Infection and Alzheimer's disease – the apoE ε4 connection and lipid metabolism

Nadezda Urosevic and Ralph N. Martins

Sir James McCusker Alzheimer Disease Research Unit, School of Psychiatry and Clinical Neurosciences, The University of Western Australia; Centre of Excellence for Alzheimer's Disease Research and Care, School of Exercise, Biomedical and Health Sciences, Edith Cowan University, Joondalup, Australia

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Abstract

Microorganisms, bacteria and viruses, may infect and cause a range of acute and chronic diseases in humans dependent on the genetic background, age, sex, immune and health status of the host, as well as on the nature, virulence and dose of infectious agent. Late onset Alzheimer's disease (AD) is a progressive neurodegenerative illness of broad aetiology with a strong genetic component and a significant contribution of age, sex and life style factors. Both infectious diseases and AD are characterised by an increased production of an array of immune mediators, cytokines, chemokines and complement proteins by the host cells as well as by changes in the host lipid metabolism. In this review, we re-examine a dangerous liaison between several viral and bacterial infections and the most significant genetic factor for AD, *APOE* $\epsilon 4$, and the possible impact of this alliance on AD development. This connection was discussed in the broader context of lipid metabolism and in the light of different capacity of various infectious agents, their toxic lipophilic products and host lipoprotein particles for binding to cell receptor(s).

I Introduction

Alzheimer's disease (AD), a slowly progressing neurodegenerative illness characterised by cognitive impairment and memory loss, is a medical condition of complex aetiology. While genetic factors play an important role, environmental factors such as life style, diet, age, brain injury and gender are also recognised as critical contributors to disease development and it is currently thought that the more common late onset form of AD is a result of interplay between these factors and genetic risk factors. In contrast, the involvement of infectious agents in disease aetiology and their role in determining disease severity, time of onset and curability has not been adequately evaluated. However, recent evidence suggests that infectious agents need serious investigation as potentially important factors that contribute to the progression, complexity and severity of AD.

In this review we wish to provide an up-to-date account of various facets of infection and its contribution to the progression of AD. While the major influence of infection on AD development is determined by the interaction between infectious agent and the host, this is further complicated by the interplay with other environmental factors and host genetic background providing additional dimensions to disease complexity. The major features of these interactions will be reviewed here and they will include: a) chronic infection at the periphery as a continuous source of persistent live microorganisms, their toxic products and stimulation of host's inflammation, b) pathogen's neuro-tropism, its persistence in the peripheral (PNS) and dissemination to the central nervous (CNS) system, c) direct pathogen-mediated injury to the CNS neurons and indirect effect via stimulation of local brain-inflammation and toxicity and d) interplay between the major AD genetic risk factor the $\epsilon 4$ allele of apolipoprotein E (*APOE* $\epsilon 4$), lipid metabolism and infection and their effect on AD development and progression.

II Chronic systemic infections and inflammation in AD

Chronic infections are a continuous source of infectious agents and their products that could disseminate around the body, stimulate host immune responses and result in chronic systemic inflammation involving CNS as well. Indeed, local brain tissue inflammation in AD has been reported to involve upregulated production of classical inflammatory mediators of innate immunity such as tumour necrosis factor α (TNF α), interleukin 1 α/β (IL-1 α/β), interleukin 6 (IL6) and complement proteins that are synthesised locally by astrocytes and microglia in the brains of Alzheimer's patients [80-82]. The additive effect of inflammation on increasing disease severity and the age at onset is well documented [99, 135]. In addition, a positive correlation between an anti-inflammatory drug therapy and a delayed onset of disease points at the significance of inflammation as the contributing factor to disease progression [11, 134].

II-1 Herpes simplex virus 1 and AD

Infectious agents reported so far to be associated with AD belong to a broad variety of microorganisms including viruses and bacteria. The common link among these is their ability to develop a persistent infection at both the periphery and in CNS with the continuous release of antigens and other stimulatory molecules involved in a sustained production of cytokines, chemokines, acute phase proteins and other mediators of inflammation and innate immunity. So far, compelling evidence has been provided for the association between infections with herpes simplex virus 1 (HSV1) and an increased risk for AD. Most people get infected with HSV1 early in life and the virus remains latent in the trigeminal ganglia (TG). A number of people carrying latent HSV1 do not develop acute infection or disease symptoms, whereas some develop cold sores upon virus reactivation. However, those who develop recurrent cold sores were found to possess with a greater frequency the genetic risk factor *APOE* $\epsilon 4$ that is

implicated in AD pathogenesis when compared with cold sore non-sufferers [25, 48]. This may either point at the common mechanism underlying virus reactivation and AD development to both of which, in addition to *APOE* genotype, inflammation and neuronal injury were also found to contribute significantly. Otherwise, it may suggest that the recurrent HSV1 virus reactivation in a form of cold sores facilitates virus dissemination to the CNS where it assumes a more direct role in the pathogenesis of AD [26]. Conversely, the likelihood for the virus to reach the brain during the lifespan is greater if the more frequent episodes of virus reactivation occur. In support of this, the same author and others reported that the latent HSV1 was found more readily in brains of elderly than young people possibly representing a cumulative effect of the recurrent HSV1 reactivations [48, 50, 132].

