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Herpes simplex virus type 1 and Alzheimer's disease: link and potential impact on treatment

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Abstract

Introduction: Alzheimer's disease (AD), the most common form of dementia worldwide, is a multifactorial disease with a still unknown etiology. Herpes simplex virus 1 (HSV-1) has long been suspected to be one of the factors involved in the pathogenesis of the disease.

Areas covered: We review the literature focusing on viral characteristics of HSV-1, the mechanisms this virus uses to infect neural cells, its interaction with the host immune system and genetic background and summarizes results and research that support the hypothesis of an association between AD and HSV-1. The possible usefulness of virus-directed pharmaceutical approaches as potential treatments for AD will be discussed as well.

Expert opinion: We highlight crucial aspects that must be addressed to clarify the possible role of HSV-1 in the pathogenesis of the disease, and to allow the design of new therapeutical approaches for AD.

Keywords: Alzheimer's disease, Human herpes simplex virus type-I, immunity, mild cognitive impairment, pharmaceutical treatments.

Article highlights

- A possible role for the reactivation of HSV-1, a virus that commonly infects humans, in the pathogenesis of AD is suggested by a string of observations.
- After the initial infection HSV-1 persists in latent state in trigeminal ganglia and, upon reactivation can reach the brain, as showed by detection of HSV-1 viral genome in brain of elderly people.
- Reactivation of HSV-1 can cause neuronal damage, directly and/or by induction of inflammation.
- Host immunity is critical to control viral reactivation and it is impaired in AD patients.
- If HSV-1 infection is a risk factor for AD, antiviral treatments could be useful in the prevention/treatment of this disease.

1. Introduction

Alzheimer's disease (AD) is a common form of senile dementia that in 2018 affects 50 million individuals around the world, and that in the near future will become one of the biggest medical issue, at least in western countries, due to the increase of life expectancy. Thus, the World Alzheimer Report predicts that in 2050 a total of 152 millions of people will suffer from AD [1]. AD is an inflammatory neurodegenerative disease characterized by progressive decline of normal cognitive abilities and of intellectual impairment, with a consequent loss of working abilities and the incapacity to perform daily living activities [2] Importantly, an intermediate stage between AD and healthy aging is Mild Cognitive Impairment (MCI), defined as a subjective and objective decline in cognitive performance that is greater than expected for individual's age and education level, but does not meet criteria for the diagnosis of dementia [3]. The brain of AD patients is mainly characterized by the presence of intraneuronal neurofibrillary tangles, formed by abnormal phosphorylated Tau protein, and extracellular senile plaques, formed by amyloid-β (Aβ), a peptide produced by the proteolysis of amyloid precursor protein [4].

The etiology of AD is still unknown: the disease is defined as multifactorial as several factors interacting with each other are suspected to be involved in its development. Amongst these factors important roles are played by genetic background, in particular the ε4 allele of the *apolipoprotein E (ApoE*) gene, infections and inflammation.

A role for pathogens in the development and progression of AD [5] has long been suspected, and human spirochetes, fungi, *Borrelia burgdorferi*, *Chlamydophila pneumomniae*, *Helicobacter pylori* and human herpes simplex virus type 1 (HSV-1) have been envisioned as possible culprits. The possibility that HSV-1 could be involved in the pathogenesis of AD, in particular, was originally hypothesized by Ball in 1982, when he proposed that "*reactivation* [of HSV-1] *travelling centripetally* [through known anatomic nerve fiber connections into the limbic areas of the brain] *might be the cause of the degenerative lesions typical both of Alzheimer's Disease and of the normal aged human brain*" [6]. After the primary infection, HSV-1 can remain latent in the nervous system; very rarely, its reactivation can result in an acute and often lethal form of encephalitis [7]. In this case, the brain area involved are the hippocampus as well as the temporal and frontal lobes; interestingly these are the same brain area that are affected in AD. This observation offered one of the first supports to the Ball hypothesis. In 1991 Jamieson and coworkers found traces of HSV-1 genome in brains of sporadic AD patients; importantly viral genome was present in those areas – hippocampus, temporal and frontal lobes – typically affected by AD [8]. The importance of

these results was somewhat diminished by the fact that HSV-1 genome was also detected in brains of non-demented elderly individuals suggesting that brain HSV-1 latent infection is a relatively frequent event. Interestingly, in 1997 Itzhaki and coworkers added a further piece to this scenario by showing that the combination of *ApoE4* allele, a genetic risk factor for AD, and HSV-1 in brain, greatly augments the risk of developing AD [9]. Some years later the same researchers proposed that recurrent reactivation of latent HSV-1 in brain results in localized neuron damage through direct and indirect toxic effects of the virus [10].

To note, the HSV-1 DNA detection in AD brain varies considerably in literature, from the absence [11] or small proportion of positivity (2%) [12], to a higher DNA presence (35%) [13], up to almost totally positive AD brain samples (70-100%)[8,9]: differences in methodological sensitivity, reduced DNA yield extraction from fixed material with long duration of storage may be the reasons of these discrepancies. Another important aspect is that not only HSV-1, but also other herpesvirus species (i.e. HHV-6A) [14] as well as bacteria (i.e. *Borrelia burgdorferi* and *Chlamydia pneumoniae*) and fungi can be detected in brain tissues [15], highlighting the need to perform larger studies to confirm these data and to analyze the presence of other uninvestigated pathogens.

Based on literature databases (up to March 2019), in this review we summarize the main findings and results that support the presence of a link between AD and HSV-1, link that, acting in synergy with other, yet unidentified factors could have a role in the onset and development of this neurodegenerative disease.

2. Herpes simplex virus

Herpes simplex virus (HSV) type 1 and type 2 are human neurotropic, host-adapted pathogens whose lifestyle is based on a long-term dual interaction with the infected host that can establish both lytic and latent infections [16]. These viruses establish latent infections in sensory ganglia; such infections can undergo reactivations that can be either asymptomatic or symptomatic. In this case, cold sores, keratitis, blepharitis, meningitis, encephalitis, genital infections or systemic and severe conditions in immune compromised patients can be observed [17]. Dissemination is very common in human communities owing to latent infection, periodic reactivation, and asymptomatic virus shedding. HSV is a highly prevalent infection worldwide: 67% of the population under the age of 50 was shown to be infected with HSV-1 and 11% harbors HSV-2 [18]. The HSV-1 infection is generally acquired during childhood, although during the past twenty years

in developed countries a decreased trend of seroprevalence has been observed in adolescent and young adults [19].

2.1 Structure of HSV-1

The HSV-1 virion includes 4 components: the outer envelope, the tegument, the capsid and the core [20] (Figure 1). The envelope consists of a lipid bilayer and anchors approximately 11 viral glycoproteins, four of which (gB, gD, gH, and gL) are essential in allowing virus entry into cells [21]. The tegument is an unstructured amorphous layer that surrounds the capsid, it includes more than 20 proteins and is important in the regulation of viral replicative cycle [22]. The capsid is composed by 162 capsomeres that are organized within an icosahedral structure. The core is the central domain of the virus and contains the linear, double stranded, 152 kbp DNA (dsDNA) genome . The HSV genome can be divided into two unique sequences, designated as unique long (UL) and unique short (US), flanked by large repeated sequences, internal (IRL and IRS) and terminal (TRL and TRS). The viral genome encodes approximately 90 unique transcriptional units, of these at least 84 encode proteins that can perform many functions in the infected cell.

