriers of the APOE- $\epsilon$ 4 allele [13]. It was suggested that certain variants in the TAP gene might affect its interaction with ICP47 and thus possessors of such variants might be less able to deal with HSV1 brain infection, resulting in AD.

Another host defence mechanism circumvented by HSV1 is the activation of protein kinase R (PKR). PKR is activated by double stranded RNA molecules and this results in the shutoff of all protein synthesis, which it mediates by the phosphorylation of the  $\alpha$  subunit of the eukaryotic initiation factor 2 (eIF2 $\alpha$ ) [86]. Although HSV1 is a double-stranded DNA virus, it encodes genes on both strands of the genome, resulting in expression of complementary RNA molecules [21]. HSV1

is, therefore, particularly susceptible to PKR-mediated protein shutoff – which it needs to prevent in order to allow synthesis of proteins for progeny virions – and as such has evolved two proteins to deal with the PKR response. One of these proteins is encoded by the US11 gene and binds to double-stranded RNA molecules, thereby preventing the activation of PKR [20]. The other protein is the product of the infected cell polypeptide 34.5 (ICP34.5) gene; this replenishes the pool of active eIF2 $\alpha$  by recruiting the cellular protein phosphatase 1 $\alpha$  to dephosphorylate eIF2 $\alpha$ , allowing protein synthesis to proceed [35]. Remarkably, there is increasing evidence to suggest that PKR activation is involved in the neuronal degeneration in AD: both activated PKR and phosphorylated eIF2 $\alpha$  are present in post mortem brain tissue from AD subjects [67] and a recent study

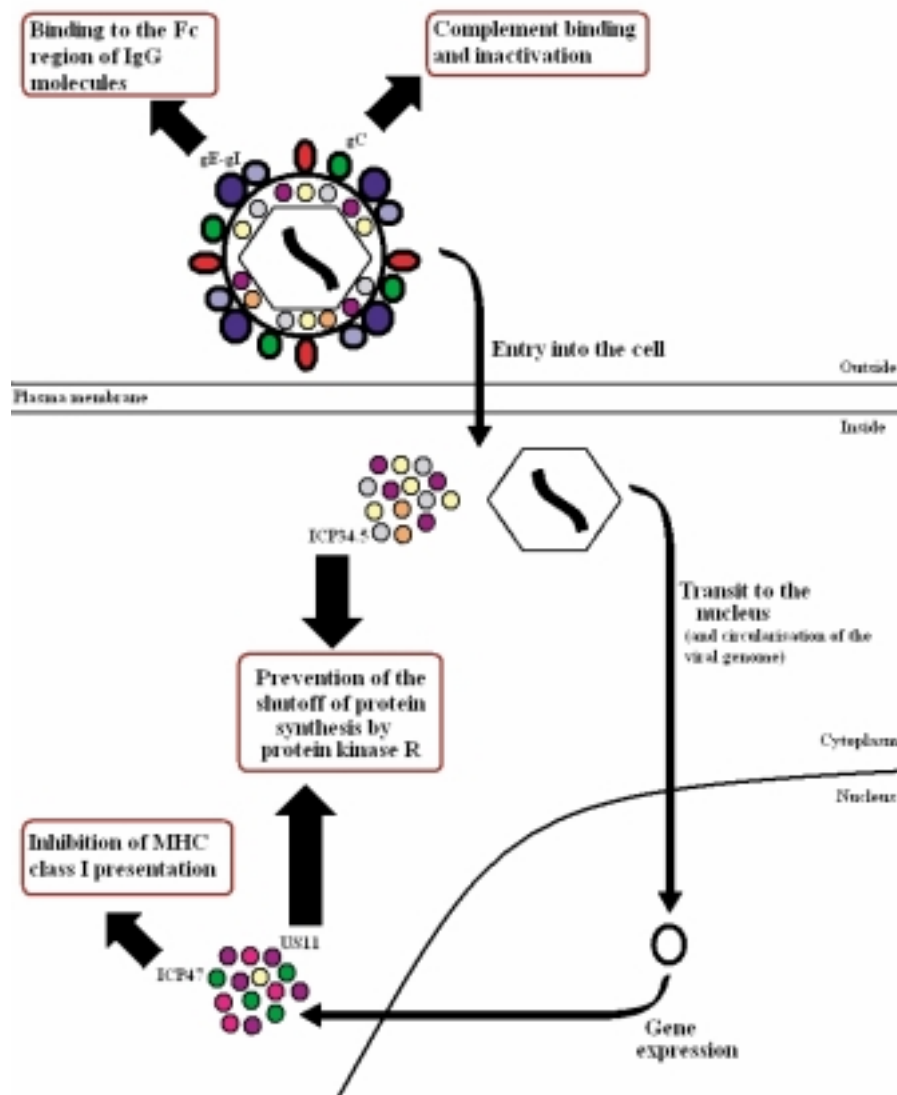


Fig. 2. Circumvention by HSV1 of host defence mechanisms (see text for explanation).

has shown a genetic association with polymorphisms in PKR in AD [14]. Although PKR activation is a component of several stress-activated pathways and its activation in AD may be a consequence of these rather than an antiviral mechanism, it is tempting to speculate that the activated PKR found in the AD brain is part of the brain's response to HSV1. The reason why PKR activation and eIF2 $\alpha$  phosphorylation are found in AD brain despite HSV1's ability to suppress these effects might be explained by the fact that both US11 and ICP34.5 are late genes and so the effects observed in the AD brain could represent the early stages of HSV1 reactivation.

