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Chlamydophila Pneumoniae and the Etiology of Late-Onset Alzheimer's Disease

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Abstract. Sporadic, late-onset Alzheimer's disease (LOAD) is a non-familial, progressive neurodegenerative disease that is now the most common and severe form of dementia in the elderly. That dementia is a direct result of neuronal damage and loss associated with accumulations of abnormal protein deposits in the brain. Great strides have been made in the past 20 years with regard to understanding the pathological entities that arise in the AD brain, both for familial AD (∼5% of all cases) and LOAD (∼95% of all cases). The neuropathology observed includes: neuritic senile plaques (NSPs), neurofibrillary tangles (NFTs), neuropil threads (NPs), and often deposits of cerebrovascular amyloid. Genetic, biochemical, and immunological analyses have provided a relatively detailed knowledge of these entities, but our understanding of the "trigger" events leading to the many cascades resulting in this pathology and neurodegeneration is still quite limited. For this reason, the etiology of AD, in particular LOAD, has remained elusive. However, a number of recent and ongoing studies have implicated infection in the etiology and pathogenesis of LOAD. This review focuses specifically on infection with *Chlamydophila* (*Chlamydia*) *pneumoniae* in LOAD and how this infection may function as a "trigger or initiator" in the pathogenesis of this disease.

Keywords: Alzheimer's disease, amyloid, animal models antibiotic, APOE, *Chlamydia pneumoniae*, etiology, infection, LOAD, neuroinflammation

OVERVIEW

The idea that rheumatoid arthritis, atherosclerosis, multiple sclerosis, and other currently idiopathic chronic diseases might be caused by, or at very least exacerbated by, long-term microbial infection is not new. However, this idea has garnered renewed interest since the conclusive demonstration that the bacterial pathogen *Helicobacter pylori* is involved in the pathogenesis of certain ulcers (reviewed in [1]). Indeed, the notion of infectious involvement in chronic disease genesis was the focus of much interest in both chronic and infectious disease research for much of the 20th century. For one example, early in the 1900's rheumatoid arthritis was considered to be an infectious disease. That explanation faded in the 1930's but re-emerged several times over the course of the century [2–5]. Similarly, it has been contended for many years that the development of multiple sclerosis involves an infectious component [6], and congruent arguments have been proposed in relation to several other idiopathic chronic diseases.

In essentially all of these cases, infectious etiology and/or involvement in chronic disease initiation have been difficult to establish and thus have not been accepted readily by either the research or clinical communities. In place of viral or bacterial agents, many alternative mechanisms have been examined to explain chronic disease generation. For example, over the past decade several large-scale studies have been undertaken to explore the possible genetic bases of rheumatoid arthritis and other chronic clinical entities, including late-onset Alzheimer's disease (LOAD). Perhaps not

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surprisingly, most such studies have indicated that disease development is not attributable to one or a few straightforward mutations or gene polymorphisms, as appears to be the case in familial Alzheimer's disease (FAD); rather, research indicates that disease genesis appears to be multifactorial, resulting from complex interactions between environmental factors and genetic background [7,8]. Many previous attempts have been made to define associations between various infectious agents and LOAD, but none of these associations proved to be either etiologic or exacerbating for LOAD-related neuropathology. Viral pathogens targeted and so far dismissed in these studies include measles virus, lentiviruses, adenovirus, and several others [9, 10]. However, interesting studies from a group in the UK have identified herpes simplex virus type 1 (HSV-1) infection as a risk factor for development of AD in people expressing *APOE* ε4 [11,12] (see also other material in this issue). In addition, various bacterial pathogens, including *Coxiella burnettii* and *Chlamydia trachomatis* have been investigated, but no relationship with AD neuropathogenesis was identified [13].

In earlier reports, we described an association between infection with the intracellular respiratory bacterial pathogen *Chlamydophila* (*Chlamydia*) *pneumoniae* and the genesis of LOAD [14,15]. In this review, we summarize our earlier work, as well as newer studies published by our group and others that implicate a possible chlamydial involvement in the genesis of LOAD. We also provide some recent but as yet unpublished observations from our group which bear on that question.

