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Infiltration of the brain by pathogens causes Alzheimer's disease

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Abstract

Despite very numerous studies on Alzheimer's disease (AD), especially on amyloid plaques and neurofibrillary tangles, little information has been obtained thus on the causes of the disease. Evidence is described here that implicates firstly herpes simplex virus type 1 (HSV1) as a strong risk factor when it is present in brain of carriers of the type 4 allele of the gene for apolipoprotein E (APOE-ε4). Indirect support comes from studies indicating the role of APOE in several diverse diseases of known pathogen cause.

A second putative risk factor is the bacterium, *Chlamydia pneumoniae*. This pathogen has been identified and localized in AD brain. Current studies aimed at "proof of principle" address the entry of the organism into the CNS, the neuroinflammatory response to the organism, and the role that the organism plays in triggering AD pathology. An infection-based animal model demonstrates that following intranasal inoculation of BALB/c mice with *C. pneumoniae*, amyloid plaques/deposits consistent with those observed in the AD brain develop, thus implicating this infection in the etiology of AD.

Keywords: Alzheimer's disease; Herpes simplex virus type 1; Apolipoprotein E; *Chlamydia pneumoniae*; Polymerase chain reaction; Intrathecal antibodies; Human herpesvirus 6; Human cytomegalovirus; Blood–brain barrier; Olfactory pathways; Chronic inflammation; Animal model

1. Introduction

The early events in the development of Alzheimer's disease (AD) and its pathological features—amyloid plaques and neurofibrillary tangles (NFT)—remain unclear. Currently, about 20 million people worldwide suffer from AD, and the numbers are increasing with the rise in numbers of those reaching old age. Thus, the need for effective treatments for, and (hopefully) prevention of, AD is becoming ever more urgent.

The possibility of an infectious aetiology of several chronic diseases, including AD, has long been debated. Koch's postulates, which can in some cases provide absolute proof that a particular microorganism causes a particular disease, have been invaluable in the prevention and treatment of many diseases as well as in advancing microbiology. However, the postulates do not hold for chronic diseases of possible microbial aetiology, particularly those occurring late in life, or for those of possible viral aetiology, or for those that are multifactorial in origin. In diseases of relatively old age, a microbe acting earlier in life might operate by a "hit-and-run" mechanism, or could eventually be present only at an extremely low level, so that searches for the organism might reveal nothing even if it had initiated damage. In viral diseases, the postulates requiring isolation and growth in pure culture cannot be met as viruses reproduce only within living cells. In multifactorial diseases, a causative organism would not be present in those whose disease is due to other factors. However, absence of evidence is not evidence of absence: in several cases when, eventually, overwhelming experimental evidence was obtained, the pathogen concept had to be accepted even though it had met with great opposition initially; two examples are the involvement of viruses in certain types of cancer, and of a bacterium in stomach ulcers.

The detailed research that has been made seeking and detecting two pathogens in brain, herpes simplex virus type 1 (HSV1), and *Chlamydia pneumoniae*, is described here. The "hit-and-run" theme is not pursued because no such studies have been made in respect to AD and indeed it is obviously very difficult to devise appropriate experiments.

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2. Herpes simplex virus type 1 and AD

A possible viral involvement in the etiopathogenesis of AD was first suggested in 1977 [17]. Later, in 1982, HSV1 was proposed as a likely candidate virus [3] because in herpes simplex encephalitis (HSE), a very rare but extremely serious acute infection of the central nervous system (CNS), the earliest and most severely affected regions are the same as those showing the earliest and most severe pathological changes in AD. Although HSE is a very different disease from AD, it leads to certain long-term after-effects such as memory loss and cognitive decline which are highly characteristic of AD; also, in both diseases, headache and seizures are a common feature, even though AD patients at an advanced stage might well under-report these (and other) symptoms [60]. Further, anecdotal evidence suggests that at least in some cases, the pathology of burnt-out HSE brain resembles that seen in AD. There have been a number of reports of mild or atypical HSE, and of chronic, relapsing or recurrent HSV infection in brain, and these are consistent with the proposal (v.i.) that HSV1 in brain is a major factor in AD, that it is normally latent when in brain, and that periodically it reactivates (reviewed in [15]).