It is obviously very difficult to compare the effects of HSV1 infection on a cell during its relatively short time course with the protracted development of AD, particularly at the earlier stages of the disease, pre-diagnosis, about which there is necessarily very little information. In fact certain cellular differences have been detected between earlier versus later stages of AD, e.g., autophagic processes (see next section) increase early in the AD brain though they are less apparent later [57], and presumably there are many other differences between the initial damaged cell and its final terminal state, in AD, and on HSV1 infection. The difficulties are compounded by the fact that the virus, on reactivi-

vation from latency, i.e., from an existence merely as a DNA molecule, requires synthesis of each component for formation of intact virions, whereas in simple acute infection, the complete pre-packaged virus already exists and on cell entry can immediately start its infectious cycle [73]. Further, the requirement of the virus at earlier stages of infection – the need to keep the cell alive while subverting its mechanisms to viral requirements, i.e., replication of all viral components – must differ from those when viral progeny have been made; at the latter stage the damaged cell is no longer needed and so it can die. Repeated reactivation compounds the problem, but meets the requirement for a long-term (potentially) damaging agent which could lead to chronic disease – a requirement which would not be met by a single acute environmental event such as brief infection or head injury.

#### **HERPES SIMPLEX VIRUS TYPE 1, ALZHEIMER'S DISEASE AND AUTOPHAGY**

Autophagy is a process that involves the lysosomal degradation of the cell's own components [57]. It is particularly important for the catabolism of aberrant proteins that the eventual malfunctions in autophagy might explain the accumulation of abnormal proteins in certain neurological diseases, including AD [65,101]. The cause and mechanism of autophagic dysfunction in AD remains unclear but it might very probably involve HSV1 since autophagy, as well as degrading host proteins, is a defence mechanism that has evolved to deal with invading pathogens. To compensate for this defence mechanism, many pathogens have co-evolved processes that counteract autophagy. In the case of HSV1, the virally-encoded ICP34.5 interferes with autophagic degradation (heterophagy) of the intruding pathogen via its modulation of PKR and eIF2 $\alpha$  [83, 84]: this process would also prevent autophagic degradation of abnormal cell proteins such as A $\beta$  [40]. Thus, as we have found [97], HSV1 infection would lead to accumulation of A $\beta$ .

#### **HERPES SIMPLEX VIRUS TYPE 1, ALZHEIMER'S DISEASE, INFLAMMATION AND OXIDATION**

Inflammation is a key event in many viral infections, including HSV1. Markers of inflammation are found in the AD brain too, and in fact several inflam-

matory mediators are common to both HSV1 infection and AD. Thus the inflammatory molecules associated with AD might reflect HSV1 infection. For example, infection with HSV1 leads to the production of tumour necrosis factor  $\alpha$ , interleukin 6, interleukin 8 and interferon-inducible protein 10 [55], all of which are elevated in the brains of AD sufferers [18]. In addition, peripheral mononuclear cells from AD patients exhibit elevated expression of RANTES and MCP-1 [39], two chemokines that are also increased in HSV1 infection [55]. Further, interleukin 1 $\beta$  (IL-1 $\beta$ ) is elevated in AD [62] and, in mice, protects against HSV1 encephalitis [78]. Perhaps in AD the brain responds to HSV1 infection by producing more IL-1 $\beta$  – which might explain the fact that full-blown encephalitis is not evident in the AD brain.

Inflammation, as well as being a consequence of HSV1 infection, can reactivate the virus [49]. (This phenomenon could account for the apparent protective effect shown in some, though not all, studies on non-steroidal, anti-inflammatory agents against AD [52]; these agents, by preventing inflammation, would stop the reactivation of HSV1 in brain, thereby reducing the damage caused by the virus.) Inflammation in the brain might originate from the periphery, since there is a growing body of evidence to suggest that peripheral events strongly influence processes in the brain relevant to AD. For example, inflammatory cytokines in the periphery, produced during systemic infection, can lead to stimulation of immune cells in the CNS and consequent inflammation which, in elderly humans, causes cognitive decline, and in animals, memory loss. Perhaps in humans the cognitive decline is caused by inflammation-induced reactivation of HSV1.

The influence of systemic infection on CNS function has been described in detail by Kongsman et al. [48]. Invasion of the body by micro-organisms triggers production of pro-inflammatory cytokines by peripheral phagocytic cells, and the resulting immune message is conveyed to the brain via neural and humoral pathways which stimulate expression of cytokines in the brain by macrophage-like cells and microglia. Interestingly, these innate immune cells in the brain increase in reactivity with age and thus aging would lead to a greater extent of inflammation in brain – which could well contribute to the development of neurological disease [31]. In fact in rodents, experimental peripheral infection or lipopolysaccharide injection causes memory defects in older but not in younger animals [5], and in humans, several epidemiological studies have shown an association between extent or number of infectious episodes



and cognitive decline in the elderly. Firstly, seropositivity for herpesviridae, APOE- $\epsilon$ 4 carriage, and low education level in elderly cardiovascular patients were found to be associated with risk of cognitive impairment [82]. Secondly, Aiello et al. [1] have shown that elderly subjects with higher levels of antibody (IgG) to another herpes virus, cytomegalovirus (CMV), suffer greater rates of cognitive decline over a four year period (no such changes in level of IgG to HSV1 were detectable – as would be expected, since the titre remains unaltered between and during periods of recrudescence [91]). Another study on elderly humans – AD patients – has shown that cognitive decline occurs for at least two months after systemic infection [37]. In younger people, in contrast, no such decline was noted after systemic infection [26]. All of these studies are consistent with a viral role in AD in that they suggest that repeated systemic infection causes inflammation in brain which reactivates latent HSV1 in brain, which could cause AD.