THE AMYLOID CASCADE HYPOTHESIS

The dominant idea that has guided studies of the origin of neuropathology in Alzheimer's disease (AD) for more than a decade is the "amyloid cascade" hypothesis. As contended by this important idea, the generation and deposition of amyloid-β (Aβ) are considered to be the critical events underlying neuronal degeneration [16]. This hypothesis is applicable to FAD, since it has been well established that this version of AD is caused by genetic mutations which result in increased amyloid formation and deposition. However, studies have demonstrated that the etiology of LOAD does not arise from identical, similar, or other genetic defects, thus calling into question the generality of Aβ as the universal causative factor in AD. Importantly for this article, the neuropathology underlying both FAD and LOAD is essentially identical, indicating that factors other than the specific genetic lesions underlying the former must exist to explain the neuropathology of LOAD. The latter disease typically presents in older age, and age is indeed the primary risk factor for its development. Recently, other risk factors have been proposed, including: atherosclerosis [17], Type 2 diabetes [18], neurotrauma [19], and infection [11,14,20]. Thus, the likely scenario for the development of LOAD appears to be an as yet poorly understood interplay between genetic risk, as exemplified by possession of the *APOE* ε4 allele, and environmental factor(s), including perhaps infection with *C. pneumoniae*.

CHLAMYDOPHILA (CHLAMYDIA) PNEUMONIAE

C. pneumoniae is an obligate intracellular bacterial pathogen of the human respiratory tract that is responsible for community-acquired pneumonia [21]. The organism was initially described as a unique chlamydial species in 1989. This organism, like all chlamydial species, infects mucosal surfaces, in this case the lung/pulmonary and nasal mucosa [21–23]. Systemic dissemination of the bacterium from the respiratory tract has been extensively documented [24], and available evidence indicates that a major vehicle of dissemination employed by the organism is the monocytic cell [25]. Epidemiologic studies demonstrate that *C. pneumoniae* is ubiquitous [26].

As with all chlamydial species, *C. pneumoniae* undergoes an unusual biphasic developmental cycle during normal growth. In the first phase, the elementary body (EB), the infectious metabolically-inactive extracellular form of the organism, attaches to a target eukaryotic host cell; these are most often epithelial cells, but other cell types can be infected, including astrocytes, microglia, and neurons [15,27,28]. The organism then is brought into a cytoplasmic inclusion within which it reorganizes into the metabolically active growth form of the organism, the reticulate body (RB). RB undergo several rounds of cell division, after which most reorganize back to the EB form.

Newly-formed EB are released from the host cell *via* lysis or exocytosis to continue propagation of the infection [27]. *C. pneumoniae* requires about 72 hr to complete passage through the cycle. Interestingly, studies have shown that under certain conditions and/or within specific host cell types the organism can and often does alter its biologic state to generate persistent,

long-term infections. Chlamydiae undergoing such infections are morphologically aberrant and display an unusual transcriptional profile [29–31]. Importantly, reports indicate that the mechanisms of pathogenesis differ between active infection and persistent chlamydial infection, and it is in the persistent state that these organisms elicit chronic disease [32,33].

C. pneumoniae has been associated with several chronic pulmonary diseases, including chronic obstructive pulmonary disease and sarcoidosis [34]. Importantly, infection with this organism has been associated with a surprisingly wide array of non-respiratory diseases, including atherosclerosis, giant cell (temporal) arteritis, inflammatory arthritis, multiple sclerosis, and others [35–38]. While some of these associations remain controversial, the role of this organism in atherogenesis has gained significant credence during the last ten years [37,39,40].