II-2 Human immunodeficiency virus and hepatitis C virus

Other human viruses implicated in dementia are human immunodeficiency virus (HIV) [19], hepatitis C virus (HCV) [32, 33], human herpesvirus 6, cytomegalovirus and others as reviewed elsewhere [3, 26]. Apart from sexual transmission, HIV may spread by parenteral routes that are also common to HCV [113]. It is not unusual that a significant proportion of HIV carriers are also seropositive for HCV, especially in intravenous drug users and other human cases of parenteral viral infection [113]. HIV and HCV are members of taxonomically different viral families, *Retroviridae* and *Flaviviridae*, respectively, characterised by distinct virion structures, mechanisms of replication and tissue tropism. Acute infection with HIV targets and destroys host's CD4⁺ T cells resulting in acquired immunodeficiency syndrome (AIDS), while HCV is a leading cause of viral hepatitis specifically targeting hepatocytes. However, both viruses may also infect and replicate at low levels in mononuclear cells of peripheral blood, monocytes and macrophages, representing a potential source of persistent virus and virus-induced cytokines [6, 90]. Other common

feature arising from the ability of both viruses to infect monocytes/macrophages is virus spread and persistence in CNS, stimulation of local brain inflammation and decline of mental functions. Mild cognitive impairment (MCI) was observed in subjects infected with HIV in the absence of opportunistic infections [19] and in those with chronic HCV infection not associated with substance abuse, concurrent depression or hepatic encephalopathy [33].

II-3 Host inflammatory responses to viral infections – relevance to AD

Early host response to virus infection is non-specific and it includes synthesis and secretion of interferons, cytokines, chemokines and complement proteins needed to encounter the virus and protect the host from the viral spread. Interferons α/β and γ are directly induced by viral proteins or viral double stranded RNA (dsRNA) and they are involved in triggering intracellular signalling cascades resulting in activation of antiviral pathways within the cell [123]. However, some of these pathways, especially the pathway mediated by dsRNA-dependent protein kinase (PKR), are also involved in regulation of cell survival and, in the presence of excessive stimulation, may lead to cell death [2]. Recently, an association between PKR and Alzheimer disease was established suggesting a relation between AD and virus infection *via* PKR, a common host factor involved in regulation of cell survival in response to both virus infection and endoplasmic reticulum (ER)-stress caused by mis-folded proteins [12]. On the other hand, virus-induced γ interferon (γ IFN) and $\text{TNF}\alpha$ are also known to stimulate the synthesis of inducible nitric oxide synthase (iNOS) in macrophages. The iNOS enzyme is involved in the production of reactive nitric oxide (NO), a small effectors molecule implicated in antiviral defence as well as in cell death [56, 58]. Amyloid β ($\text{A}\beta$) peptide, a major disease marker in AD, was also shown to enhance iNOS in conjunction with $\text{TNF}\alpha$ [138]. Cytokines such as $\text{IL}1\alpha$ and β , IL6, $\text{TNF}\alpha$ and γ IFN that are part of the host systemic inflammatory response to virus infections are also major constituents of systemic

and local inflammatory reactions in AD [82]. Although evident, the impact of allelic polymorphisms in genes for IL1 [93], IL6 [97], TNF α [66] and some other inflammatory mediators such as the C reactive protein (CRP) [126], is not as strong as the effect of the late onset AD risk factor *APOE* $\epsilon 4$ judged by the odds ratio (OR) values, though a synergistic interaction between the latter gene and the cytokine genes can not be ruled out. However, in addition to the fact that old age is the major risk factor for late-onset AD, it also coincides with an increased immune senescence that may facilitate the spread of infection to CNS and result in a pathogen-driven upregulation of the inflammatory response adding additional weight to the role of infection in AD [99, 107].

II-4 Chronic bacterial infections and inflammation – a path to AD?

In addition to viruses, bacteria have also been implicated in chronic systemic infections with the prospect of CNS involvement and association with AD. So far, infections with two spirochetes *Treponema* and *Borrelia*, were identified as potentially important players in the aetiology of AD. These gram-negative bacteria were isolated from either the blood or brains of people with AD and they are implicated in chronic infectious diseases such as periodontal disease, ulcerative gingivitis, syphilis and Lyme disease [85-88, 110]. Oral *Treponemas* sp. *pectinovorum* and *socranskii* involved with periodontal diseases were also detected in saliva and TG of both healthy controls and AD patients alike. However, they were found in the CNS of AD patients more often than in healthy controls possibly following dissemination by axonal route [110]. A causative agent of syphilis, *Treponema pallidum*, transmitted by sexual contact, was also found to persist in CNS during the tertiary stage of disease causing dementia, cortical atrophy, microgliosis and amyloid depositions [3]. Another spirochete, *Borrelia burgdorferi*, a causative agent of Lyme disease, was detected in CNS associated with the development of neurologic complications known as Lyme