2.1. Experimental evidence for a role for HSV1

Support for the involvement of HSV1 comes from the fact that the virus is ubiquitous—a necessary characteristic, in view of the high proportion of people who succumb to AD. (At least until recent decades, about 90% of the population was infected with this virus in infancy; with rising socio-economic level, the age of primary infection has been increasing.) Also, HSV1 remains life-long in the infected host, in a latent state in peripheral neurons, reaching the brain in old age (v.i.), consistent with the onset of AD usually in the elderly.

The first main discovery vis-à-vis HSV1 involvement in AD was that the viral DNA is present (in latent form, as shown by detection of latency associated transcripts [29]) in a high proportion of elderly brains (as shown by polymerase chain reaction, PCR) [30,31], in the regions most affected by AD—the temporal and frontal cortices. It should be stressed that great care was taken to preclude contamination during preparation of tissue specimens, of DNA from them and during PCR; these have been described previously in detail [30,34]. Detection of HSV1 DNA in a high proportion of elderly control brains as well as AD brains does not vitiate the proposition that the virus plays a role in AD; the extent of damage caused by most (perhaps all) infectious agents is probably determined by host genetic factors; for example, only 20–40% of the population suffer from herpes labialis (cold sores), a disorder of the peripheral nervous system (PNS) known to be caused by HSV1, despite the fact that a very high proportion harbor the virus in their PNS. The second major discovery was that HSV1 confers a high risk of AD when in brain of those with the type 4 allele of the apolipoprotein E (APOE) gene [28,34]. AD is almost certainly multi-factorial, and consistently, the combination of virus and genetic factor was found in about 60% of the AD patients; the risk factors in the other 40% are not known. The results also showed that the AD brain is not predisposed to HSV1 infection: the proportion of elderly controls harboring the viral DNA in brain is similar to that of AD patients. Similarly, carriers of an ε4 allele are not predisposed to HSV1 infection: very few infected controls have an ε4 allele. Thus, APOE appears to affect the extent of viral damage, not susceptibility to the virus.

In younger people, the virus is almost always absent in brain ([31]; Wozniak, Mann, Cairns, Lin, Itzhaki, to be published). It was therefore suggested that HSV1 reaches the brain in older age, as the immune system declines, and resides there in latent form, and that under conditions of stress or immunosuppression it reactivates (as is the case in the PNS) and then causes an acute but localized infection—a "mild" encephalitis—the damage being more serious in those people who possess an APOE-ε4 allele. At the molecular level, apoE protein (and its truncated forms) and HSV1 both attach to cells using heparan sulphate proteoglycans (HSPG) [32,63] so a tentative explanation is that the two might compete for attachment and hence for entry into brain cells, with apoE4 competing less well; thus apoE4 would allow more viral entry, spread and hence consequent damage. Alternatively, apoE4 might repair viral damage less well [28,34].

2.2. Antibodies to HSV are present in CSF

PCR detection of HSV1 DNA in brain is strongly supported by the discovery that over 50% of AD patients and of age-matched controls have antibodies to HSV1 in their cerebrospinal fluid (CSF) (these antibodies are known to persist for up to several years after HSE); the antibodies are not due to leakage across the blood–CSF barrier, and they are absent in the CSF of children (in whose brains no HSV1 would be expected) (Wozniak, Combrinck, Wilcock, Itzhaki, submitted). These results thus indicate that the whole viral genome is present in brain (as did the detection previously, by RT–PCR, of latency-associated transcripts), and that an acute infection must have occurred there—and perhaps recurred.

2.3. Confirmation of detection of HSV1 in brain and its association with APOE-ε4 in AD

Four other groups have confirmed the detection of HSV1 DNA in brain [5,7,19,52]. As to APOE-ε4 and HSV1 in AD, there have been only two other adequate studies; one obtained results similar to the above [26], and the other showed a similar trend [6]. Recently, two groups claimed that there was no association between HSV1 and APOE-ε4 in AD but as each detected HSV1 DNA in only one brain (out of 34 and 15, respectively) the interpretation seems unwarranted

[22,43], especially as one study made no checks for contamination [27]. Thus the evidence from several laboratories for HSV1 presence in brain is strong (and vitiates the need to invoke the possibility of a "hit-and-run" mechanism).