C. PNEUMONIAE AND ALZHEIMER'S DISEASE

In our initial report of an association of *C. pneumoniae* with AD, we demonstrated that the DNA of the organism was present in 90% of postmortem, sporadic, LOAD brain samples by polymerase chain reaction (PCR) [14]. In contrast, we found that only 5% of postmortem, age-matched, non-AD, control brain samples contained *C. pneumoniae* DNA. PCR was performed using highly specific and sensitive systems targeting sequences on the *C. pneumoniae* chromosome [35]. Brain tissues from areas that typically display the neuropathology characteristic of AD were analyzed, including temporal cortex, hippocampus, parietal cortex, and pre-frontal cortex. As an internal control, areas less often demonstrating AD pathology, such as the cerebellum, also were included. In 17 of 19 LOAD brains, positive samples were obtained from at least one area demonstrating neuropathology, and in four cases, from the cerebellum. In the brains from which the cerebella were positive, we noted that severe neuropathology existed throughout the brain, including the cerebella, while in the two LOAD brains that were PCR-negative, extremely mild pathology was observed [14].

Additional experimental methods were employed to determine if *C. pneumoniae* antigens or viable organisms were present in the LOAD and control brain tissue samples. Samples from PCR-positive brains were analyzed by immunohistochemistry and electron microscopy. LOAD samples contained antigens from *C. pneumoniae*, particularly in areas of the temporal cortex, hippocampus, parietal cortex, and pre-frontal cortex; perivascular macrophages, microglia, and astroglial cells all were immuno-positive for the organism. With regard to the single (parietal cortex) sample from a control brain which alone was weakly but repeatably PCR-positive, we found no *C. pneumoniae* antigens. Electron microscopy of LOAD brain tissue samples revealed chlamydial inclusions containing EB and metabolically active RB. Immunoelectron microscopy demonstrated labeling of the organism with a monoclonal antibody to an outer membrane protein (OMP). Immunoelectron microscopy was negative in comparable control sections that were negative by PCR.

Frozen brain tissue samples were analyzed by reverse transcriptase-PCR (RT-PCR) to determine whether bacterial RNA could be identified. This analysis successfully targeted two messengers, one encoding the KDO transferase, and the other an $\sim$376 kDa protein specific to *C. pneumoniae*. Since transcripts were obtained from frozen tissues, we hypothesized that we also could obtain cultures of *C. pneumoniae* from these tissues. Tissue homogenates of representative PCR and RT-PCR positive samples were prepared and incubated with a human monocyte cell line (THP-1) in culture. Recovery of viable bacteria was successful from two different AD brains and negative from two control brains [14]. Thus, our analyses confirmed that *C. pneumoniae* DNA and antigens were present in areas of AD neuropathology, and that the organism remained viable within frozen AD brain tissues.

Analyses of LOAD brain tissue samples revealed that 11 of the PCR-positive samples had at least one allele for the apoE4 isoform (64%), consistent with apoE4 being a risk factor for development of LOAD [41]. A separate study in individuals with reactive arthritis showed that in patients who had *C. pneumoniae* DNA in their synovial tissues, 68% had at least one copy of the *APOE* ε4 allele [42]. Given the high percentage of individuals in these two studies who were both infected with *C. pneumoniae* and *APOE* ε4 positive, we argued that some relationship between that allele and *C. pneumoniae* exists; we further contended that both factors confer risk for chronic diseases that have been associated with both traits [14,42].

RELATIONSHIP BETWEEN C. PNEUMONIAE, APOE, AND LOAD

Many chronic clinical entities with which *C. pneumoniae* infection has been associated also are associ-

ated with possession of the ε4 allele type at the *APOE* locus on human chromosome 19 [6]. Recent work from our group has provided evidence of a clinically meaningful relationship between possession of the *APOE* ε4 allele and infection with *C. pneumoniae* in LOAD. *In situ* hybridization analyses indicated that the number of *C. pneumoniae*-infected cells in affected brain regions of ε4-bearing LOAD patients was higher overall than that in congruent brain regions from LOAD patients lacking that allele [43]. Further real time PCR analyses of brain tissue samples targeting sequences on the *C. pneumoniae* chromosome demonstrated that the bacterial burden in samples lacking the ε4 allele varied widely, as expected, but that samples from ε4-bearing patients had significantly higher bacterial loads overall than did congruent samples from patients without the allele [43]. These data inform previous observations that ε4-bearing individuals have a higher risk of developing LOAD, and that those ε4-bearing patients progress more rapidly to cognitive dysfunction than do individuals lacking this allele.