2.2 Virus attachment and entry

To initiate infection, HSV-1 binds at least three different classes of cell-surface receptor and fuses its envelope with the plasma membrane. The entry of HSV-1 into epithelial cells is a complex process [23]. Its envelope contains 11 glycoproteins that are very important in mediating the initial steps of viral attachment and entry into the cell as well as facilitating cell-to-cell spread of the virus [21]. The virus enters by fusion with the plasma membrane or via endosomes through an orchestrated process that requires gB, the most ubiquitous envelope glycoprotein in human herpesviruses, and three other essential envelope glycoproteins (gD and gH/gL), activated in a cascade fashion.

Glycosaminoglycan-chains (GAGs) are expressed on the host cell; among these, heparan sulfate (HS) proteoglycans (HSPGs) are the primary attachment receptors for HSV gB and gC. The interaction of gC and gB with HS receptor is labile and is reinforced by the participation of gD in the process [24,25]. After the viral and the host membranes are brought into close vicinity, gD interacts with one or more cellular receptors [25] inducing a conformational change to the heterodimer gH/gL, modifying it into a form that interacts and triggers the fusogenic activity of gB [23]. By the interaction of gH/gL with gB, the prefusion glycoprotein shifts to a post fusion

conformation that is capable of forming the fusion pore.

Fusion pore completes the fusion process; at this point the virion particles, along with the tegument, enter the cytoplasm. After penetration into the cytoplasm, some tegument proteins remain in the cytoplasm while other are transported to the nucleus or remain associated with the capsid that travels via microtubule network, to the nucleus [26]. The processes of transcription and replication of the viral genome, as well as the assembly of progeny capsids, take place within the nucleus.

2.3 Lytic cycle

Ones inside the nucleus, viral DNA is rapidly circularized and viral genes are expressed in a tightly regulated, interdependent temporal sequence. The lytic cycle of HSV-1 can be divided into three phases, which involve the expression of three groups of viral genes: α or Immediate Early (IE), β or Early (E) and γ or Late (L) [20]. IE genes are first expressed, about 2-4 hours post-infection, by the combined action of the tegument viral protein (VP) 16, known also as α-TIF (α-trans-inducing factor) with at least two cellular proteins, the octamer-binding protein (Oct-1) and the host cell factor 1 (HCF-1), that targets the TAATGARAT motif upstream of the IE promoters stimulating the transcription of five IE genes [27]. During this early stage the corresponding proteins are synthesized: infected-cell polypeptide (ICP)4, ICP27, ICP22, ICP0 and ICP47. ICP4, an essential viral protein, is a DNA binding protein that interacts with basal transcription factors, such as TATA-binding protein (TBP), TFIIB, TFIID and TAF250 [28]. This interaction activates most E and L genes and represses the transcription of other IE genes [28]. ICP27, in particular, is responsible for the post-transcriptional modifications that control viral mRNA splicing, represses the expression of some IE and E proteins and induces L protein expression [29]. ICP27 also contributes to the decrease of cellular genes expression and is an important regulator of host cell fate [29]. ICP22 plays an important role in replication and pathogenicity of HSV-1 since his function is crucial to allow the optimal expression of E and L genes, to promote the formation of functional virions composition, and to permit capsid nuclear egress [30]. ICP47 binds to the transport proteins TAP1 or TAP2, preventing the transport of viral peptides to the endoplasmic reticulum [31]. ICP0 is a transactivator that promotes transcription of many viral and cellular genes during the lytic infection and is essential for latency reactivation [32]. ICP0 is a RING finger protein and exhibits two distinct ubiquitin ligase activities that interact with different cellular E2 ubiquitin-conjugating enzymes and therefore targets different cellular substrates [32]. The major mechanism of action of

ICP0, thus, may be the degradation of specific cellular proteins via the ubiquitin-proteasome pathway [32]. After IE gene transcription and expression, the E phase starts, this leads to the production of proteins responsible for viral DNA synthesis and packaging. Expression of E genes requires at least the presence of functional ICP4 and reaches a peak at 4-8 hours post-infection. During this phase, proteins mostly act as enzymes and are responsible for the replication of the viral genomes that are produced. Among them important roles are played by DNA polymerase (UL30/UL42 complex), thymidine kinase (TK), single-stranded DNA (ssDNA) binding protein (SSB), also known as ICP8, a DNA helicase-primase, and UL9 [20,21]. Finally, viral DNA replication stimulates the transcription of L genes that mainly consist of structural proteins of the virion, such as tegument and envelope proteins, and proteins responsible for the assembly of the viral particle. The assembly of the viral particle starts in the nucleus: a procapsid, a spherical fragile intermediate is formed, the DNA is then packaged and undergoes a morphological change to become a mature icosahedral capsid [20,21].

2.4 HSV-1 infection in neuronal cells and latency establishment

The initial site of entry within neurons is usually at the axon terminus near peripheral epithelial cells [33]. The entry mechanism in sensory nerves depends on the cell type and on the interaction of viral glycoproteins with the cellular receptors [33]. During infection of neurons, HSV-1 causes a biphasic remodeling of the actin cytoskeleton by first inactivating and then reactivating cofilin-1, resulting in F-actin assembly and disassembly during early and late stages of infection [33]. After the fusion, the capsid is transported retrogradely to the nucleus along the microtubules; this transport involves the dynein/dinactin complex [34]. Release of the viral genome in the neuronal nucleus results in its rapid association with histones to create a circular episomal DNA. Viral genes are not expressed at this stage with the exception of LAT gene which is expressed in high abundance [35]. The functions of LATs transcripts are not completely clear, but they have a fundamental role in maintaining viral latency which can silence lytic gene expression and block apoptosis [36]. During latency, chromatin plays an important role: in fact, the histones associated with the latent viral genome are often modified [35]. Experimental animal model have been used to show that in the trigeminal ganglia of infected mice the expression of genes IE, E and L occurs in the first 24-72 hours after infection, whereas in the following period their expression decreases and LAT transcripts accumulate: this results in the establishment of latent infection [37]. Generally sensory neurons do not express lytic proteins, but several stress stimuli may induce the

reactivation of the virus in these neurons. The assembled capside exits the nucleus through the inner nuclear membrane and merges with the outer nuclear membrane. Viral capsids containing inner tegument proteins with or without an envelope, and vesicles associated with glycoproteins and tegument proteins are targeted for active transport along microtubules in axons using the neuronal secretory pathway. The virus is then transported in anterograde manner to the area of primary infection where a new productive infection starts [38].

3. Virus-host interaction

3.1 HSV-1 and apoptosis

Apoptosis, also called programmed cell death, is an important innate mechanism that eliminates pathogen-infected cells. This mechanism has a crucial role in limiting viral replication and transmission emphasizing the importance of this host-interaction process in viral pathogenesis. HSV has evolved in the manner to modulate apoptosis in different cell types either with anti-apoptotic genes to promote the generation of new viral progenies or with pro-apoptotic genes to promote cell death to favor viral release and shedding. LAT, US3 and many other HSV-1 genes, including ICP4, ICP34.5, UL54, US1, US5 and US6 [39] are suspected to regulate apoptosis. The ability of LAT to interfere with apoptosis correlates with its ability to promote spontaneous reactivation [40]. LAT inhibits multiple steps in apoptotic cascades and inhibit dephosphorylation of pAKT levels to promote cell survival and indirectly to control caspase 3 and block apoptosis [41]. The two small RNAs encoded by the initial part of LAT gene are involved in the inhibition of caspase 8- and caspase 9-induced apoptosis [42]. The US3 is a multifunctional protein kinase that plays various roles in the viral life cycle by phosphorylating a number of viral substrates [43]. US3, can block apoptosis, as it activates antiapoptotic substrates targeted by the cellular cyclic adenosine monophosphate (cAMP)-dependent protein kinase [44]. Benetti and Roizman have demonstrated that Us3 blocks the proteolytic cleavage of caspase 3 inhibiting its activation and consequently the apoptotic event [45]. Many other viral proteins have a role in blocking apoptosis (summarized in table 1), in particular, US5 that encodes for the non-essential glycoprotein J (gJ) inhibits caspase 3/8 activation through Fas-mediated or granzyme B induced apoptosis [46,47].