neuroborreliosis that may progress to encephalitis, meningitis and dementia [3, 83, 85-88]. Lyme disease is a multi-systemic flu-like illness transmitted by a bite of an infected tick and if not treated by antibiotics, the infection may spread and persist in the skin, joints, heart and nervous system producing characteristic disease symptoms. It is noteworthy that besides HSV1, spirochetes were the most studied infectious microorganisms associated with AD [72-73, 83, 85-89].

Strong evidence was presented for the presence of intracellular bacterium *Chlamydia pneumoniae* (Cpn) in brains of AD patients [7, 35]. Although usually acquired at mucosal surfaces causing acute respiratory infections, Cpn may also reach CNS *via* infected mononuclear cells following the breach of blood-brain-barrier (BBB) [74, 91]. Despite some inconsistency regarding the Cpn detection in AD brains [36, 109], this pathogen was shown to induce AD-like amyloid deposits in mouse brain upon injection [9, 68]. Interestingly, the severity of AD-like brain pathology in Cpn-infected BALB/c mice appears to be influenced by the Cpn strain and propagation history suggesting for the first time that strain-dependent bacterial factors may determine the rate of AD pathogenesis [9, 68]. Furthermore, the presence of the host's *APOE* $\epsilon 4$ allele coincided with increased bacterial loads and greater numbers of infected cells than in *APOE* $\epsilon 3$ carriers signifying the importance of *APOE* genotype in susceptibility to Cpn [34]. Of other bacterial pathogens, association of *Helicobacter pylori* with MCI is worth mentioning as another example of potentially harmful infection that may influence a development of dementia [62].

Persistent localised or systemic infections with bacteria provide a continuous supply of bacterial toxins, such as a constituent of bacterial cell wall of gram-negative bacteria, the endotoxin lipopolysaccharide (LPS), and other toxic bacterial lipoproteins. LPS and bacterial lipoproteins possess very strong immuno-stimulatory properties and they are the major cause of systemic inflammatory reaction following bacteremia in acute infections. LPS binds to the

CD14/TLR4/MD2 receptor complex at the cell surface of macrophages or brain microglia and stimulates production of pro-inflammatory cytokines such as TNF α that is the crucial factor of inflammation [114] and which is also known to stimulate production of beta amyloid (A β) considered as a key player in the pathogenesis of AD [66]. Systemic LPS administration to mice stimulated microglia activation and increased the production of TNF α for up to 10 months following the initial administration [104]. This also resulted in a significant reduction in numbers of dopaminergic neurons in substantia nigra [104]. In addition, TNF α was shown to be the key cytokine that causes excitoneurotoxicity by stimulating glutamate release from activated microglia [117]. In a parallel study, a single intraperitoneal treatment with LPS caused oxidative injury in the brain as estimated by the detection of reactive oxygen species, NO production and lipid peroxidation accompanied by alterations in spatial learning using Y-maze test [95]. Persistent bacterial infections continuously release low doses of LPS and other stimuli of chronic inflammation such as TNF α that eventually leads to body wasting or cachexia characterised by body fat, muscle and weight loss [54]. Body wasting was also observed to precede dementia suggesting involvement of common etiological factors in AD as in chronic inflammatory diseases [59, 101].

III Pathogen's neuro-tropism and persistence in CNS – a precondition for AD?

Although chronic infections with viruses and bacteria at the periphery are sufficient to produce the systemic inflammatory response as described above, they are usually not confined to the periphery and they frequently spread to the brain. Cytokines such as TNF α that are produced at the periphery may destabilise and permeabilise BBB, a tight junction between brain endothelial cells that separates and protects the brain from the peripheral

influences [96]. However, the breach of BBB may occur following the acute infection at the periphery facilitating the import of microorganisms to the CNS, as recently shown for the spread of the flavivirus West Nile encephalitis (WN) [129]. Hematogenous route of virus spread to CNS was also observed for HSV1 in both vertical (mother-to-child) and horizontal transmissions [13, 15, 16]. Interestingly, the major genetic risk factor for AD, *APOE ε4*, was found to contribute to HSV1 neuroinvasiveness and colonisation of CNS in mice [14, 17] that proceeded along the neural paths known to be involved in AD progression: hippocampus, temporal and frontal cortex [48]. Consistent with studies in humans that revealed latent HSV1 in brains of elderly but not young people, latent CNS infection with HSV1 in mice was detected at late stages of disease following intraperitoneal inoculation [17]. Furthermore, the spread of both latent and active virus in the brain was observed within the same regions that are usually affected in AD. A majority of people who carry latent virus in the brain are asymptomatic and only a very small proportion may develop herpes simplex encephalitis (HSE), a very acute life-threatening condition that results in permanent disturbances in memory, cognition and personality [25]. Acute HSV1 infection in CNS causing HSE creates overly severe condition in order to be directly associated with the occurrence of AD. However, mild HSE were also shown to result in a slight loss of memory despite a nearly complete recovery. It is not clear how the latent virus would be responsible for the disease development unless sporadic virus reactivations occur resulting in mild HSE that may remain undiagnosed in most of the cases [25, 26]. The latter was confirmed by virus reactivation in primary cultures of hippocampal neurons following short exposures to hyperthermia [16]. However, even latent HSV1 was shown to induce oxidative damage to neurons [124].