2.4. APOE modulates outcome of infection

Strong support for the concept that HSV1 and APOE confer together a major risk for AD comes from the discovery that APOE-ε4 is a risk factor for cold sores [28,34], and that (surprisingly), APOE-ε2 is a risk factor for HSE [37]. Further support—even though more indirect—comes from studies showing that APOE determines the extent of damage in liver caused by a very different virus—hepatitis C virus [62], and that it influences damage in the CNS and PNS in HIV-infected people pre-AIDS [11]. Thus, APOE governs the outcome of several diseases that are known to be caused by viruses (very diverse ones). There is also preliminary evidence that APOE influences susceptibility to infection by the malaria protozoon [61]. The common factor amongst these pathogens is that they bind to HSPG in the cell surface—as does apoE, and/or they enter cells via one of the apoE receptors. Thus, one explanation for the involvement of apoE is that it competes with the pathogen for entry into cells, the isoform with the greatest affinity for the relevant cell type competing best and thus being the most protective.

The combined effect of HSV1 in brain and APOE-ε4 is shown also in a different type of investigation–infection of APOE–transgenic mice by HSV1 [10]. It was found that a few days after infection, the amount of virus in brain was much greater in APOE-ε4 than in APOE-ε3 mice. If in humans HSV1 reaches the brain at an earlier age or in a larger amount in APOE-ε4 carriers than in APOE-ε3 carriers, this could account for the earlier age of onset and the greater amyloid load in brain of the former.

2.5. HSV1 and amyloid and tau

Inflammatory processes may lead to increased production of β-amyloid (Aβ) protein and its deposition in the characteristic senile plaques seen in AD brain, which may play a primary role in the development of AD. Soluble protofibrillar oligomers of Aβ may also be important; for example, in mice over-expressing forms of the amyloid precursor protein (APP) associated with familial AD, subtle behavioral and physiological changes occur prior to or even independently of the development of amyloid plaques [45]. Thus any process increasing the levels of Aβ (or of Aβ 1–42, the form of Aβ which aggregates more readily, and which is considered to be more toxic than the normal Aβ 1–40), may contribute directly to the development of AD. Such processes would include various neurological insults leading to damage or stress in neuronal tissues (including head injury and stroke), which are known to cause deposition of β-amyloid [48,51]. Similarly, in HIV-infected brain, the level of APP mRNA increases in neurons [55], and amyloid plaques have been detected [16]. Reactivation of HSV1 within brain might well cause changes in APP processing and upregulation of Aβ secretion, which could contribute directly to AD via amyloid deposition or inflammatory cascades. In fact preliminary data show that HSV1 infection of human neuroblastoma cells in culture causes an increase in a 55 kDa C-terminal fragment of APP; levels of full length APP decrease—as expected from the known reduction in synthesis of host cell proteins caused by the virus [14].

Interestingly, a direct connection between HSV1 and β-amyloid has been shown in that there is marked homology between a sequence in the latter and in HSV1 glycoprotein B [12]. Also, a study of HSV1 transport along the giant axon of the squid [53] has shown that a very large number of APP molecules associate with HSV1 virions (the latter prepared from Vero cells and hence containing Vero APP) and are protected against degradation until the synapse is reached. If this occurs in human CNS neurons, it might affect APP axonal transport as well as hydrolysis of this molecule, thus causing synaptic and neuronal dysfunction.

Homology has also been reported between the virus and tau protein—the main component of the other common neuropathological feature of AD, neurofibrillary tangles [57]. Incidentally, in another disease of known viral cause—sub-acute sclerosing panencephalitis (caused by measles virus)—neurofibrillary tangles are often seen in patients many years after a symptom-free latency period following initial infection [4].