3.2 HSV-1 and host immunity

Multiple innate immune pathways cooperate to form a barrier to viral infection: particular receptors (called pattern recognition receptors, PRRs) survey cells surface and intracellular compartments for specific pathogen-associated patterns (PAMPs), including viral DNA, RNA and

proteins. Toll like receptor-2 (TLR2) recognizes HSV-1 envelope glycoproteins (gD, gH, gL and gB), whereas on TLR9 and TLR3 detect respectively viral GC-rich/AT-repeated DNA regions and double strand RNA (dsRNA) [48]. TLR3, in particular, has an important protective role against HSV-1, as witnessed by the observation that patients with TLR3 dominant negative mutations are more susceptible to herpes simplex encephalitis [49].

A number of cytosolic receptors have been suggested to sense HSV-1 nucleic acid as well. Thus, RIG-1 and MDA-5 sense dsRNA whereas other receptors (cGAS, DNA-PK, DAI, IFI16, DDx41, DHx9, DHx36) are activated by viral DNA, although for many of these receptors the precise mechanism remains to be investigated [48].

After the binding of viral ligands to receptors, adaptors (such as MyD88, TRIF, MAVs, STING) are recruited, kinase proteins (as IKK, TBK) are activated and, after nuclear translocation of transcription factors (NF-Kb; IRF3; IRF7), type I IFNs (IFN-α and IFN-β) are generated; IFNs are crucial for the control of infection as they production induces the expression of hundreds of responsive genes (ISGs), among which pro-inflammatory cytokines (IL-6, IL-12, TNFα) and chemokines (CXCL-10, CXCL-9) that promotes viral clearance through the recruitment of specialized immune cells.

The inflammasome is activated as well by HSV-1: this process is started by DNA binding to sensor molecules (IFI16 and NOD-like receptor family pyrin domain containing 3 or NLRP3) and results in the production of the pro-inflammatory cytokines IL-1β and IL-18 [50]. IFI16 (IFNγ-inducible protein 16), in particular, is another important player in the immune response to HSV-1. Thus, IFI16 binds the viral genome in the cytoplasm of the infected cell through STING/IRF3 proteins [51]; this leads to type 1 IFNs expression, and, through the activation of the inflammasome to IL-1β and IL-18 release. Notably this proteins has a nuclear action as well, as in the nucleus it promotes heterocromatin assembly on HSV-1 DNA and silences HSV-1 expression [52].

Another central element of innate immunity is the complement system**,** whose components (in particular C1q e C3b) can directly bind to HSV-1. This leads to antibody (Ab)-mediated neutralization of the virus, inhibition of receptor binding on the surface of cells, reduction of the infectivity, and lysis of infected cells. The classical pathway of the complement is also triggered in an antibody-independent manner when C1 directly binds to virions or to infected cells. The complement system is also essential in the regulation of adaptive responses by enhancing T and B lymphocytes-mediated responses.

The production of cytokines and chemokines induces adaptive antigen-specific cell response. Viral antigens, bound to MHC-I and presented by antigen presenting cells (APC), are recognized by CD8+T lymphocytes, this results in the activation of their effector mechanisms and the establishment and maintenance of memory T cells. Granzymes and perforins induce the lysis of infected cells; IFN-γ production by activated T lymphocytes enhances the processing of viral peptides that will be presented by MHC, thus expanding the potency of cells-mediated immune responses.

Overall, if the innate immunity system mainly controls the initial phase of HSV-1 replication, cell-mediated adaptive immunity play a major role in preventing reactivation from latency and limiting of viral spread [53].

A novel population of T cells known as tissue-resident memory CD8+ (TRM) T cells has been recently described, these cells are critical for peripheral immune surveillance and protection against viral infection [54]. Following a primary HSV-1 infection, CD8+TRM cells are generated and retained for long time in non-lymphoid tissue, including ganglia and mucosa [55]. CD8+TRM survey latently infected niches, if infected cells are detected, IFN-γ and granzyme B will promptly be produced. The regulation mechanisms for TRM cell during viral reactivation in central nervous system (CNS) remain unclear but it is conceivable that vaccines boosting the resident CD8+TRM cells could be a viable option for protection against HSV-1 infection and/or reactivation.

$CD4^{+}$T cells are critically important for the prevention of HSV genital infection [53] and they are responsible for herpes stromal keratitis, following the production of Th1 cytokines. Notably, these cells are also present in sensory ganglia and spinal cord, where they mediate clearance of HSV-1 from neural tissue and persist for a long time after infection.

The Ab response to HSV-1 infection is broad, polyclonal and is mainly directed towards envelope glycoproteins as well as toward tegument and capsid proteins [56]. During infection gD and gB are the most important viral proteins in stimulating the production of IgGs; these Ab will then prevent the interaction between HSV-1 and its cell receptors, neutralizing viral infectivity. The role of humoral immunity in protection against HSV-1 infection is however still controversial [57]: naturally induced Abs are not able to protect from viral reactivation and to completely avoid virus transmission. This is at least partially the consequence of the ability of the virus to develop immune evasion strategies to inhibit neutralizing Ab response, including transmission through the cell-to-cell spread that protects the virus from immune surveillance.

Interestingly, HSV-1-specific cell-mediated immune responses can be detected in HSV-1 seronegative individuals [58]; this could be a consequence of HSV-1 cross-reactivity with other herpesviruses, including varicella zoster virus (VZV): these two viruses in fact present homologies in numerous genes; immunity related to VZV infection or vaccination could thus modulate HSV-1 or HSV-2 infection and vice versa [59].

Notably attempts to obtain an effective vaccines based on induction of Ab response by gB/gD envelope glycoproteins, showed only limited efficacy in humans, suggesting that a more complete protection against HSV-1 infection might be obtained upon stimulation of high titers of neutralizing Abs and, likely, by designing vaccines that will preferentially stimulate HSV-1-specific cell -mediated immunity (in particular tissue resident memory T-cells [60].