Another phenomenon common to HSV1 and AD is oxidative damage. In AD, there is evidence that oxidation of the major cell macromolecules – nucleic acids, proteins and lipids – occurs at an early stage in the disease, preceding the main neuropathological changes seen in AD brain, and such changes are also displayed in animal models of the disease and in biological fluids of AD patients. It is suggested that aggregation of A $\beta$  and tau are compensatory responses to oxidative stress; indeed, cell culture studies show that oxidative stress causes intracellular A $\beta$  accumulation and tau phosphorylation [66]. Similarly, the direct effects of viruses, including HSV1, on cells plus the host's inflammatory responses to viral infection can lead to increased formation of reactive oxygen and reactive nitrogen species [90]. These species in turn can regulate host inflammatory and immune responses as well as causing oxidative damage in nucleic acids and proteins and in lipid-containing structures to which, the nervous system, being cholesterol and poly-unsaturated lipid-rich, is particularly susceptible [90]. Even during latent HSV1 infection (of mice) there is evidence of oxidative damage and of persistent inflammation, and it is suggested on the basis of detection of oxidation products in different types of cell, that not only the latently infected neurons but also uninfected neurons and glial cells are damaged [90]. However it should be mentioned that if a very low level persistent infection i.e., virus replication, were to occur, it might be below the limits of detection by convention techniques and therefore would not be distinguishable from latent infection.

## **HERPES SIMPLEX VIRUS TYPE 1, ALZHEIMER'S DISEASE AND CHOLESTEROL**

Several studies have shown that statins, drugs that lower cholesterol, reduce the risk of developing AD, and other reports have shown that high levels of cholesterol fed to animals or given to cell cultures promote the formation of A $\beta$  from A $\beta$ PP by regulating secretase activity [94]. These data have led to diets rich in cholesterol being strongly implicated in AD – although conclusions from the epidemiological studies remain somewhat controversial. Interestingly, HSV1 infection leads to a marked accumulation of cholesterol, partly due to decreased hydrolysis of cholesterol esters and decreased efflux of cholesterol [38]. Thus, in HSV1-infected cells in the brain there might be an accumulation of cholesterol. The increase in cholesterol levels caused by HSV1 infection might partly account for our recent discovery that HSV1 causes an increase in A $\beta$  peptides in infected neural cells and mouse brain [97].

A further connection between HSV1, cholesterol and AD has been postulated by Hill and colleagues [36]. They suggest that statins disrupt the formation of lipid rafts, thereby inhibiting the spread of the virus through the nervous system. Lipid rafts, of which cholesterol is an essential component, are a requirement for entry of HSV1, as shown recently by Bender et al. [7]. However, the concept depends on whether statins are definitely protective against AD and this is still uncertain. A possible role for APOE in this mechanism – determining the level of cholesterol in lipid rafts (as it does in plasma) – is suggested by the finding in APOE-transgenic mice that the amount of cholesterol is about two-fold higher in certain components of the plasma membrane in APOE- $\epsilon$ 4 than in  $\epsilon$ 3 animals [34]. If this is true of humans also, HSV1 might enter and spread more readily in brain of APOE- $\epsilon$ 4 carriers.

## **CONCLUSION**

In summary, we propose that during events such as stress and peripheral infection, latent HSV1 reactivates (as in the PNS) and causes an acute but localised infection, perhaps a “mild”, variant type of encephalitis, causing greater damage – both direct, and indirect via inflammatory processes – in APOE- $\epsilon$ 4 carriers, and eventually, AD. (Cases of mild – and of recurrent – HSE have been reported: these perhaps occur relatively often but are under-diagnosed [47,89].) APOE might

act via the immune system, or by affecting the spread of virus in brain by apoE isoform-specific competition with HSV1 for entry into cells, or by determining the extent of repair of virally-damaged tissue, although our data favour the second explanation. The actual damage might well comprise the effects of the virus, described above, on A $\beta$ , tau, and on the cell cycle, which strikingly resemble those occurring in AD. We hope to examine such possibilities by investigating the effects of HSV1 infection of APOE-transgenic mice, and in particular, to find if APOE- $\epsilon$ 4 animals develop AD-like pathology.

At present, treatment of AD is palliative at best; our data provide a strong rationale for a completely different approach, namely, antiviral therapy, and perhaps in future, vaccination against HSV1 early in life. Unfortunately, no clinical trials of antiviral drugs – which are readily available and of low cost – have yet been set up for AD, even though such trials have been held for multiple sclerosis sufferers [6], despite the less strong evidence of a herpesvirus involvement in the latter. We very much hope that pharmaceutical companies will not be deterred from establishing such trials for AD patients merely because of the older age of the patients compared to MS sufferers.