The involvement of HSV1 in AD points to the use of vaccination against the virus for prevention of at least some cases of AD as well as of HSE—this seems more likely now that the age of primary infection is rising, and is supported by animal studies showing that HSV1 infection of mice results in an acute infection of their brains followed, eventually, by the establishment there of latent viral infection (detected by PCR) in about 40% of the animals, and that vaccination against HSV1 with mixed viral glycoproteins protects the mice against establishment of latent brain infection [35].

3. Other herpesviruses and AD

A role for other herpesviruses in AD has been investigated also. Human herpesvirus 6 (HHV6) was found in brain of a much higher proportion of sufferers than aged normals suggesting that this virus might be a risk [36]; however, an equally valid conclusion is that AD brains are more susceptible to the virus. Nevertheless, animal models have shown that HHV6 enhances the damage caused by HSV1, suggesting that HHV6 might enhance the damage caused by HSV1 in AD brain. Other viruses (herpes simplex virus type 2 [36], varicella zoster virus [33] and cytomegalovirus (CMV) [36]) were sought but they were either absent or present at relatively low amounts. In the case of vascular dementia, CMV was found in brain of a greater proportion of patients than of

elderly normals, suggesting that this virus might play a role in this disease [38]; however, as with HHV6 it is uncertain whether its presence is a cause or an effect. Interestingly, however, recent studies have implicated it as a causal factor in coronary heart disease.

4. Epidemiological studies

Three recent studies of a very different type support a viral role in AD, even if indirectly: (a) AD patients showed a decline in cognitive function for at least two months after a systemic infection, associated probably with microglial cell activation caused by entry of cytokines into the brain [23]; (b) cognitive impairment in elderly cardiovascular patients was associated with viral pathogen burden (HSV1 alone and with HSV2 and cytomegalovirus) [56]; and (c) vaccination against various viruses was protective against AD [59]. All these are consistent with the proposal that a peripheral infection, by giving rise to cytokine production and entry into brain with consequent brain inflammation, would reactivate HSV1 in brain (while vaccination, by preventing infection, would reduce inflammation events).

5. *Chlamydia pneumoniae and AD*

Another organism that has been linked to AD is *C. pneumoniae*. In this case, the bacterial DNA has been found (in some studies) to be present in brain of a very high proportion of AD patients but in only very few age-matched normals, indicating a greater susceptibility to entry and infection of brain by the bacterium (and possibly also to damage by the latter) in AD patients rather than, as with HSV1, a greater susceptibility to damage of the nervous system.

5.1. Experimental evidence for a role of C. pneumoniae in AD

The first report of an association of *C. pneumoniae* with AD demonstrated by PCR that the DNA of the organism was present in 90% of postmortem brain samples examined from late-onset AD [2]. As compared to these results, only 5% of postmortem brain samples from age-matched, non-AD, control individuals contained DNA from *C. pneumoniae*. In this study, PCR was conducted using highly specific and sensitive probes for sequences of *C. pneumoniae* chromosomal DNA [54]. PCR positivity was detected in samples obtained from at least one area demonstrating neuropathology (e.g., temporal cortices, hippocampus, parietal cortex, pre-frontal cortex) as well as, in four cases, areas less often demonstrating AD pathology (e.g., cerebellum). Interestingly, in the latter four cases, severe neuropathology was observed throughout, while in the two AD brains that were PCR-negative, very mild pathology was observed [2].

In addition to PCR, other techniques such as immunohistochemistry and electron microscopy were used to determine if *C. pneumoniae* antigens or the organism itself were present in the brain tissues [2]. These analyses demonstrated in AD samples, but not in control samples, that antigens for *C. pneumoniae* were apparent within perivascular macrophages, microglia, and astroglial cells in areas of the temporal cortices, hippocampus, parietal cortex, and pre-frontal cortex. Electron microscopy revealed chlamydial inclusions that contained elementary and reticulate bodies. Immunoelectron microscopy verified *C. pneumoniae* in the samples following labeling of the organism with a monoclonal antibody to an outer membrane protein. Immunogold labeling was not evident in the comparable control sections negative by PCR. Frozen tissue samples were analyzed by RT–PCR to determine whether RNA transcripts from *C. pneumoniae* could be identified. With this analysis, two transcripts were identified, one for KDO transferase and the other for a 76 kDa protein [2]. Given that the RNA for these transcripts was recoverable from the frozen tissues, homogenates of representative PCR and RT–PCR positive samples were prepared for culturing in THP-1 monocytes. Culturing was successful from two different AD brains and negative from two control brains [2]. Taken together, these data suggested that *C. pneumoniae* was present in areas of AD neuropathology, was viable from AD brain tissues, and was capable of being cultured from those tissues.