3.3 Evasion from host immunity

HSV-1 has evolved several mechanisms to counteract the host immune response, allowing its persistence in infected hosts humans (reviewed in 48 and 61 and summarized in Table 2). Usually the host uses the xenophagy - *i.e.* the autophagic degradation of intracellular pathogens - to block HSV-1 infection [62]. HSV-1 xenophagy is stimulated by type 1 IFNs and is mediated by antiviral proteins, including the double stranded RNA-dependent protein kinase R (PKR). PKR is activated upon binding a double-stranded RNA; this precludes protein synthesis in virus-infected cells by phosphorylation of the α subunit of eukaryotic initiation factor 2 (eIF-2α). HSV-1 produces two different proteins to evade this defense mechanism: US11 and ICP34.5. In particular, US11 directly binds to PKR, blocking its phosphorylation and inhibiting its activity, whereas ICP34.5 recruits phosphatase proteins of the host to phosphorylate eIF2α with consequent translation blockage. Both these two viral proteins are required for full resistance to type 1 IFN-mediated immune response, as both inhibit autophagic degradation of HSV-1 proteins [63]. To note, ICP34.5 is able to inhibit autophagy also in an alternative way, binding the essential autophagy protein beclin 1. LAT also can interfere with the type 1 IFN pathway, as it was demonstrated in animal models that this transcript regulates the expression of IFN in neurons [64]. Another HSV-1 key protein is the US3 tegument protein. US3 dampens the IFN-β signaling and reduces IFN–γ production by cytotoxic T lymphocytes, protecting the infected cells from lysis [65]. US3 can also modulate TLR responses, inhibiting the TLR2 and TLR3 signaling, and, in association with gB, interfering with the activity of natural killer cells, by inhibiting CD1d antigen presentation and their consequent activation [66].

Finally, HSV-1 can also evade the complement-dependent host immune response by binding the viral Fc receptor (vFcR) to the Fc end of IgGs; this results in the suppression of complement-dependent neutralization and the survival of infected cells.

3.4 Control of HSV-1 reactivation

Normally the nervous system activates effective innate and adaptive immune responses to contain HSV-1 infection [67]. In particular, upon HSV-1 infection human microglia cells produce a number of pro-inflammatory cytokines and chemokines [68], with a concomitant activation of lymphocytes to control viral replication [69]. Therefore, a delicate balance between host surveillance and viral immune evasion mechanisms drives HSV-1 into latency. The virological mechanisms, related to latency and reactivation, have been extensively investigated [16,69]. The absence of viral product during latency in neurons lead to hypothesize that cellular factors act as "trigger" for HSV-1 reactivation, but we have only a partial knowledge of these factors. Reactivation can be induced in humans by environmental stress (UV radiation, fever, fatigue, hormonal change, cranial trauma, immunesuppression) and by other stimuli (i.e. NGF deprvation or histone deacetilase inhibition), as observed in cultured neurons or using animal models [16]. It is important to underline that these experimental approaches are often imperfect and they don't completely represent the natural occurring reactivation in humans, where probably other characteristics (related to cell type, species, or viral strain) can determine the results of this complex mechanism. Other concomitant infections, as i.e. with human Cytomegalovirus (CMV) [70], can be considered as well important factors inducing HSV-1 reactivation. The current understanding of the factors regulating latency reactivation is limited yet and much more knowledge should be gained before to have a clearer picture of viral reactivation process.

In vivo studies in the murine model showed that HSV-1-infection of trigeminal ganglia results in the expression of major histocompatibility complex (MHC) class II antigens and triggers the production of pro-inflammatory and neuroinflammatory cytokines and proteins [71]. Interestingly, in the case of encephalitis, disease severity and the disruption of the blood-brain barrier are the consequence of the pro-inflammatory response to the virus [72], and immune markers of lymphocyte activation remain increased in cerebrospinal fluid for many months after the resolution of infection [73].

It is believed that reactivation can be controlled mainly by an efficient generation of $CD8^+$ and to a lesser extent of CD4+T lymphocytes. Most of the CD8+T cells infiltrating trigeminal ganglia (TG) are

activated and secrete IFN-γ or TNF-α, but only few also express granzyme B. IFN-γ inhibits the expression of ICP0, a potent transactivator of viral genes that promote lytic cycle and granzyme B can degrade the IE protein ICP4, inhibiting viral replication in the absence of neuronal apoptosis [74].

Induced regulatory T cells (iTreg*)* were recently indicated to be an HSV-1 "latency switch" through the regulation of HSV-1 specific -$CD8^+$T cell. Thus, after acute primary infection, iTregs increase, this facilitates HSV-1 latency by suppressing cytotoxic response. Environmental stresses, on the other hand, result in an increase of iTreg cells and modulate glucocorticoid expression; this results in a decrease of $CD8^+$T cell surveillance and HSV-1 reactivation [75]. Although further study are needed to better understand the fine mechanism of their interaction with $CD8^+$T cells, manipulation of iTreg-cell-based could help in the prevention of damages derived from HSV-1 reactivation.

Most of HSV-1 infected individuals are "asymptomatic", as viral reactivation is infrequent. In addition, only a minority of "symptomatic" subjects shows evident symptoms and recurrence of disease. The complex mechanisms that lead asymptomatic subjects to be "naturally protected" from clinical disease, are not completely known, and seem to derive from variations in the number and nature of the HSV-1 antigens (mainly gB and gD) that are targeted by cell immunity [76]. The identification of these "protective" epitopes, that characterize naturally protected individuals, represents a remarkable advance in the understanding of the immunological control of HSV-1 reactivation. Studies on whole profiles of "protective" or "pathogenic" HSV-1 antigens will help to develop new effective vaccine strategies. Moreover, the phenotypical and functional characterization of the viral epitopes that are presented to T lymphocytes in relation to different HSV-1 clinical manifestation will further clarify the nature of the relationship between host immunity and pathogenesis.

4 The interplay between HSV-1 and AD

4.1 HSV-1 specific immunity in AD patients

Evidences of microglia-mediated inflammation in AD brain have been reported by many authors. Thus, in AD brains, TLR expression is upregulated and pro-inflammatory cytokines and chemokines are produced [77,78]. Although it is hard to know if these features are a consequence of the disease or contribute to its pathogenesis, a realistic hypothesis is that a vicious cycle is created, where IFN-γ-producing T cells infiltrate the CNS [79], driving Aβ deposition/accumulation, while Aβ

activates glial cells to produce others inflammatory mediators, leading to chronic process of neuronal dysfunction and cellular death.

In a recent work, De Chiara and co-authors have established a mouse model of HSV-1 infection where they show AD-like phenotype in animal brains after multiple viral reactivation after thermal stress. In this work they demonstrated that the accumulation of Aβ, hyperphosphorilated Tau and cognitive deficit was proportional to the numbers of HSV reactivations [80]. HSV-1 repeated reactivations from latency are likely to have a significant impact on the pathogenesis of AD [6] as such reactivations would concur to neuronal damage directly, via viral action and indirectly as a consequence of the upregulation of neuroinflammation. Several authors investigated the possible role of HSV-1 in the pathogenesis of AD by analyzing HSV-1-specific humoral immunity [reviewed in 81]. After a first important prospective study correlating the presence of HSV-1 specific IgM (an indicator of episodes of HSV-1 reactivation) with risk to develop AD [82], other authors observed that elevated HSV-1-IgG Ab titers are significantly more frequent in patients compared to age-matched healthy controls [83]. A positive correlation between HSV-1-specific IgG titers and the cortical volumes of brain regions typically affected in AD was also described in mild AD patients [84]; this data indicate a possible protective role of HSV-1-specific humoral immunity in the early phase of AD. Notably, this effect is specific for HSV-1, as no relations were observed between CMV- and human herpesvirus 6 (HHV-6)-specific Abs and either magnetic resonance imaging (MRI) or clinical parameters in AD patients [84,85].

At least two other experimental observations favor the hypothesis that HSV-1-specific Abs have a protective effect against AD development. Thus: 1) AD incidence increases with age, possibly as a consequence the natural decline of the potency of immune responses seen in senility [86]; 2) age-dependent BBB injuries, although detected in normal brain, are more pronounced in MCI subjects compared to age-matched normal subjects [87]: consequently the high concentration of HSV-1 specific Ab could limit viral reactivation in that brain regions where the BBB is disrupted.