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## References

- [1] A. Aiello, M. Haan, L. Blythe, K. Moore, J.M. Gonzalez and W. Jagust, The influence of latent viral infection on rate of cognitive decline over 4 years, *J Am Geriatr Soc* **54** (2006), 1046–1054.
- [2] M.J. Ball, Limbic predilection in Alzheimer dementia: is reactivated herpesvirus involved? *Can J Neurol Sci* **9** (1982), 303–306.
- [3] C. Bancher, H. Leitner, K. Jellinger, H. Eder, U. Setinek, P. Fischer, J. Wegiel and H.M. Wisniewski, On the relationship between measles virus and Alzheimer neurofibrillary tangles in subacute sclerosing panencephalitis, *Neurobiol Aging* **17** (1996), 527–533.
- [4] J.R. Baringer and P. Pisani, Herpes simplex virus genomes in human nervous system tissue analyzed by polymerase chain reaction, *Ann Neurol* **36** (1994), 823–829.
- [5] R.M. Barrientos, E.A. Higgins, J.C. Biedenkapp, D.B. Sprunger, K.J. Wright-Hardesty, L.R. Watkins, J.W. Rudy and S.F. Maier, Peripheral infection and aging interact to impair hippocampal memory consolidation, *Neurobiol Aging* **27** (2006), 723–732.
- [6] E. Bech, J. Lycke, P. Gadeberg, H.J. Hansen, C. Malmstrom, O. Andersen, T. Christensen, S. Ekholm, S. Haahr, P. Hollsberg, T. Bergstrom, B. Svennerholm and J. Jakobsen, A randomized, double-blind, placebo-controlled MRI study of anti-herpes virus therapy in MS, *Neurology* **58** (2002), 31–36.
- [7] F.C. Bender, J.C. Whitbeck, M. Ponce de Leon, H. Lou, R.J. Eisenberg and G.H. Cohen, Specific association of glycoprotein B with lipid rafts during herpes simplex virus entry, *J Virol* **77** (2003), 9542–9552.
- [8] L. Benetti and B. Roizman, Herpes simplex virus protein kinase US3 activates and functionally overlaps protein kinase A to block apoptosis, *Proc Natl Acad Sci USA* **101** (2004), 9411–9216.
- [9] P. Bertrand, D. Guillaume, L. Hellauer, D. Dea, J. Lindsay, S. Kogan, S. Gauthier and J. Poirier, Distribution of herpes simplex virus type 1 DNA in selected areas of normal and Alzheimer's disease brains: a PCR study, *Neurodegeneration* (1993), 201–208.
- [10] P.S. Bhattacharjee, D.M. Neumann, D. Stark, H.W. Thompson and J.M. Hill, Apolipoprotein E modulates establishment of HSV-1 latency and survival in a mouse ocular model, *Curr Eye Res* **31** (2006), 703–708.
- [11] K. Blennow, M.J. de Leon and H. Zetterberg, Alzheimer's disease, *Lancet* **368** (2006), 387–403.
- [12] T.M. Block, S. Deshmane, J. Masonis, J. Maggioncalda, T. Valyi-Nagi and N.W. Fraser, An HSV LAT null mutant reactivates slowly from latent infection and makes small plaques on CV-1 monolayers, *Virology* **192** (1993), 618–630.
- [13] M.J. Bullido, A. Martinez-Garcia, M.J. Artiga, J. Aldudo, I. Sastre, P. Gil, F. Coria, D.G. Munoz, V. Hachinski, A. Frank and F. Valdivieso, A TAP2 genotype associated with Alzheimer's disease in APOE4 carriers, *Neurobiol Aging* **28** (2007), 519–523.
- [14] M.J. Bullido, A. Martinez-Garcia, R. Tenorio, I. Sastre, D.G. Munoz, A. Frank and F. Valdivieso, Double stranded RNA activated EIF2 alpha kinase (EIF2AK2; PKR) is associated with Alzheimer's disease, *Neurobiol Aging* (2007).
- [15] J.S. Burgos, C. Ramirez, I. Sastre, M.J. Bullido and F. Valdivieso, ApoE4 is more efficient than E3 in brain access by herpes simplex virus type 1, *Neuroreport* **14** (2003), 1825–1827.
- [16] J.S. Burgos, C. Ramirez, I. Sastre, M.J. Bullido and F. Valdivieso, Involvement of apolipoprotein E in the hematogenous route of herpes simplex virus type 1 to the central nervous system, *J Virol* **76** (2002), 12394–12398.
- [17] J.S. Burgos, C. Ramirez, I. Sastre and F. Valdivieso, Effect of apolipoprotein E on the cerebral load of latent herpes simplex virus type 1 DNA, *J Virol* **80** (2006), 5383–5387.
- [18] M. Cacquevel, N. Lebeurrier, S. Cheenne and D. Vivien, Cytokines in neuroinflammation and Alzheimer's disease, *Curr Drug Targets* **5** (2004), 529–534.
- [19] D. Carpenter, C. Hsiang, D.J. Brown, L. Jin, N. Osorio, L. Benmohamed, C. Jones and S.L. Wechsler, Stable cell lines expressing high levels of the herpes simplex virus type 1 LAT are refractory to caspase 3 activation and DNA laddering following cold shock induced apoptosis, *Virology* (2007).
- [20] K.A. Cassady and M. Gross, The herpes simplex virus type 1 U(S)11 protein interacts with protein kinase R in infected cells and requires a 30-amino-acid sequence adjacent to a kinase substrate domain, *J Virol* **76** (2002), 2029–2035.
- [21] Y.E. Chang, L. Menotti, F. Filatov, G. Campadelli-Fiume and B. Roizman, UL27.5 is a novel gamma2 gene antisense to