The hematogenous route of viral spread to the CNS is also exploited by other viruses especially those that hijack mononuclear blood cells, monocytes and macrophages, as previously reported for the HIV and HCV viruses [6, 90, 105]. Unlike HSV1, HIV and HCV

do not infect neurons nor develop latency in the brain. In contrast, HIV and HCV produce active infection in macrophages that is sometimes difficult to detect by conventional approaches [39]. Whereas infected macrophages may not be a significant source of infectious virus due to the low level of infection, they indisputably serve as a significant source of pro-inflammatory cytokines or other toxic products of viral origin (HIV protein *tat*) the increase of which is an additional and important risk factor for AD development.

Spirochetes were isolated from blood and brains of AD patients suggesting that their transmission to CNS may occur by both neuronal and hematogenous routes [88, 110]. Furthermore, human neuronal, endothelial and glial cell lines were shown to internalise *B. burgdorferi* *in vitro* providing compelling evidence for the *in vivo* invasion of CNS as a possible means of escaping immune surveillance at the periphery [70, 89]. In CNS, the bacteria were hypothesised to spread by axonal transport from the hippocampus as the major ‘entrance portal’ up to the higher brain centres similar to the stages of AD development [72, 73]. Indeed, *B. burgdorferi* was found to be associated with amyloid plaques in brains of AD patients [88]. Furthermore, extended exposures of organotypic foetal rat telencephalon tissue cultures to *B. burgdorferi* or LPS resulted in increased levels of amyloid precursor protein (APP), *tau* protein hyperphosphorylation and in formation of amyloid deposits *in vitro* [89].

IV APOE, lipids and infection

IV-1 Apolipoprotein E

Apolipoprotein E (apoE) is a plasma lipid transport protein implicated in regulation of lipid metabolism and associated with the risk for developing several medical disorders. In humans there are three isoforms, apoE ϵ 2, ϵ 3 and ϵ 4 that differ in two amino acids at positions 112 and 158. The structural polymorphism in apoE modulates the conformation and influences the quality and stability of the protein’s binding to lipoprotein particles [38],

cellular receptors [45, 75] and amyloid β peptides [118, 136]. In addition, a number of other physiological properties of apoE, such as its anti-oxidant [65], anti-apoptotic [52, 53], immuno-modulatory [18, 71], and atheroprotective capacity [23] are influenced significantly by the presence of either *arg* or *cys* at positions 112 and 158. This creates conditions for isoform-specific effects on development of a number of chronic diseases, or more specifically, for *APOE* ϵ 4-associated risk of developing atherosclerosis [4, 60], stroke [111], Alzheimer disease [76, 79] and related disorders. Characteristically, the common molecular basis of these diseases is thought to be determined by isoform specific differences of apoE on lipid metabolism though for AD our understanding of these molecular mechanisms is inadequate.

IV-2 Lipids, apoE and AD

Lipids are very important biomolecules with a range of physiological roles in the body from serving as the cellular nutrients and body's energy storage depot to being structural components of cell membranes and metabolic precursors in steroidogenic pathways. The liver as a major metabolic organ has a special position in lipid metabolism. It plays a key role in cholesterol and triglyceride synthesis as well as in catabolism of lipids and their biliary excretion. The lipids make up metabolic energy depots that are stored in adipose tissues in the form of triglycerides and used according to metabolic requirements of the body. However, the major supply of lipids comes from the dietary intake and they are delivered to other peripheral organs *via* circulation in the form of large lipid droplets called chylomicrons. In contrast, in diseases, especially infectious diseases and AD, lipid metabolism is affected leading to lipid depletion and body wasting [40, 54, 59].

The distribution and transport of lipids among different body compartments occurs *via* blood by means of lipoprotein particles of different densities. There are four major classes

of lipoprotein particles in human plasma: very low density lipoprotein particles (VLDL), intermediate density lipoprotein particles (IDL), low density lipoprotein particles (LDL) and high density lipoprotein particles (HDL). However, in cerebrospinal fluid (CSF) only HDLs of different densities (HDL1, HDL2 and HDL3) are found [61]. The protein components of plasma and CSF lipid particles are specialised amphipathic proteins, apolipoproteins that are involved in transport of hydrophobic lipids and their delivery to peripheral and CNS cells. In addition to proteins, lipoprotein particles may also carry a number of different lipophilic compounds including vitamins, hormones, peptides or viruses that are involved in a regulation of tissue metabolism, signalling, metabolite disposal or, as in the case of viruses, they just simply hijack the lipid particles.