The efficacy of humoral responses is also modulated by Ab avidity, that is the relative strength with which Abs bind antigens. A significantly increase of the HSV-1 IgG avidity index was described in MCI compared to AD individuals [88]; additionally another study showed that HSV-1-specific Ab avidity was significantly higher at baseline in MCI-non-converters compared to those MCI who did develop AD [89]. Notably, in that study, a positive correlation was observed between avidity of Abs and cortical volumes (MRI analyses). However, other experiments are needed to better understand the role of Ab avidity in neurodegeneration.

The biological properties of Abs are different in different IgG subclasses. A comparative analysis of the distribution of the four HSV-1-specific IgG subclasses showed a significantly increased frequency of HSV-1-specific IgG3, the subclass with the strongest complement activation ability, in MCI compared to AD and healthy controls (HC) [90,91]. It is also known that at least three HSV-1 envelope glycoproteins regulate complement system and are able to prevent or reduce phagocytosis-mediated virus neutralization: the heterodimer gE-gI binds Fc portion of Ab molecule and function as IgG Fc receptor (vFcγR) [92]. Moreover gC directly binds complement components (C3 and C5), inhibiting complement activation and virus neutralization as well as complement mediated lysis of infected cells [93]. It is important to note that HSV-1 specific IgG3 Abs also have the highest neutralizing capacity of all Ab subclasses [94]. The development of an IgG3 response in MCI patients could thus be interpreted as an attempt to prevent HSV-1 reactivation. Finally, the protective effect of Abs in the early phase of AD could be also due to $CD4^+$T lymphocytes functional impairments: a recent paper showed that Ab access to neuronal tissues is controlled by local secretion of IFN-γ from $CD4^+$ memory T cells in a mouse model of genital HSV infection [95]. To note, other viruses are suggested to be involved in cognitive impairment, as Epstein Barr virus (EBV) and CMV [96]: new researches are surely needed to better understand if one or several microbes are involved in AD.

4.2 HSV-1, amyloid beta and calcium

One of the major hallmark of AD is the presence of cortical senile plaques in the brain of affected patients [4]. The major component of these plaques is Aβ, that derives from the cleavage of the ubiquitous membrane protein amyloid precursor protein (APP) that in central nervous system (CNS) is expressed by neurons, astrocytes and microglia in 8 different isoforms. Its primary physiological function is not known, but it seems to be involved in neuronal survival, synaptic plasticity and cell adhesion [97]. In healthy individuals APP cleavage is mainly mediated by β-secretase, while in AD patients γ pathway is more enhanced resulting in overproduction of Aβ, in particular its fragments 1-40 and 1-42, which are derived from the cleavage of precursor protein (APP) by β- (BACE-1) and γ-secretases. A direct relation between Aβ and HSV-1 was demonstrated in an *vitro* study showing that HSV-1 infection of human neuroblastoma cells reduces APP levels and increases the APP 55 KDa C-terminal fragment. Moreover, HSV-1-infected human neuronal and glia cells were shown to be characterized by an increase of BACE-1 and γ-secretases, leading a concomitant intracellular increase of Aβ 1-40 and 1-42 [98]. The brain accumulation of Aβ 1-42

was then confirmed by studies performed in HSV-1 infected BALB/c mice [98] as well as in HSV-1 infected human and rat neuronal cells [99]. Data in rat cortical cells indicated that HSV-1 plays a role in the dualism between Ca2+ and Aβ, since the Ca2+ signalling attendant to viral attachment and entry induce modifications in APP that lead to its cleavage and the consequent formation of Aβ 1-42 accumulation [100]. These findings are particularly interesting as AD mice are characterized by elevated levels of Ca^{2+} in neurons [101], and genes involved in calcium signaling are deregulated in neurons of AD brains [102]. Furthermore, the link among HSV infection and AD amyloid plaques formation are associated also to the homology between the internal amino acid sequence of HSV-1 glycoprotein B (gB) and the carboxyl-terminal region of Aβ; therefore the intracellular processing of gB in neurons can lead to the generation of amyloid fragments that accelerates *in vitro* Aβ aggregation [103]. Moreover it was found that the Us11 HSV-1 protein ligates a microtubule-binding protein involved in APP trafficking [104], possibly altering its cellular distribution [105].

The co-localization of HSV-1 DNA and amyloid plaques in AD patients' brain, besides being a strong evidence of a possible relation between HSV-1 and AD, supports the idea that Aβ could have an antimicrobial role and could be secreted to protect neurons from injuries [106]. In the case of HSV-1, in particular, *in vitro* and animal studies demonstrated that repeated viral reactivations can result in APP processing and accumulation of Aβ and other APP fragments, although a direct effect of HSV-1 on Aβ accumulation remains to be confirmed in humans in vivo. Aβ anti-infective activity has been recently showed, even if its accumulation as oligomer results neurotoxic and can cause the destruction of brain structure and functionality [107].

4.3 HSV-1 and tau protein

Another hallmark of AD pathology is the hyperphosphorylation of tau protein. The main function of this protein is to stabilize microtubules, a process regulated by its phosphorylation. The normal level of tau phosphorylation is a consequence of dynamic regulation of tau kinases and tau phosphatases.

In AD, the hyperphosphorylated tau is aggregating in paired helical filaments (PHF) and neurofibrillary tangles (NFT) and can no longer perform this role. It has been proposed that this fact induces microtubules disintegrations dismantling cytoskeleton and thus neuronal transport [108]. This may first affect communications between neurons and finally lead to cell death [109]. Thus, HSV-1 infection causes an increase of hyperphosphorylated tau protein in murine cells [110],

and in neuroblastoma cells [111]. Wozniak and co-workers have demonstrated that HSV-1 phosphorylates several sites of tau by inducing over-expression of two enzymes: GSK3β and PKA which are involved in protein phosphorylation [112]. To note, treatment of neuronal cells with antiviral drugs prior to HSV-1 infection prevents tau protein hyperphosphorylation [110].

4.4 HSV-1 and autophagy

Autophagy is the physiological mechanism used by cells to disassemble and degrade unnecessary or dysfunctional components. AD is associated with a deregulation of such mechanism; this results in a decreased clearance of Aβ-containing autophagic vacuoles, and Aβ accumulation [113]. An *in vitro* study on human neuron cells demonstrated that HSV-1 directly impairs autophagy, increasing the intracellular accumulation of autophagosomes [114] and reducing Aβ autophagic degradation [115].

4.5 HSV-1 and oxidative stress

The reciprocal balance between free radicals and antioxidants is altered as well in AD, where oxidative stress with a consequent damage to cellular molecules is present. Thus, DNA, RNA and proteins that are damaged as a result of oxidative stress are observed in the AD brain [116]; of note, oxidative stress is one of the main culprits for neuroinflammation and neurodegeneration. *In vivo* studies have proven that HSV-1 can cause oxidative stress and neuronal damage in rabbits [117] as well as in mice [118]; these findings were confirmed in human neuronal cells, where oxidative stress was shown to also result in intracellular accumulation of Aβ [119]. Notably, HSV-1 can induce oxidative stress via mitochondrial damage, another cellular alteration seen in AD [120], can interfere with axonal transport of mitochondria in rat neurons *in vitro* [121] and can induce the degradation of mitochondrial DNA and mRNA in cell lines [122].