Pneumonia as a cause of death for AD patients in the original study of *C. pneumoniae* associated with AD was documented in 4 of 19 cases, with one of the four expiring from aspiration pneumonia [2]. Although pneumonia is a fairly common cause of death among AD patients, there were few differences between the AD and control populations available to the investigators. In addition, of the few cases for which pneumonia was a cause of death in both the populations, in no instance was *C. pneumoniae* documented as the etiologic agent. Thus, it appears unlikely that *C. pneumoniae*'s presence in AD tissues related to the patient's having a pneumonia caused by this organism at the time of death. Other factors may predispose a patient to long-term infection: these include the intracellular nature of the organism and the ease with which *C. pneumoniae* infections can become chronic and/or persistent in the body. As evidence indicated that the organism resided within cells in the parenchyma of AD brains and that some profiles of the organism showed atypia, these infections, most likely, were persistent in the brains of these individuals.

The association of the APOE-ε4 genotype with *C. pneumoniae* infection in AD was addressed in the first report of the association of *C. pneumoniae* in sporadic AD [2]. In that report, 11 of 19 (58%) had at least one ε4 allele, and in all 11 cases, *C. pneumoniae* was identified from those brains. The relationship of these factors also has been studied in reactive arthritis (ReA) patients who often demonstrate infectious agents in synovial tissues. In brief, results from one ReA study indicated that 68% of patients who were PCR positive for *C. pneumoniae* DNA in their synovia possessed at least one copy of the APOE-ε4 allele (see [24]). The APOE-ε4

gene product may actually promote aspects of *C. pneumoniae* pathobiology in both diseases. Further studies are required to determine whether APOE-ε4 and *C. pneumoniae* infection are directly related, and whether co-incidence of the two should be considered as a risk factor.

The uniqueness of the early study and the importance of replicating these findings have led others to attempt to identify and associate the presence of *C. pneumoniae* with sporadic AD. There have been mixed results reported from these attempts. Two reports from work presented at two different scientific meetings identified *C. pneumoniae* in brain samples from late-onset sporadic AD [42,47]. One of these reports employed replicate PCR assays and probit regression analyses in demonstrating that DNA from *C. pneumoniae* was present in 85% of AD brain samples [42], but not in control samples. The second report used immunohistochemical analyses to identify *C. pneumoniae* antigens in 11 of 12 paraffin embedded AD brain samples, but found no evidence for these antigens in controls (0 of 15) [47]. In contrast, three early reports, and one more recently, found no or minimal association of *C. pneumoniae* with AD [18,46,50,58]. These studies also used PCR and immunohistochemistry screening of AD and control tissues, although the techniques differed somewhat from those of the original work. In review of the literature, there have been discrepancies in clearly identifying an association of *C. pneumoniae* with other diseases as well. At this time, multiple reasons for all these discrepancies could be given, including sampling error, methodology, and absence of standardized techniques. This conundrum has led to a more specific evaluation in vitro and in animal models of the interrelationship of infection with *C. pneumoniae* and the known pathological processes identified in sporadic AD. In this regard, studies have been employed to determine how *C. pneumoniae* can enter the CNS, stimulate a neuroinflammatory process, and act as a "trigger" for amyloid processing and deposition into amyloid plaques, a hallmark of AD pathology.