Among a number of apolipoproteins involved in lipid transport, apoE ϵ 4 has a special position due to its association with the risk for atherosclerosis, stroke, AD and with the poor recovery from head injury and hypoxia [4, 60, 76, 79, 111]. Importantly, all of these conditions are connected to disturbances in plasma triglyceride and cholesterol levels. The association between *APOE* and disease development originates from a disparity in binding of different apoE isoforms to lipid particles or cellular receptors involved in their clearance resulting in inability to clear extracellular lipids by intracellular uptake and degradation within the tissues. While *APOE* ϵ 2 has a strong association with the type III hyperlipoproteinemia due to a poor binding of lipid particles containing apoE ϵ 2 to LDL receptor (LDLr) [10], *APOE* ϵ 4 has been associated with the poor clearance and recycling of lipoprotein particles carrying cholesterol and A β peptides. Inability to clear and degrade plasma lipids, or more specifically LDL-bound cholesterol, leads to increased levels of serum cholesterol and increased risk for developing atherosclerosis and cardiovascular diseases. However, the accumulation of soluble A β in brain tissue and CSF as well as an increased deposition of fibrillary A β in brain parenchyma and in the walls of small blood vessels in

CNS are hallmarks of AD and cerebral amyloid angiopathy (CAA), respectively. The common denominator for both peripheral and CNS lipid disorders is apoE ϵ 4 and its inability to maintain lipid homeostasis particularly after an environmental insult and with this, a homeostasis of lipophilic compounds associated with the lipid particles. There is some evidence to indicate that the major lipophilic compounds associated with lipid particles in CNS are A β peptides and their clearance/deposition in the brain is heavily dependent on lipid clearance [79].

IV-3 Lipids and infection

Host response to infections in addition to cytokine release involves changes in lipid metabolism and plasma lipid levels. These plasma lipid changes were shown to be mediated by the major pro-inflammatory cytokines TNF α , IL-1 and IL-6 that may induce an increase in serum content of triglycerides and VLDL [40-42, 122], a decrease in serum cholesterol, HDL and LDL [102, 125] or increased serum levels of triglycerides and cholesterol [31, 84], dependent on the nature of the infectious agent. There are several mechanisms involved in the infectious agents and/or cytokines induced changes in lipid metabolism including the regulation of lipid/lipoprotein production by liver, lipoprotein lipase activity that controls lipolysis in serum, lipoprotein clearance at the periphery *via* common cellular receptors as well as the regulation of BBB functionality and reverse flow of lipoproteins from serum to CSF [98]. Some of the changes imposed on lipid metabolism by viruses and bacteria are part of their host invasion programme while the others represent the elements of the host's innate defence against infection.

Lipoproteins and lipids present in human serum and milk contribute to the host's innate immunity against viruses since they were shown to possess direct antiviral properties [30, 115]. Antiviral properties of human plasma derived HDL were studied against a number

of enveloped and non-enveloped viruses in cell culture and they demonstrated a broad non-specific antiviral activity [115]. This activity was possibly contributed by the apolipoprotein component of the lipid particles since both protein components of HDL, apoA-I, apoE and their amphipathic peptide derivatives or analogues were shown to express antiviral properties *in vitro* [27, 57, 116]. The antiviral property of apolipoprotein-derived peptides was mapped to the region of the protein that is responsible for binding the cell receptor [27, 116]. Furthermore, this antimicrobial effect of apolipoproteins was not limited to viruses only. Similar studies were performed in which the toxic effect of LPS and the intensity of endotoxemia were neutralised by apoE enriched lipid particles [8, 108, 127]. This neutralising effect was again traced to the same region of apoE that directs binding to the cell receptors suggesting that the viruses and bacterial cell wall products bind to the same receptors on cell surface as apoE (Fig. 1) [27, 48, 57]. The anti-infective effect of apoE peptides was shown to occur at the cell attachment stage for a number of viruses including HSV1, HSV-2, HIV and bacteria such as *Plasmodium aeruginosa*, *P. berghei* and *Staphylococcus aureus* [57]. Furthermore, *in vivo* studies using *APOE* deficient mice confirmed the role of *APOE* in host susceptibility to endotoxemia and bacterial infection [22], while mice expressing human *APOE* $\epsilon 3$ and *APOE* $\epsilon 4$ genes revealed apoE isoform-specific effect on the systemic and CNS-based pro-inflammatory response to LPS [71]. However, when viral (HIV *tat*) and bacterial (LPS) products reach CNS, their neutralisation by apoE-enriched lipoprotein particles becomes very important for the prevention of their cytotoxic effect on neurons (Fig. 1). As shown on Fig. 1, apoE $\epsilon 3$ enriched lipoprotein particles possess the ability to neutralise both LPS and HIV product *tat* and prevent oxidative stress on neurons, while in the presence of apoE $\epsilon 4$, no neutralisation of toxic effects of *tat* and LPS occurs [27].