- the herpes simplex virus 1 gene encoding glycoprotein B, *J Virol* **72** (1998), 6056–6064.
- [22] E.H. Corder, K. Robertson, L. Lannfelt, N. Bogdanovic, G. Eggertsen, J. Wilkins and C. Hall, HIV-infected subjects with the E4 allele for APOE have excess dementia and peripheral neuropathy, *Nat Med* **4** (1998), 1182–1184.
- [23] E.H. Corder, A.M. Saunders, W.J. Strittmatter, D.E. Schmechel, P.C. Gaskell, G.W. Small, A.D. Roses, J.L. Haines and M.A. Pericak-Vance, Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families, *Science* **261** (1993), 921–923.
- [24] D.H. Cribbs, B.Y. Azizeh, C.W. Cotman and F.M. LaFerla, Fibril formation and neurotoxicity by a herpes simplex virus glycoprotein B fragment with homology to the Alzheimer's A beta peptide, *Biochemistry* **39** (2000), 5988–5994.
- [25] P. Delobel, S. Flament, M. Hamdane, C. Mailliot, A.V. Sambro, S. Begard, N. Sergeant, A. Delacourte, J.P. Vilain and L. Buee, Abnormal Tau phosphorylation of the Alzheimer-type also occurs during mitosis, *J Neurochem* **83** (2002), 412–420.
- [26] F.B. Dickerson, J.J. Boronow, C. Stallings, A.E. Orioni, S. Cole, B. Krivogorsky and R.H. Yolken, Infection with herpes simplex virus type 1 is associated with cognitive deficits in bipolar disorder, *Biol Psychiatry* **55** (2004), 588–593.
- [27] M.M. Esiri, S.C. Biddolph and C.S. Morris, Prevalence of Alzheimer plaques in AIDS, *J Neurol Neurosurg Psychiatry* **65** (1998), 29–33.
- [28] H.M. Friedman, L. Wang, N.O. Fishman, J.D. Lambris, R.J. Eisenberg, G.H. Cohen and J. Lubinski, Immune evasion properties of herpes simplex virus type 1 glycoprotein gC, *J Virol* **70** (1996), 4253–4260.
- [29] R.J. Geraghty, C. Krummenacher, G.H. Cohen, R.J. Eisenberg and P.G. Spear, Entry of alphaherpesviruses mediated by poliovirus receptor-related protein 1 and poliovirus receptor, *Science* **280** (1998), 1618–1620.
- [30] S.I. Gerber, B.J. Belval and B.C. Herold, Differences in the role of glycoprotein C of HSV-1 and HSV-2 in viral binding may contribute to serotype differences in cell tropism, *Virology* **214** (1995), 29–39.
- [31] J.P. Godbout and R.W. Johnson, Age and neuroinflammation: a lifetime of psychoneuroimmune consequences, *Neurol Clin* **24** (2006), 521–538.
- [32] L. Gordon, S. McQuaid and S.L. Cosby, 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** (1996), 33–40.
- [33] A. Gupta, J.J. Gartner, P. Sethupathy, A.G. Hatzigeorgiou and N.W. Fraser, Anti-apoptotic function of a microRNA encoded by the HSV-1 latency-associated transcript, *Nature* **442** (2006), 82–85.
- [34] H. Hayashi, U. Igbavboa, H. Hamanaka, M. Kobayashi, S.C. Fujita, W.G. Wood and K. Yanagisawa, Cholesterol is increased in the exofacial leaflet of synaptic plasma membranes of human apolipoprotein E4 knock-in mice, *Neuroreport* **13** (2002), 383–386.
- [35] B. He, M. Gross and B. Roizman, The gamma(1)34.5 protein of herpes simplex virus 1 complexes with protein phosphatase 1alpha to dephosphorylate the alpha subunit of the eukaryotic translation initiation factor 2 and preclude the shutoff of protein synthesis by double-stranded RNA-activated protein kinase, *Proc Natl Acad Sci USA* **94** (1997), 843–848.
- [36] J.M. Hill, I. Steiner, K.E. Matthews, S.G. Trahan, T.P. Foster and M.J. Ball, Statins lower the risk of developing Alzheimer's disease by limiting lipid raft endocytosis and decreasing the neuronal spread of Herpes simplex virus type 1, *Med Hypotheses* **64** (2005), 53–58.
- [37] C. Holmes, M. El-Okil, A.L. Williams, C. Cunningham, D. Wilcockson and V.H. Perry, Systemic infection, interleukin 1beta, and cognitive decline in Alzheimer's disease, *J Neurol Neurosurg Psychiatry* **74** (2003), 788–789.
- [38] H.Y. Hsu, A.C. Nicholson, K.B. Pomerantz, R.J. Kaner and D.P. Hajjar, Altered cholesterol trafficking in herpesvirus-infected arterial cells. Evidence for viral protein kinase-mediated cholesterol accumulation, *J Biol Chem* **270** (1995), 19630–19637.
- [39] C. Iarlori, D. Gambi, F. Gambi, I. Lucci, C. Feliciani, M. Salvatore and M. Reale, Expression and production of two selected beta-chemokines in peripheral blood mononuclear cells from patients with Alzheimer's disease, *Exp Gerontol* **40** (2005), 605–611.
- [40] R.F. Itzhaki, S.L. Cosby and M.A. Wozniak, Herpes simplex virus type 1 and Alzheimer's disease: the autophagy connection, *J Neurovirol* In proof.
- [41] R.F. Itzhaki, W.R. Lin, D. Shang, G.K. Wilcock, B. Faragher and G.A. Jamieson, Herpes simplex virus type 1 in brain and risk of Alzheimer's disease, *Lancet* **349** (1997), 241–244.
- [42] G.A. Jamieson, N.J. Maitland, G.K. Wilcock, J. Craske and R.F. Itzhaki, Latent herpes simplex virus type 1 in normal and Alzheimer's disease brains, *J Med Virol* **33** (1991), 224–227.
- [43] G.A. Jamieson, N.J. Maitland, G.K. Wilcock, C.M. Yates and R.F. Itzhaki, 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** (1992), 365–368.
- [44] Z.S. Ji, W.J. Brecht, R.D. Miranda, M.M. Hussain, T.L. Innerarity and R.W. Mahley, Role of heparan sulfate proteoglycans in the binding and uptake of apolipoprotein E-enriched remnant lipoproteins by cultured cells, *J Biol Chem* **268** (1993), 10160–10167.
- [45] R.T. Johnson, *Viral Infections of the Nervous System*, Second ed., Philadelphia: Lippincott-Raven Publishers, 1998.
- [46] D.Y. Kim, L.A. Ingano and D.M. Kovacs, Nectin-1alpha, an immunoglobulin-like receptor involved in the formation of synapses, is a substrate for presenilin/gamma-secretase-like cleavage, *J Biol Chem* **277** (2002), 49976–49981.
- [47] P.E. Klapper, G.M. Cleator and M. Longson, Mild forms of herpes encephalitis, *J Neurol Neurosurg Psychiatry* **47** (1984), 1247–1250.
- [48] J.P. Kohnsman, P. Parnet and R. Dantzer, Cytokine-induced sickness behaviour: mechanisms and implications, *Trends Neurosci* **25** (2002), 154–159.
- [49] J.D. Kriesel, The roles of inflammation, STAT transcription factors, and nerve growth factor in viral reactivation and herpes keratitis, *DNA Cell Biol* **21** (2002), 475–481.
- [50] C. Krummenacher, A.V. Nicola, J.C. Whitbeck, H. Lou, W. Hou, J.D. Lambris, R.J. Geraghty, P.G. Spear, G.H. Cohen and R.J. Eisenberg, Herpes simplex virus glycoprotein D can bind to poliovirus receptor-related protein 1 or herpesvirus entry mediator, two structurally unrelated mediators of virus entry, *J Virol* **72** (1998), 7064–7074.
- [51] A.D. Kwong and N. Frenkel, Herpes simplex virus-infected cells contain a function(s) that destabilizes both host and viral mRNAs, *Proc Natl Acad Sci USA* **84** (1987), 1926–1930.
- [52] G. Li, E.B. Larson, J.A. Sonnen, J.B. Shofer, E.C. Petrie, A. Schantz, E.R. Peskind, M.A. Raskind, J.C. Breitner and T.J. Montine, Statin therapy is associated with reduced neuropathologic changes of Alzheimer disease, *Neurology* **69** (2007), 878–885.