5.2. Entry of *C. pneumoniae* into the CNS

Infection of the oral and nasal mucosae of the respiratory tract by *C. pneumoniae* is considered to be the normal route of entry for this obligate, intracellular pathogen into the body [20]. However, the exact mechanisms by which the organism becomes a systemic pathogen are not well-defined. Two routes by which *C. pneumoniae* may enter the CNS are thought to be intravascular and olfactory. Evidence for these routes has been obtained in studies of the association of this organism with AD [2,24]. *Chlamydia pneumoniae* infected glial cells, perivascular macrophages, and monocytes have been identified around blood vessels in the AD brain [2,40], and in olfactory bulbs by PCR [24]. As the monocyte may be the principal peripheral blood cell in which *C. pneumoniae* is harbored [1] and in which the organism gains initial entry to the circulation [8], the monocyte may very well be the vehicle for trafficking *C. pneumoniae* across the blood–brain barrier (BBB) [40].

5.2.1. *C. pneumoniae* and the blood–brain barrier

There is precedence for chronic persistent infection of monocytes with *C. pneumoniae* [1], and this could facilitate systemic and CNS infection with this organism. Recent evidence implicates monocytes and human brain microvascular endothelial cells (HBMECs) in the entry of *C. pneumoniae* through an in vitro model of the blood–brain barrier [40]. *Chlamydia pneumoniae* infection of HBMECs increased their expression of the surface adhesion molecules, intercellular adhesion molecule-1 (ICAM-1) and vascular cellular adhesion molecule-1 (VCAM-1). In a similar manner, *C. pneumoniae* infection of THP-1 monocytes resulted in increased surface expression of the β integrins LFA-1 and MAC-1, the ligands for ICAM-1, and the α4β1 integrin VLA-4, the ligand for VCAM-1. With increased expression of the surface adhesins on the endothelial cells and the integrins on the monocytes, there was a three-fold increase in transmigration of the monocytes through the in vitro barrier in comparison to transmigration of uninfected monocytes through an uninfected endothelial barrier. Thus, brain microvascular endothelial cells and peripheral monocytes could play a major role in promoting the entry of *C. pneumoniae* into the CNS.

In conjunction with these studies, junctional protein expression was examined to determine how the zonula adherens and zonula occludens junctions were affected following endothelial and monocyte infection with *C. pneumoniae* [41]. Analysis of surface-to-junction cross-talk examined the role that cadherins, catenin, and occludin play in maintaining the endothelial junctional integrity following infection. Results from this study demonstrated that infection of HBMECs with *C. pneumoniae* led to up regulation of VE-cadheren, N-cadheren, and β-catenin [41]. In contrast, infection of the HBMECs with *C. pneumoniae* resulted in the down-regulation of the tight junctional protein, occludin, at 36–48 h post-infection, with recovery of occludin expression at 72 h post-infection [41]. These data suggest that a compensatory response occurred at the level of the adherens junction to maintain barrier integrity during the down-regulation of tight junctional proteins at which time barrier permeability increased. Occludin expression returned to control levels at 72 h post-infection, which suggests that the permeability changes were transient. These transient changes would increase the likelihood that transmigration of monocytes through the HBMEC barrier would occur. The alteration in the blood–brain barrier transport mechanism could therefore lead to increased immune cell infiltration and pathogen entry into the brain. The influx of activated monocytes infected with *C. pneumoniae* through the blood–brain barrier could have dire consequences in the brain. An example of this is shown with brain infection with HIV-1 in which infected perivascular monocytes infiltrate the brain resulting in subsequent symptoms of dementia [9]. As perivascular

macrophages, pericytes, microglia, and astroglia have been shown to be infected with *C. pneumoniae* in the AD brain [2], this infection could account for a significant proportion of neuroinflammation and underlying pathology in the brain.

5.2.2. *C. pneumoniae and the olfactory pathway*

An alternative route of entry for *C. pneumoniae* into the CNS is through the olfactory pathways. Since *C. pneumoniae* readily infects epithelial cells and has direct access to the olfactory neuroepithelium of the nasal olfactory system, this route of infection would be likely given that *C. pneumoniae* is a respiratory pathogen. Examination of the olfactory bulbs obtained at autopsy from two AD cases revealed by PCR and RT–PCR that *C. pneumoniae* genetic material was present in these structures [24]. Some of the earliest pathology observed in AD occurs in the olfactory and entorhinal cortices, in particular layers II and III of the entorhinal cortex of the parahippocampal gyrus from which neural projections of the perforant pathway arise to innervate the hippocampal formation [25]. Earlier studies found evidence of the organism in the entorhinal cortex, hippocampus, and temporal cortex [2]. These findings bring into question how infection, inflammation, and/or damage of the olfactory bulbs could lead to damage in deeper cortical and limbic structures in the AD brain.