IV-4 Cellular receptors for lipids and viruses

The cell surface receptors involved in cellular uptake of APOE and lipoprotein particles are members of the LDL receptor family, the most important being LDL (LDLr) itself and LDLr-related protein 1 (LRP-1) [92]. These two receptor types are implicated in a cell type-specific and apoE isoform-specific endocytosis of lipoprotein particles in liver and CNS [5, 67] and also, together with apoE isoforms, in regulating cellular cholesterol homeostasis and clearance/degradation of A β [45, 67, 69]. In addition, there are other binding sites and receptors on the cell surface that are involved in different stages of lipoprotein metabolism such as a broad spectrum receptor, heparan sulphate proteoglycans (HSPG) involved in cellular attachment of apoE enriched lipoproteins [51], and scavenger receptor class B type I (SR-BI), physiologically relevant HDL receptor involved in selective cholesterol uptake by liver and bidirectional flux of cholesterol between HDL and cells [63].

Interestingly, some of these receptors involved in apoE and lipoprotein cellular binding and uptake are also implicated in virus entry and in neutralising bacterial LPS. Many viruses, including HSV, use HSPG as a docking site on the cell surface for the attachment that facilitates their binding to specific cell receptors and subsequent fusion with the cell membrane and virus internalisation [26, 49]. This mode of virus entry is heavily dependent on the cell membrane's micro-domains enriched in cholesterol (lipid rafts) [43]. In the case of HCV, it may also depend on the initial viral attachment to the SR-BI receptor prior to the binding to the specific HCV receptor, CD81 [55]. For this mode of virus entry, the hematogeneous route of virus transmission, as shown for HSV1 [13, 16], and/or an association of the virus with plasma VLDL, LDL and HDL, as demonstrated for HCV [24, 77, 94, 128] appears to be critical for viral infectivity. Furthermore, the HDL-mediated virus entry via SR-BI receptors is an alternative route used by the HCV virus that is responsible for

the observed inability of high concentrations of neutralising antibodies to prevent and clear the HCV infection [28].

In addition to broadly specific HSPG and SR-BI receptors, lipoprotein-associated viruses such as HCV or virus derived proteins, such as *tat* of HIV virus, may also bind to LDLr and LRP1 receptors, respectively [1, 24, 29]. The latter interaction was shown to promote apoptosis in human mixed cultures of neurons and astrocytes [29]. Neutralisation of bacterial endotoxin LPS occurred by binding to either HDL, LDL or VLDL resulting in a lipoprotein-mediated redirection of LPS uptake from Kupffer cells to parenchymal liver cells where it is targeted for inactivation and disposal [108, 127]. As a result of this, macrophages become less activated and produce less pro-inflammatory cytokines [8].

V Viruses and APOE $\epsilon 4$ – a dangerous liaison

Apolipoprotein E is the major protein component of VLDL, LDL and HDL and it is actively involved in lipid transport, cellular uptake and catabolism. As reviewed above, the genetic variant *APOE $\epsilon 4$* that codes for *arg* residues at the positions 112 and 158 is associated with an increased risk for developing AD, stroke, atherosclerosis and related cardiovascular conditions. At the cellular level, the presence of the *APOE $\epsilon 4$* allele and protein predispose carriers to a greater vulnerability to A β -induced oxidative damage [65], lysosomal leakage [52, 53] and A β -induced cytotoxicity [130], a greater microglia activation, increased secretion of inflammatory mediators [18], less efficient A β binding to cellular receptor(s) [45] and decreased cellular uptake of A β [137]. Many of these effects were also observed *in vivo* at the systemic level [44, 71] suggesting a broad scope and great complexity of processes regulated by *APOE*. In addition, significant evidence was presented that point to the

regulatory role of *APOE* $\epsilon 4$ in infection as well as to the synergistic action of *APOE* $\epsilon 4$ and infection on the increased risk for AD.