4.6 HSV-1 and host genetics

The major AD-associated genetic risk factor is *ApoE* [123]. *ApoE* codes for ApoE protein, a fat-binding 299 amino acid glycoprotein component of lipoproteins that plays a fundamental role in the maintenance and homeostasis of neurons. Three different isoforms of ApoE exist, and each of them differs in the ability to accomplish these critical tasks. In brain tissues, ApoE is produced by astrocytes and microglial cells, and is involved in different pathways, including lipid transport, lipid metabolism regulation, synaptic plasticity, cell signaling and neuroinflammation. Interestingly,

ApoE2 and ApoE3 isoforms are very effective in maintaining and repairing neuronal cells, whereas ApoE4 works less efficiently. Several studies showed that *ApoE4* is the major known genetic risk factor for AD [reviewed in 123]. Although the exact mode of *ApoE4* action in AD is unknown, higher HSV-1 viral titers and an increased expression of HSV-1 IE genes were detected in brains of cognitive deficits-affected mice carrying *ApoE4* genotype [124].

A working hypothesis tying together AD, *ApoE4* and HSV-1 is that ApoE4 competes with HSV-1 for attachment to the viral entry receptors HPSGs less effectively than ApoE3 and ApoE2 [125]. Other susceptibility genes for AD were identified in genome-wide association studies (GWAS); these include phosphatidylinositol binding clathrin assembly protein (PICALM) and nectin 2 (NC-2). Both these genes produce proteins that are associated with HSV-1 lifecycle: in particular PICALM is involved in the viral exit from the nucleus [126], whereas NC-2 codes for the HVEb adhesion molecule, one of the receptors for the entrance of HSV-1 into host cells [127].

Another group of susceptibility genes for AD are genes involved in the host immune response against infection. In particular: 1) clusterin inhibits the formation of the membrane attack complex (MAC), usually activated by infection, by interacting with several of its components [128]; 2) complement receptor 1 (CR1) binds complement C3 components, blocking the complement pathway and preventing the formation of MAC [129]. Interestingly, the HSV-1 glycoprotein C is a CR1 mimics and it binds the complement C3 components, turning off the complement pathway.

Another gene involved in AD development is cholesterol 25-hydroxylase (*CH25H*). This protein regulates lipid metabolism, and is increased in the temporal cortex and the hippocampus of AD patients; notably high levels of Aβ deposits were observed to be associated with specific single nucleotide polymorphisms (SNPs) of the *CH25C* gene [130]. *CH25H* is an interferon-stimulated gene involved in the host immune response against viruses, HSV-1 included. In fact, CH25H interacts with 25-hydroxycholesterol (25OHC), a protein that prevents HSV-1 infection by blocking the virus-cell fusion [131]. Moreover it was demonstrated that chronic upregulation of 25OHC due to infections causes the accumulation of non-soluble cholesteryl esters in the brain, leading the cerebral vessel atherosclerosis with vascular occlusion, which contributes to AD pathology [132].

Other results showed an association between IFN-ʎ pathway genes SNPs and AD. IFN-ʎ, includes four structurally related IFN-ʎ molecules (ʎ1, ʎ2, ʎ3 and ʎ4) endowed with potent antiviral activities. Interestingly, specific SNPs on *IFNL3* and on *IRF7*, a fundamental transcriptional regulator of IFN-dependent immune responses, are associated with HSV-1 antibody titers in AD and MCI patients [133]. A very recent study showed that G78R, a particular variant of the paired

Immunoglobulin-like type 2 receptor alpha (PILRA) a negative regulator of inflammation in myeloid cells, is protective against AD [134]. HSV-1 uses PILRA as an entry receptor [135], and G78R down regulates the ability of PILRA to bind endogenous and exogenous ligands, HSV-1 included. Finally, because several IL-10 SNPs were suggested to be a risk factor for AD [136], and because IL-10 plays an important role in HSV-1 reactivation [137], it cannot be excluded that SNPs interfering with IL-10 production, modulate HSV-1 reactivation.

5. Pharmaceutical treatment: HSV-1 and Alzheimer's Disease

If HSV-1 is a risk factor for AD, the use of antivirals should be considered in this disease, especially when one considers that current AD therapies are only marginally efficacious [138]. Thus, cholinesterase inhibitors and memantine are the only FDA-approved medications for AD but their effect is extremely limited as they do not alter the course of the disease [138,139]. *In vitro* studies in which anti-HSV-1 antiviral agents, including acyclovir, penciclovir, and foscarnet were analyzed showed that these drugs reduce HSV-1 particles as well as Aβ and P-tau accumulation [140]. Notably, a recent retrospective study performed in a large Taiwanese cohort [141] suggested that HSV infection is associated with an increased risk of dementia and that this risk decreased after treatment with anti-herpetic drugs. Two other articles studied the possible relation between dementia and reactivation in older age of another herpetic virus, the varicella zoster virus (VZV), reporting an increased risk of cognitive decline after herpes zoster ophthalmicus (HZO) [142] and a decrease in incidence of dementia in HZO subjects treated with anti-herpetic antiviral [143]. The use of antivirals, notably, could down modulate CNS inflammation, reducing the production of pro-inflammatory molecules, Aβ and hyperphosphorylated tau proteins. Early combination of neuroprotective and anti-inflammatory agents may represent an efficacious approach to AD. Natural products, and more specifically *polyphenols*, have been reported as promising antiviral and agents for treatment of neurodegenerative disease [144]. Phytochemicals including *flavonoids, alkaloids, terpenoids* and *phenols* are of considerable interest for the treatment of such diseases [144]. *Flavonoids* are naturally occurring, biologically active, and therapeutically effective polyphenols endowed with antiviral, anti-allergic, anti-inflammatory, antitumoral, and antioxidant activities [145]. They can cross the BBB and may exhibit neuropharmacological activities, influencing the protein function and gene expression. *Genistein* is phytoestrogen in soybean and proficiently mimics the pharmacological functions of estrogen. It can act as estrogen receptors (ERs) agonist, and could reduce Aβ-induced toxicity [146]. *Daidzein*, another flavonoid, binds ERs

in the brain and, because of its structural similarity with estrogen, it acts as a neuroprotective agent antagonizing the action of estrogens [146]. *Luteolin* has anti-inflammatory and anti-oxidative properties, and its protective effect on the hippocampus structure and learning flaws has been studied in a AD rat model [147]. *Apigenin* and *acacetin* could inhibit the activation of pro-inflammatory cytokines and nitric oxide (NO) production, protecting AD neurons from inflammatory-induced stress [148]. *Epigallocatechin gallate (EGCG)* acts as a potent anti-oxidant agent and prevents the hippocampal neuronal cell death [149]. *Cyanidin-3-glucoside (C3G)* is a naturally occurring anthocyanin. Results obtained in SK-N-SH neuroblastoma cell line showed that *C3G* reduces Aβ 1-42 accumulation, H_2O_2-induced neurotoxicity, ROS production and dampens the Aβ-induced expression of ER stress proteins [150]. *Pelargonidin* (*PEL*), an anthocyanin, crosses the BBB, inhibits inducible nitric oxide synthase (iNOS), a pivotal driver of oxidative stress, as well as NO production and NF-κB expression [151]. All these products can modulate molecular pathways that are altered by HSV infection.