5.3. *Inflammation and C. pneumoniae*

Immunopathogenesis from inflammation is a hallmark of Chlamydia-induced disease. Chlamydial infections in vivo typically result in chronic inflammation characterized cellularly by the presence of activated monocytes and macrophages [49]. At sites of chlamydial infections, pro-inflammatory cytokines (IL-1β, IL-6, TNFα) and TH1-associated cytokines (IFNγ and IL-12) have been identified [49]. Promotion of any or all of these responses could be evoked by chronic or persistent infection with *C. pneumoniae* as well as by chlamydial products such as lipopolysaccharide (LPS), heat shock proteins, and outer membrane proteins. The expression of LPS alone by this organism could account for numerous aspects of AD pathology. Previous work by others [21] demonstrated that *E. coli* LPS injected at low dose directly into the brains of rats resulted in inflammation characterized by increased cytokine production and microglial activation. In addition, pathology comparable to that observed in AD was observed in the rat temporal lobe as demonstrated by the induction of the amyloid precursor protein. These studies suggest that products of infection, produced by the infectant or by the host in response to infection, may stimulate inflammation in the brain resulting in neuropathology.

5.4. *C. pneumoniae and an animal model of AD*

Proof of principle for the involvement of infection in chronic disease often uses animal models to determine how infection may promote the pathology consistent with a particular disease entity. In this regard, a mouse model has been developed to test whether *C. pneumoniae* can be a primary trigger for AD pathology following a non-invasive route of inoculation [39]. Since normal, young, non-transgenic mice do not develop AD pathology, they are a suitable host for analyzing whether inoculation with this organism would lead to any pathological change in the brain. Normal, young (3 months), female BALB/c mice were inoculated intranasally with an isolate of *C. pneumoniae* obtained from an AD brain. Uninfected control mice received vehicle only which consisted of Hank's balanced salt solution. The intranasal route of inoculation was used for the following reasons: the recognized olfactory deficits in AD [25], the previous PCR findings of *C. pneumoniae* in human olfactory bulbs [24], and the respiratory nature of this pathogen providing direct access to the nasal olfactory neuroepithelium. Mice were sacrificed at 1, 2, and 3 months post-inoculation by perfusion fixation and the brain and olfactory bulbs were collected. Representative areas of the brain and olfactory bulbs were analyzed for the presence of *C. pneumoniae*, inflammation, and pathology characteristic of AD, in particular amyloid plaques and neurofibrillary tangles.

Light microscopy and electron microscopy revealed the presence of *C. pneumoniae* in the olfactory bulbs of animals 1–3 months post-infection [39]. Upon labeling for typical pathology in areas of the brain associated with AD pathology (e.g., hippocampus, entorhinal cortex) and in a region less affected in AD (e.g., cerebellum), the most striking finding was the presence of Aβ 1–42 immunoreactive amyloid plaques. Few plaques were first observed at 1 month post-infection in all regions. Increased numbers of plaques were observed at 3 months post-infection in areas typically affected in AD. With regard to the cerebellum at 3 months post-infection, there were some plaques apparent, but these were much fewer in this area and not apparent in all cerebella examined. Evidence of inflammation was observed infrequently with reactive astrocytes located in some areas of amyloid plaque deposition as well as around some blood vessels. Analysis of the amyloid plaques following thioflavin S staining revealed fibrillogenesis in a few plaques. Fibrillogenesis of amyloid peptides is a common finding in mature plaques in the AD brain. These data suggest that mature amyloid plaque formation in the brain is a consequence of infection with *C. pneumoniae*; however, it is unknown whether inflammation and/or direct turn on of amyloid production/processing in the neurons is responsible for amyloid deposition and plaque formation. In some regions of the brain, intracellular immunolabeling for Aβ 1–42 in pyramidal neurons was observed. Intracellular processing of the amyloid precursor protein into Aβ 1–42 recently has been recognized in the pyramidal neurons in the entorhinal cortex of AD brains, and is speculated to account for a portion of the dense-core amyloid plaques observed in AD cortices [13]. The animal data suggest that the initial intracellular accumulation of Aβ 1–42 may give rise to the extracellular

accumulation of Aβ 1–42. Whether this intracellular accumulation could also give rise to other neuropathology remains to be determined.