V-1 HSV1 and *APOE* $\epsilon 4$

The initial evidence for the role of *APOE* $\epsilon 4$ in infection were obtained by Itzhaki and colleagues [48] who observed that *APOE* $\epsilon 4$ was a risk factor for cold sores caused by the reactivation of HSV1 virus. Furthermore, they found out that *APOE* $\epsilon 4$ was more frequently associated with the presence of HSV1 DNA in the brains of elderly AD patients compared to non - $\epsilon 4$ carriers suggesting the cumulative contribution of both *APOE* $\epsilon 4$ and HSV1 to the increased risk for AD development [47, 48]. These findings were further supported by studies in animal models using transgenic mice humanised for *APOE* $\epsilon 3$ or *APOE* $\epsilon 4$ genes where the presence of *APOE* $\epsilon 4$ coincided with the greater risk for HSV1 spread to the brain [14] and for establishing virus latency in CNS [17]. Since both acute and latent HSV1 virus infections caused oxidative damage to neurons and focal chronic inflammation in the brains of infected mice, this further supported the proposition that the cumulative risk for AD development was conveyed by both *APOE* $\epsilon 4$ and the virus [124]. A plausible explanation for a greater HSV1 spread and latency in the brains of *APOE* $\epsilon 4$ than of *APOE* $\epsilon 3$ carriers was that the virus competed better against apoE $\epsilon 4$ - than against apoE $\epsilon 3$ -enriched lipoprotein particles for binding to the cell receptor and intracellular internalisation (Fig. 2) [49]. However, additional mechanisms of HSV1 pathogenesis in AD not associated with *APOE* genotype have also been proposed such as a direct HSV1 virus particle binding to APP and kinesin during anterograde axonal transport of HSV1 affecting the APP processing and increasing A β production [112], as well as a direct contribution of viral glycoprotein B to the

senile plaque generation by forming neurotoxic fibrils homologous to amyloid fibrils of A β peptide [20].

V-2 HIV and *APOE* $\epsilon 4$

In addition to HSV1, HIV virus can also invade the CNS though rarely causing acute encephalitis but rather more often dementia (19). The involvement of host genetic factors in vulnerability to HIV infection of nervous system has also been investigated pointing at the contribution of *APOE* $\epsilon 4$. Subjects infected with HIV that are carriers of *APOE* $\epsilon 4$ were found to be at the higher risk for developing dementia and peripheral neuropathy than HIV-positive subjects who were *APOE* $\epsilon 4$ negative [19]. In addition, HIV-positive *APOE* $\epsilon 4$ carriers with dementia show a deregulated lipid and sterol metabolism [21], while the dementia patients positive for HIV present increased oxidative stress and lipid peroxidation in brain and CSF [120]. Unlike HSV1, HIV virus does not infect or replicate in neurons and it does not cause a direct cytopathic effect. In contrast, the virus reaches the brain by infected macrophages which support HIV virus replication and shed the viral protein *tat*, a neurotoxic product associated with oxidative injury and neuronal death *via* LRP1 receptor and TNF α , respectively [64, 69]. The susceptibility of *APOE* $\epsilon 4$ positive neurons to HIV was revealed *in vitro* where apoE $\epsilon 4$ was unable to protect neurons from oxidative insult of *tat* in contrast to the significant protective effect of apoE $\epsilon 3$ (Fig. 1) [100]. Furthermore, the cytotoxic effect of *tat* on human neurons was exacerbated by the co-treatment with morphine suggesting an additional risk for development of dementia in HIV-infected *APOE* $\epsilon 4$ -positive opiate drug users [121]. In contrast, treatment with the natural compounds, diosgenin and L-deprenyl found in oriental spices, exerted a protection against toxic effects of *tat* and opiates [121]. The synergistic effect of the host apoE $\epsilon 4$ and the viral protein *tat* on the increased neurotoxicity *in vitro* and *in vivo* appears to be a result of the increased *tat* binding,

intracellular internalisation and activation of downstream signalling pathways within the cell, due to a reduced ability of apoE ϵ 4-containing lipoproteins to compete with *tat* for entry [120, 121].

V-3 HCV and APOE ϵ 4

The role of chronic virus infection in cognitive impairment and cerebral dysfunction is less obvious with HCV since this virus is very rarely found extra-hepatically [32, 39]. However, there is evidence of virus crossing the BBB possibly by infected monocytes/macrophages [105, 106] that may secrete excess cytokines and cause excitotoxicity in CNS [33]. An association of *APOE* ϵ 4 with neuropsychiatric symptoms induced by IFN I treatment of chronic HCV infection have been reported [37], although no effect of the apoE ϵ 4 phenotype on HCV presence in CNS or severity of CNS symptoms has been determined to date. In contrast, the degree of liver damage caused by HCV was shown to be negatively correlated with the presence of *APOE* ϵ 4 suggesting a protective rather than a harmful role for apoE ϵ 4 in liver disease [119, 131]. Furthermore, it was the presence of *APOE* ϵ 3, but not *APOE* ϵ 2 or *APOE* ϵ 4 that predisposed the carriers for the chronic HCV infection of the liver [103]. This intriguing finding suggests complex interactions between the HCV virus, lipoprotein particles and cellular receptors in determining the outcome of liver infection. The previous knowledge of the apoE isoform-specific clearance of lipid particles by liver cells is critical for better understanding of these interactions. Since apoE ϵ 2-enriched lipoprotein particles show decreased affinity of binding to LDLr on liver cells [10], their effect on virus entry may only occur if both apoE ϵ 2 and virus bind the same VLDL particles resulting in reduced viral attachment to LDLr. Indeed, virus production and infectivity are dependent on VLDL particle assembly and the presence of apoE [46]. Likewise, apoE ϵ 4-enriched VLDL were shown to bind LDLr with an increased affinity, although this property