- [53] W.R. Lin, J. Graham, S.M. MacGowan, G.K. Wilcock and R.F. Itzhaki, Alzheimer's disease, herpes virus in brain, apolipoprotein E4 and herpes labialis, *Alzheimer's Reports* **1** (1998), 173–178.
- [54] W.R. Lin, M.A. Wozniak, M.M. Esiri, P. Klenerman and R.F. Itzhaki, Herpes simplex encephalitis: involvement of apolipoprotein E genotype, *J Neurol Neurosurg Psychiatry* **70** (2001), 117–119.
- [55] J.R. Lokensgard, S. Hu, W. Sheng, M. vanOijen, D. Cox, M.C. Cheeran and P.K. Peterson, Robust expression of TNF-alpha, IL-1beta, RANTES, and IP-10 by human microglial cells during nonproductive infection with herpes simplex virus, *J Neurovirol* **7** (2001), 208–219.
- [56] B.J. Marshall and J.R. Warren, Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration, *Lancet* **1** (1984), 1311–1315.
- [57] M. Martinez-Vicente and A.M. Cuervo, Autophagy and neurodegeneration: when the cleaning crew goes on strike, *Lancet Neurol* **6** (2007), 352–361.
- [58] N. McGrath, N.E. Anderson, M.C. Crosson and K.F. Powell, Herpes simplex encephalitis treated with acyclovir: diagnosis and long term outcome, *J Neurol Neurosurg Psychiatry* **63** (1997), 321–326.
- [59] R.M. Miller and H.J. Federoff, Isoform-specific effects of ApoE on HSV immediate early gene expression and establishment of latency, *Neurobiol Aging* (2006), doi:10.1016/j.neurobiolaging.2006.09.006.
- [60] R.I. Montgomery, M.S. Warner, B.J. Lum and P.G. Spear, Herpes simplex virus-1 entry into cells mediated by a novel member of the TNF/NGF receptor family, *Cell* **87** (1996), 427–436.
- [61] I. Mori, Y. Kimura, H. Naiki, R. Matsubara, T. Takeuchi, T. Yokochi and Y. Nishiyama, Reactivation of HSV-1 in the brain of patients with familial Alzheimer's disease, *J Med Virol* **73** (2004), 605–611.
- [62] R.E. Mrak and W.S. Griffin, Potential inflammatory biomarkers in Alzheimer's disease, *J Alzheimers Dis* **8** (2005), 369–375.
- [63] Z. Nagy, M.M. Esiri and A.D. Smith, The cell division cycle and the pathophysiology of Alzheimer's disease, *Neuroscience* **87** (1998), 731–739.
- [64] A.V. Nicola, A.M. McEvoy and S.E. Straus, Roles for endocytosis and low pH in herpes simplex virus entry into HeLa and Chinese hamster ovary cells, *J Virol* **77** (2003), 5324–5332.
- [65] R.A. Nixon, J. Wegiel, A. Kumar, W.H. Yu, C. Peterhoff, A. Cataldo and A.M. Cuervo, Extensive involvement of autophagy in Alzheimer disease: an immuno-electron microscopy study, *J Neuropathol Exp Neurol* **64** (2005), 113–122.
- [66] A. Nunomura, R.J. Castellani, X. Zhu, P.I. Moreira, G. Perry and M.A. Smith, Involvement of oxidative stress in Alzheimer disease, *J Neuropathol Exp Neurol* **65** (2006), 631–641.
- [67] A.L. Peel, PKR activation in neurodegenerative disease, *J Neuropathol Exp Neurol* **63** (2004), 97–105.
- [68] G.C. Perng, C. Jones, J. Ciacci-Zanella, M. Stone, G. Henderson, A. Yukht, S.M. Slanina, F.M. Hofman, H. Ghiasi, A.B. Nesburn and S.L. Wechsler, Virus-induced neuronal apoptosis blocked by the herpes simplex virus latency-associated transcript, *Science* **287** (2000), 1500–1503.
- [69] L. Pulliam and H. Rempel, HIV-TAT inhibits neprilysin and elevates amyloid beta, *Neurobiol Aging* **25** (2004), S460–S461.
- [70] N. Ray and L.W. Enquist, Transcriptional response of a common permissive cell type to infection by two diverse alpha-herpesviruses, *J Virol* **78** (2004), 3489–3501.