Silver staining (Gallyas) and immunolabeling for the tau protein failed to demonstrate at 1, 2, and 3 months post-infection that neurofibrillary tangle pathology was evident in the mouse brain. It is possible that the amount of time post-infection was not sufficient to initiate this type of pathology in mouse, and thus, experiments have been initiated to investigate whether long-term infection (e.g., >1 year) will result in tangle formation and increased plaque formation consistent with the progressive nature of AD. Alternatively, immunosenescent aged animals may be more susceptible to tangle formation following acute or chronic Chlamydial infection. Studies to address these questions are now underway. The development of this unique model should provide a foundation for testing how sporadic AD may be a consequence of infection as well as testing intervention strategies designed to eradicate the infection, diminish inflammation, and improve/restore cognitive function.

6. Other bacteria and AD

In the brains of neuropathologically confirmed cases of AD, bacteria other than *C. pneumoniae* have been found [44]. In particular, spirochetes were detected in the blood and cerebrospinal fluid from AD patients. Furthermore, these organisms were isolated and cultured from AD patients [44]. Ultrastructural analysis indicated that taxonomically these organisms were in the order of *Spirochaetaes*. Intriguingly, the tick-borne pathogen *Borrelia burgdorferi* is the major human spirochetal infection in the US and Europe and neurologic involvement can occur in up to 40% of symptomatic infections. Whether this organism has a direct relationship to the pathogenesis of AD remains to be determined. As an aside, in the original study of the association of *C. pneumoniae* and AD [2], PCR for DNA against *B. burgdorferi*, as well as that for other organisms such as *Chlamydia trachomatis*, *Mycoplasma pneumoniae* and *Mycoplasma hominis* was all negative. Negative findings from those brains, however, do not necessarily rule out the involvement of spirochetes in AD. There may be population differences, technical issues, as well as strain variants of *Borrelia* and/or other spirochetes that compromise the ability to detect infection in brain tissues. Obviously, this scenario may be quite similar to that relating to detection of *C. pneumoniae*, as both positive and negative findings have been reported in the analysis of *C. pneumoniae*'s involvement in AD.

7. Epilogue

There are defined technical difficulties in consistently determining infection associated with chronic disease throughout the body. A relatively primitive understanding of issues of latency of infection, dissemination of infection throughout the body, and strain heterogeneity of a variety of unique microorganisms requires tempered conclusions at this time. Nonetheless, a microbial involvement in AD points to the use of well-established anti-microbial agents for the treatment of the disease. In the case of *C. pneumoniae*, common antibiotics such as tetracyclines, rifampin and the fluoroquinolones might be used whereas with HSV1, acyclovir or its derivatives could be employed. In addition, vaccinations might be used as a preventative measure. In any event, given the current evidence for the involvement of pathogens in the etiology of AD, infection as a significant component in the pathogenesis of this neurodegenerative disease warrants serious consideration—and greatly expanded investigation.

Note added in proof

A recent epidemiological study on bipolar disorder patients, relevent to HSV1 and AD, shows that in middle-aged control subjects, cognitive decline is not associated with systemic infection [Dickerson FB, Boronow JJ, Stallings C, Origoni AE, Cole S, Krivogorsky B, Yolken RH. Infection with herpes simplex virus type 1 is associated with cognitive deficits in bipolar disorder. Biol Psych 2004;55:588–593.]. This is very consistent with the absence of HSV1 DNA in brain of younger people ([31]; Wozniak, Mann, Cairns, Lin, Itzhaki, to be published).Thus, inflammation alone in brain might not affect cognitive function but might require another factor such as HSV1 presence.

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