was not positively correlated with the apoE ϵ 4 capacity to clear plasma VLDL and cholesterol [60, 78]. This outcome is influenced by either down regulation of LDLr on the cell surface or decreased internalisation of the bound VLDL [57, 78]. The consequence of either scenario on HCV virus entry would be a decreased uptake of apoE ϵ 4-VLDL-HCV enriched complexes by hepatocytes (Fig. 3). In this regard, viral complexes with apoE ϵ 3 but not with apoE ϵ 4 would preserve optimal capacity for the receptor binding and virus entry (Fig. 3) in agreement with the published data [103, 119, 131].

VI Conclusion

The molecular interactions between infectious agents and their toxins and apoE-enriched lipoprotein particles in CNS and at the periphery may modulate the outcome, severity and persistence of infection. In addition, some of these interactions may also be implicated in AD development, progression and severity. The molecular mechanisms are not fully elucidated although some evidence point at the competition for the receptor binding and internalization between the viruses and Apo E containing lipoprotein particles (Fig. 2), as well as neutralisation of viruses/bacteria and their toxins by apoE-enriched lipoprotein particles (Figs. 1 and 3). In addition, infection may cause effects on cellular membranes leading to upregulation of β/γ -secretase activities and resulting in accumulation of cellular A β deposits [133]. Although the presence of different apoE isoforms creates conditions for an impaired cholesterol metabolism in CNS and at the periphery predisposing for a number of diseases including AD, atherosclerosis, stroke and others, the consequences may be wider and unpredictable when other actors, such as viruses and bacteria, come into play. As reviewed above, these other actors may become associated with the lipoprotein particles and apoE. Being also lipophilic, they may further compete or interact with the internally occurring lipophilic compounds, A β peptides, and prevent their clearance and disposal. Very

little evidence is available regarding interactions between infections and A β metabolism and their consequences. However, some epidemiological findings indicate that these aspects need thorough investigation in order to clearly determine the contribution these infectious agents play in the pathogenesis of AD.

The ability of the host to handle an infection depends on the host genetic background, age, immune status, diet as well as on the dose and virulence of the infectious agent. Infectious agents have also developed very sophisticated strategies to escape immune surveillance of the host of which their spread to the brain as an immune-privileged organ is the one. Both viruses and bacteria are able to persist latent in neurons or replicate at a very low level in neuroglia. During their persistence, microorganisms may continuously release toxic products and induce pro-inflammatory cytokines at low levels. These products become an additional burden to the host already challenged by the effects of ageing, poor diet, lack of exercise and/or genetic factors. Therefore, the use of anti-infective therapies to keep this burden in CNS low is highly attractive once definitive evidence has been obtained. Furthermore, the host also uses lipid particles for neutralising and disposing off the infectious agents and their toxic products. In this process the liver has a central position as the major organ of detoxification and clearance. In this regard, an infection-free liver may be a part of the preventative therapy for AD which will be greatly enhanced by a better understanding of the mechanism of action of the major genetic risk factor *APOE $\epsilon 4$* in the pathogenesis of this devastating disease.

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Figure legends

Figure 1 – Proposed apoE isoform-specific neutralisation of viral and bacterial toxins with apoE-enriched lipoprotein particles in CNS. Neurotoxic product of HIV virus, *tat*, released from infected macrophages and bacterial endotoxin LPS, derived locally or from the periphery, are neutralised by binding to apoE ϵ 3-enriched particles (E3) (A). APOE ϵ 4-(E4) enriched lipid particles (LP) exhibit reduced binding to neuronal LRP1 receptor as well as poor neutralisation of *tat* and LPS (B). In the absence of neutralisation, *tat* and LPS binding to LRP1 receptors may trigger intracellular signalling leading to neuronal injury by oxidative stress.

Figure 2 – Receptor binding and competition between HSV1 and apoE-enriched lipoprotein particles in CNS (modified from Itzhaki et al, 2006). Lipoprotein particles (LP) enriched with apoE ϵ 3 (E3) may show stronger binding to HSPG than HSV1 resulting in prevention of HSV1 adhesion (A), while apoE ϵ 4-enriched (E4) particles may have less affinity for binding to HSPG allowing HSV1 adhesion and entry into neurons (B) resulting in an increased infection and neuronal damage.

Figure 3 – Proposed model for VLDL-mediated HCV virus entry into hepatocytes. Possible coating of HCV virus nucleocapsids with apoE ϵ 3-enriched (E3) VLDL allows better viral entry into hepatocytes and better infection in liver (A), while coating with apoE ϵ 4-enriched (E4) VLDL prevents virus entry and liver tissue damage (B).

Figure 1

Figure 2

Figure 3