- [71] A. Reske, G. Pollara, C. Krummenacher, B.M. Chain and D.R. Katz, Understanding HSV-1 entry glycoproteins, *Rev Med Virol* **17** (2007), 205–215.
- [72] J.D. Rodriguez, D. Royall, L.T. Daum, K. Kagan-Hallet and J.P. Chambers, Amplification of Herpes simplex type 1 and Human Herpes type 5 viral DNA from formalin-fixed Alzheimer brain tissue, *Neurosci Lett* **390** (2005), 37–41.
- [73] B. Roizman and A.E. Sears, The replication of Herpes simplex viruses, in: *Fields' Virology*, 3rd ed., B.N. Fields, D.M. Knipe, P.M. Howley, R.M. Chanock, M.S. Hirsch, J.L. Melnick, T.P. Monath and B. Roizman, eds, New York: Lippincott-Raven Press, 1996, pp. 2231–2295.
- [74] A.D. Roses, Apolipoprotein E alleles as risk factors in Alzheimer's disease, *Annu Rev Med* **47** (1996), 387–400.
- [75] M.M. Saksena, H. Wakisaka, B. Tijono, R.A. Boadle, F. Rixon, H. Takahashi and A.L. Cunningham, Herpes simplex virus type 1 accumulation, envelopment, and exit in growth cones and varicosities in mid-distal regions of axons, *J Virol* **80** (2006), 3592–3606.
- [76] P. Satpute-Krishnan, J.A. DeGiorgis and E.L. Bearer, Fast anterograde transport of herpes simplex virus: role for the amyloid precursor protein of Alzheimer's disease, *Aging Cell* **2** (2003), 305–318.
- [77] L.W. Sequiera, L.C. Jennings, L.H. Carrasco, M.A. Lord, A. Curry and R.N. Sutton, Detection of herpes-simplex viral genome in brain tissue, *Lancet* **2** (1979), 609–612.
- [78] Y. Sergerie, S. Rivest and G. Boivin, Tumor Necrosis Factor-alpha and Interleukin-1 beta Play a Critical Role in the Resistance against Lethal Herpes Simplex Virus Encephalitis, *J Infect Dis* **196** (2007), 853–860.
- [79] S.J. Shipley, E.T. Parkin, R.F. Itzhaki and C.B. Dobson, Herpes simplex virus interferes with amyloid precursor protein processing, *BMC Microbiol* **5** (2005), 48.
- [80] D. Shukla, J. Liu, P. Blaiklock, N.W. Shworak, X. Bai, J.D. Esko, G.H. Cohen, R.J. Eisenberg, R.D. Rosenberg and P.G. Spear, A novel role for 3-O-sulfated heparan sulfate in herpes simplex virus 1 entry, *Cell* **99** (1999), 13–22.
- [81] E.R. Sprague, W.L. Martin and P.J. Bjorkman, pH dependence and stoichiometry of binding to the Fc region of IgG by the herpes simplex virus Fc receptor gE-gI, *J Biol Chem* **279** (2004), 14184–14193.
- [82] T.E. Strandberg, K.H. Pitkala, K.H. Linnavouri and R.S. Tilvis, Impact of viral and bacterial burden on cognitive impairment in elderly persons with cardiovascular diseases, *Stroke* **34** (2003), 2126–2131.
- [83] Z. Talloczy, W. Jiang, H.W. Virgin, D.A. Leib, D. Scheuner, R.J. Kaufman, E.L. Eskelinen and B. Levine, Regulation of starvation- and virus-induced autophagy by the eIF2alpha kinase signaling pathway, *Proc Natl Acad Sci USA* **99** (2002), 190–195.
- [84] Z. Talloczy, H.W. Virgin and B. Levine, PKR-dependent autophagic degradation of herpes simplex virus type 1, *Autophagy* **2** (2006), 24–29.
- [85] G.R. Taylor, T.J. Crow, D.A. Markakis, R. Lofthouse, S. Neeley and G.I. Carter, Herpes simplex virus and Alzheimer's disease: a search for virus DNA by spot hybridisation, *J Neurol Neurosurg Psychiatry* **47** (1984), 1061–1065.
- [86] S.S. Taylor, N.M. Haste and G. Ghosh, PKR and eIF2alpha: integration of kinase dimerization, activation, and substrate docking, *Cell* **122** (2005), 823–825.