Other natural products with an antiviral action and the ability of inducing protection from neurodegeneration are *Resveratrol* (*RSV*) and *Quercetin,* which are known activators of Sirt1 (NAD^+-dependent deacetylase sirtuin 1) and AMPK (5'AMP-activated protein kinase) [151-154]. Several studies have demonstrated that, during neuronal infection, HSV-1 modulates the AMPK/Sirt1 axis. Particularly, AMPK is down-regulated during early infection; on the other hand, the levels of Sirt1 increase, suggesting that the AMPK/Sirt1 axis is differentially modulated by the virus during infection [155]. *RSV* and *Quercetin* activate the AMPK/Sirt1 axis and induce neuroprotective and antiviral effects in HSV-1-infected neuronal cultures [156]. Other studies have demonstrated the capacity of *RSV* and *Quercetin* to delay axonal degeneration after injury [155], to block accumulation of Aβ peptide *in vitro* [156], to reduce BACE-1, which mediates the APP cleavage [155], and to provide protection from brain ischemia in both adult and neonatal rodents [157,158]. *Palmitoylethanolamide* (*PEA*), an endogenous lipid mediator is also endowed with anti-inflammatory and neuroprotective effects [159]. A murine model showed that PEA counteracted the activation and inflammation seen in AD-like mouse astrocytes and promoted neuronal viability [160]. Table 3 summarizes the drugs discussed in this paragraph.

6. Conclusions

Reports investigating a possible role for HSV-1 in the pathogenesis of AD pathogenesis are accumulating and are making increasingly clear that HSV-1 infection is a very likely co-factor of this

neurodegenerative disease (see Figure 2). Notably, this does not preclude a role for other microorganisms that, acting through peripheral infection and/or inducing the reactivation of latent viruses, can contribute to chronic inflammation in the brain and the consequent neuronal damage. The smoking gun is missing and much work remains to be performed to clarify the possible mechanisms of viral contribution to AD neurodegeneration, and to verify whether the use of anti-virals could be useful in preventing AD and/or reducing its progression. It is nevertheless important to remember the story of Barry J. Marshall and J. Robin Warren who won the 2005 Nobel prize for the discovery of the bacterium *Helicobacter pylori* and its role in gastritis and peptic ulcer disease. The hypothesis was first published in 1984 [161], it took these scientists a long time for their discoveries to be accepted by the scientific community. Antibiotic drugs are nowadays current treatment in gastric and peptic ulcers.

7. Expert opinion

The discouraging results obtained in clinical trials for AD, trials that mainly focus on Aβ accumulation and its elimination, suggest the need for alternate strategies to fight this cruel and devastating disease. Although a general erroneous idea often identifies viruses with acute damage only, many examples (e.g. HIV, measles, JCV) exist in human pathology indicating that viruses can persist in the body throughout life and can result in diseases many years after the primary infection.

A large number of evidences suggests a possible link between HSV-1 and AD, and results obtained *in vitro* and in animal models indicate that HSV-1 infection can result in anatomical and cellular abnormalities that resemble those seen in AD.

However, several important points must be underlined:

1) HSV-1 is a very common and widespread virus; fortunately nevertheless the majority of HSV-1-infected individuals does not developed AD. HSV-1, thus, can't be the only causative factor of AD, but it is a risk factor that, among others, as genetics, inflammatory status, or other infections, can favor the onset and development of the disease. The main question within this hypothesis is if AD can develop in the absence of HSV-1 *i.e.* whether the presence of HSV-1 is a necessary and mandatory factor in AD pathogenesis. As other pathogens have been detected in brain of AD subjects and the disease can also develop in HSV-1 seronegative subjects, in future it will be interesting to analyze the entire microbiome.

2) If HSV-1 is one possible risk factor for AD, as the distribution of HSV-1 seroprevalence with age is changing, with an increased number of younger subjects susceptible to HSV infection compared to twenty years ago, we cannot exclude that this epidemiological change can have future effect on the risk of dementia. Although the outcome of infection is determined by several combined factors (i.e. genetic of the host, biology of virus, other environmental agents), to better understand this aspect it will be important to perform larger epidemiological studies and to monitor in the next years the possible change of HSV-1-associated dementia risk.

3) The presence of viral DNA in the human brain of at least a part of elderly people has been repeatedly shown in autoptic analyses, but the frequency and effects of viral reactivation in the CNS is not known and is extremely difficult to investigate. As of today it is impossible to measure HSV-1 reactivation in brain *in vivo*, thus no final evidences linking HSV-1 reactivation with AD development can be drown. Analyses performed on CSF are an acceptable proxy of what goes in CNS, but it is unethical to think of performing repeated lumbar punctures in elderly people for research purposes, and no peripheral biomarkers of asymptomatic viral reactivation in the CNS have been identified. The development of probes specific for viral reactivation that could be used for imaging techniques and/or the identification of novel peripheral biomarkers, including pathology-specific microRNA could be an interesting way to allow noninvasive longitudinal monitoring of viral replication in brain.

4) If HSV-1 reactivation is indeed linked to the development of AD, then antiviral drugs should be used for its prevention. But, whom should we treat? All HSV-1 seropositive subjects or, rather only those HSV-1 infected individuals who are characterized by an unfavorable genetic or immune background or in whom a familiarity for AD is known? And when should therapy be started: at the first symptoms of cognitive decline or should we consider the possibility of a life-long therapy? An intense effort to develop basic translational and clinical research needs to be envisioned to try and find a cure for AD, a disease whose prevalence is constantly increasing and for which no therapies are currently available.

The idea that HSV-1 infection is associated with/responsible for Alzheimer's disease has been investigated starting from the mid 80's; this hypothesis has gained strength and has recently been supported by a string of experimental and clinical results. Many hints, thou, do not add up to a

fully convincing proof: the smoking gun is still missing. Data stemming from a long term follow-up study of patients showed that episodic memory impairment is associated with HSV-1 infection, especially among *ApoE4* carriers [162]; this leads to the design of a pilot study based on the use of valaciclovir (VALZ-Pilot, NCT02997982), an antiviral, in AD patients and MCI individuals. Results of this clinical trial, now in Phase II, should help clarifying whether antivirals can modulate the progression of cognitive decline. Bigger trials performed in larger cohorts that include different ethnic groups and take into account the variety of environmental and genetic factors suspected to be involved in the pathogenesis of AD will nevertheless be needed to definitely verify whether antiviral drugs can have a preventative and/or therapeutic effect in AD [see also ref.].

The unequivocal identification and characterization of those HSV-1 epitopes that elicit immune response in Alzheimer's patients, in HSV-1-infected asymptomatic individuals and in HSV-1-exposed uninfected individuals, will be extremely useful in designing efficient vaccines.

Finally, as it is known that HSV-1 can persist in enteric neurons as well, it will be interesting to study the effect of viral reactivation in this compartment, and to analyze if interventions aimed at modifying the microbioma could have a beneficial effects in AD secondary to the prevention of HSV-1 reactivation in the gastro enteric tract.

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Figure Legend

Figure 1: A schematic representation of the HSV-1 structure with lipid membrane envelope with viral surface glycoproteins, tegument layer containing viral proteins, icosahedral capsid shell and linear double-stranded DNA, is shown.

Figure 2: HSV-1 lytic infection in the epithelial cells, the process that the virus follows to infect the sensory ganglion, is shown. Retrograde transport from the sensory nerve terminus to the neuronal cell body is also shown. Factors disrupting the homeostasis that maintains the latency state results in HSV-1 reactivation; HSV-1 lytic genes in particular are reactivated. Note that sensory ganglion projects two processes: one in the epithelial tissue, the other in the brain. Newly replicated virions traffic back by anterograde axonal transport mechanisms, to re-establish infection at epithelial tissues or brain. HSV-1 enters the brains of elderly people as their immune system declines with age could contribute to the development of AD.