- [87] R.L. Thompson and N.M. Sawtell, The herpes simplex virus type 1 latency-associated transcript gene regulates the establishment of latency, *J Virol* **71** (1997), 5432–5440.
- [88] R. Tomazin, A.B. Hill, P. Jugovic, I. York, P. van Endert, H.L. Ploegh, D.W. Andrews and D.C. Johnson, Stable binding of the herpes simplex virus ICP47 protein to the peptide binding site of TAP, *Embo J* **15** (1996), 3256–3266.
- [89] K.L. Tyler, D.G. Tedder, L.J. Yamamoto, J.A. Klapper, R. Ashley, K.A. Lichtenstein and M.J. Levin, Recurrent brainstem encephalitis associated with herpes simplex virus type 1 DNA in cerebrospinal fluid, *Neurology* **45** (1995), 2246–2250.
- [90] T. Valyi-Nagi and T.S. Dermody, Role of oxidative damage in the pathogenesis of viral infections of the nervous system, *Histol Histopathol* **20** (2005), 957–967.
- [91] J.P. Vestey, M. Norval, S. Howie, J. Maingay and W.A. Neill, Variation in lymphoproliferative responses during recrudescence of orofacial herpes simplex virus infections, *Clin Exp Immunol* **77** (1989), 384–390.
- [92] I. Vincent, G. Jicha, M. Rosado and D.W. Dickson, Aberrant expression of mitotic cdc2/cyclin B1 kinase in degenerating neurons of Alzheimer's disease brain, *J Neurosci* **17** (1997), 3588–3598.
- [93] I. Vincent, M. Rosado and P. Davies, Mitotic mechanisms in Alzheimer's disease? *J Cell Biol* **132** (1996), 413–425.
- [94] S. Wahrle, P. Das, A.C. Nyborg, C. McLendon, M. Shoji, T. Kawarabayashi, L.H. Younkin, S.G. Younkin and T.E. Golde, Cholesterol-dependent gamma-secretase activity in buoyant cholesterol-rich membrane microdomains, *Neurobiol Dis* **9** (2002), 11–23.
- [95] M.A. Wozniak, E.B. Faragher, J.A. Todd, K.A. Koram, E.M. Riley and R.F. Itzhaki, Does apolipoprotein E polymorphism influence susceptibility to malaria? *J Med Genet* **40** (2003), 348–351.
- [96] M.A. Wozniak, R.F. Itzhaki, E.B. Faragher, M.W. James, S.D. Ryder and W.L. Irving, Apolipoprotein E-epsilon 4 protects against severe liver disease caused by hepatitis C virus, *Hepatology* **36** (2002), 456–463.
- [97] M.A. Wozniak, R.F. Itzhaki, S.J. Shipley and C.B. Dobson, Herpes simplex virus infection causes cellular beta-amyloid accumulation and secretase upregulation, *Neurosci Lett* **429** (2007), 95–100.
- [98] M.A. Wozniak, S.J. Shipley, M. Combrinck, G.K. Wilcock and R.F. Itzhaki, Productive herpes simplex virus in brain of elderly normal subjects and Alzheimer's disease patients, *J Med Virol* **75** (2005), 300–306.
- [99] M.A. Wozniak, S.J. Shipley, C.B. Dobson, S.P. Parker, F.T. Scott, M. Leedham-Green, J. Breuer and R.F. Itzhaki, Does apolipoprotein E determine outcome of infection by varicella zoster virus and by Epstein Barr virus? *Eur J Hum Genet* **15** (2007), 672–678.
- [100] D. WuDunn and P.G. Spear, Initial interaction of herpes simplex virus with cells is binding to heparan sulfate, *J Virol* **63** (1989), 52–58.
- [101] W.H. Yu, A. Kumar, C. Peterhoff, L. Shapiro Kulnane, Y. Uchiyama, B.T. Lamb, A.M. Cuervo and R.A. Nixon, 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** (2004), 2531–2540.
- [102] R.Y. Zhao and R.T. Elder, Viral infections and cell cycle G2/M regulation, *Cell Res* **15** (2005), 143–149.