Table 1: Summary of principal HSV-1 gene products with anti-apoptotic function.

	Genes product	Role	Effects	References
LATENCY	**LAT** *(Latency-associated transcript)*	Enhances the establishment of latency Protect the host cells from apoptosis	Inhibits apoptosis blocking GrB Modulates Bad-Bax	[163]
IMMEDIATE EARLY	**ICP4**	Regulates the gene expression cascade which controls viral infection. Anti-apoptotic	Inhibits apoptosis	[164]
	ICP27	Multifunctional regulatory protein Pro and anti-apoptotic	Inhibits apoptosis by NFkB JNk- Bcl-2- Bax-Bid	[163]
	ICP 22	Regulator of viral gene expression Promote and Inhibits apoptosis	Inhibits Caspase 8-9 AKT- NFkB	[165]
	ICP0	Anti-apoptotic	Inhibits caspase 8 Inhibits apoptosis TNF-a	[164]
LATE	**US3** (Tegument Protein)	Regulates the biological function of the virus and the host cells Anti-apoptotic	Inhibits apoptosis phosphorylating BAD Inhibits Caspase 3 Bcl2-AKT-BAX-BAD NFkB	[40,163]
	US5 (gJ) (Envelope)	Anti-apoptotic	Inhibits Fas Caspase 3-8	[163]
	US6 (gD) (Envelope)	Anti-apoptotic	Inhibits by Fas-NFkB	[163,164]

Table 2: Summary of the principal HSV-1 proteins involved in immunoevasion.

Phase	Gene product	ORFs	Host target	Inhibited host mechanism
IMMEDIATE EARLY	**ICP0**	RL2	MyD88	TLRs signaling/IFN response
			TIRAP	TLRs signaling/IFN response
			p65	NF-kB signaling/IFN response
			STING	DNA sensor signaling /IFN response
			IFI16	inflammasome
			DNA PK	DNA damage response
			ND10 /Sp100 nuclear bodies	chromatin epigenetic regulation
	ICP27	UL54	IRF3	TLRs signaling/IFN response
			STAT1	JAK/STAT signaling/IFN response
	ICP47	US12	TAP	MHC- I antigen presentation
EARLY / LATE	**tegument protein**	UL36USP	TRAF3	TLRs signaling/IFN response
	Vhs	UL41	mRNA host	host translational arrest
			IRF7	TLRs signaling/IFN response
			JAK/STAT signaling	IFN response
			Viperin	interferon-stimulated genes
			ZAP	interferon-stimulated genes
			Tetherin	interferon-stimulated genes
LATE	**US11**	US11	RIG-1	RLRs signaling/IFN response
			MDA5	RLRs signaling/IFN response
			2’5’OAS	interferon-stimulated genes
			PKR	interferon-stimulated genes
	ICP34.5	RL1	TBK1	TLRs signaling/IFN response
			Beclin	autophagy
			PKR	NF-kB signaling/IFN response
	VP16	UL48	IRF3	TLR3 /IFN response
	gC	UL44	C3b	complement activation
	gE/gI	US8/US7	IgG	neutralization/Ab dependent cytotoxicity
	gM	UL10	Tetherin	interferon-stimulated genes
	gB	UL27	PERK protein kinase	host translational arrest
	ICP8	UL29	Stress granules	host translational arrest
	Ser/Thr Protein kinase	US3	IRF3	TLRs signaling /IFN response
			TRAF6	TLRs signaling/IFN response
			STING	DNA sensor signaling

For more details, see references 48 and 61.

	Class of Drugs	Effect on HSV-1	Effect on AD	General effect	References
Chemical Products	**Aciclic-guanosine analogues:** *Acyclovir, Ganciclovir, Penciclovir, Valaciclovir, Famciclovir*	Reduction of HSV-1 particles	Reduction of Aβ, decrease of P-tau accumulation	Reduction of disorders of the micro-circulation Bradycardia and treatment of pain Bell's palsy (cranial nerve lesion)	[140-143, 166,167]
	Pyrophosphate analogues: *Foscarnet*	Reduction of HSV-1 particles	Reduction of Aβ, decrease of P-tau accumulation	Alters antidiuretic hormone mediated transport water and urea	[140-143]
	Acyclic nucleotide analogues: *Cidofovir, Adefovir*	Reduction of HSV-1 particles	Reduction of Aβ, decrease of P-tau accumulation	Inhibits mitochondrial DNA synthesis Antiproliferative agents	[140-143, 168,169]
Natural Products	**Phytochemical:** *Flavonoids, Alkaloids, Terpenoids and phenols*	Anti-viral Anti-inflammatory	Protective role in nervous system disorders	Anti-inflammatory Anti cancer	[145]
	Flavonoids: *Flavanols, flavones, Flavonols, isoflavones, anthocyanidins*	Anti-viral Anti-oxidant	Cross BBB Neuropharmacological activities Influencing protein fusion and gene expression	Anti-oxidant Anti cancer Antiangiogenic	[145]
	Isoflavones: *Genistein, Daidzein*	Molecular pathways altered during HSV-1 infection	Neuroprotective agent, antagonizing the action of estrogens	Osteogenic function	[146]
	Flavones: *Luteolin, Apigenin, Acacetin*	Anti-inflammatory Anti-oxidative Anti-viral	Inhibit the activation of proinflammatory cytokines, Protection AD neurons	Anti-inflammatory Anti-oxidative	[147,148]
	Flavanols: *Epigallocatechin(EGCG)*	Molecular pathways altered during HSV-1 infection	Prevent neuronal cell death	Cardiovascular function	[149,170]
	Anthocyanidin: *Cyanidin-3-glucoside (C3G)*	Molecular pathways altered during HSV-1 infection	Neutralize the level of Aβ 1-42 peptides	Renal protective effect	[150,171]
	Flavonols: *Quercitin* **Polyphenol:** *Resveratrol*	Sirt1 and AMPK pathways modulated by HSV-1 Anti-viral effects	Neuroprotective effects, block accumulation of Aβ peptides *in vitro,* mediate the cleavage of APP	Anti-inflammatory Anti-oxidative Anti- apoptotic	[152-156, 158]
	Endogenous fatty acid amide (class of nuclear factor agonists)**:** *Palmitoylethanolamide (PEA)*	Anti-Inflammatory	Neuroprotective effects	Neurophatic pain	[159]

Table 3: Classes of drugs for the treatment of HSV-1 infection and Alzheimer Disease

Figure 1

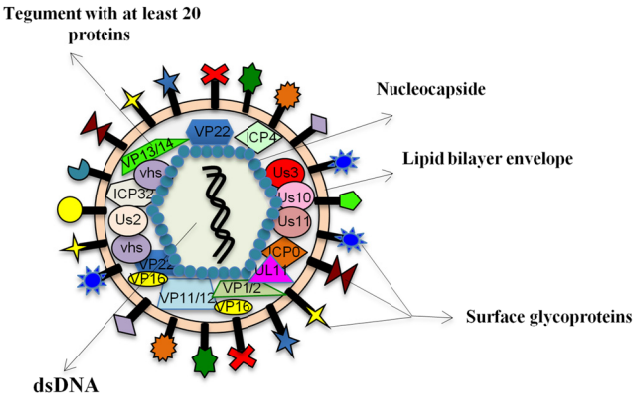

Figure 2