Other recent studies have begun to elucidate the basis of the relationship between the APOE ε4 gene product (i.e., apolipoprotein E, apoE), infection by *C. pneumoniae*, and disease genesis in several contexts. In all its forms, apoE is a secreted glycoprotein, and it was not obvious how any allelic product of the *APOE* locus could influence chlamydial pathogenesis. In studies currently underway, apoE4, unlike other products from that locus, has been demonstrated to enhance attachment of *C. pneumoniae* EB to target host cells, including astrocytes and microglial cells, by about 3-fold over levels observed in the absence of that specific allelic product; interestingly, apoE4 does not enhance attachment of EB from the sister species *C. trachomatis* to its host cells [44]. Importantly, apoE4 adherent to the EB surface retains its ability to attach to its normal receptor on the surface of host eukaryotic cells; that receptor is the LDL receptor and other members of that receptor family [45, and Gerard et al., manuscript in preparation]. Thus, while we do not yet fully understand all details concerning how apoE4-enhanced host cell attachment is accomplished for *C. pneumoniae*, these observations do provide a link between infection, the product of the *APOE* ε4 allele, and a number of clinical entities associated with both, including LOAD.

INITIAL CHARACTERIZATION OF C. PNEUMONIAE IN THE LOAD BRAIN

As indicated above, *C. pneumoniae* can be cultured from LOAD brain tissues. We recently prepared two such AD brain isolates and determined standard molecular genetic and cell biological characteristics for each. Not surprisingly, both isolates were found to be genetically diverse (i.e., not clonal), as with respiratory isolates studied by us and others [46]. Extensive analyses for single nucleotide polymorphisms (SNPs) around the 1.3 mbp chromosome indicated several differences from standard respiratory isolates and strains, although comparisons failed to identify specific genetic attributes that would indicate a neurotropism for the brain. The SNP analyses clearly indicated that the brain-derived strains were more closely related to respiratory strains of *C. pneumoniae* than to atherogenic strains [Dreses-Werringloer et al, unpublished observations]. The chromosome of one brain isolate is currently undergoing extensive DNA sequence analyses, but no data are available as yet from those studies. Cell biological studies demonstrated standard inclusion morphology and chlamydial morphology of organisms of both isolates in human epithelial cells (HEp-2), astrocytes (U-87 MG), and microglial cells (CHME-5), as in previously published studies from this group [28]. Studies are now underway to assess the molecular genetic and cell biological characteristics of the two AD brain isolates in human neuronal cell lines.

ENTRY OF C. PNEUMONIAE INTO THE CENTRAL NERVOUS SYSTEM

A question of major interest concerns the route by which a respiratory pathogen such as *C. pneumoniae* reaches the CNS. The olfactory pathway may be the most vulnerable area of the body by which chlamydiae gain access to the brain. As *C. pneumoniae* is a respiratory organism infecting epithelial cells on mucosal surfaces, it contacts the olfactory neuroepithelium of the nasal olfactory system upon inspiration. Examination of the olfactory bulbs obtained at autopsy from LOAD cases revealed by PCR and RT-PCR that *C. pneumoniae* genetic material was present in these structures [4]. Intriguingly, some of the earliest pathology observed in AD occurs in the olfactory bulbs [48]. These appear to be damaged earlier than the entorhinal cortex, suggesting that olfaction is damaged preclinically in AD [48]. Following damage to the bulbs, the entorhinal cortices demonstrate neurofibrillary tangles, 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. We previously showed the or-

ganism in the entorhinal cortex, hippocampus, and temporal cortex [14]. These findings bring into question how specific infection(s), inflammation, and/or damage of the olfactory bulbs could lead to damage in deeper cortical and limbic structures, thereby resulting in symptoms of LOAD.

C. pneumoniae also may cross the blood brain barrier (BBB) to enter the brain following uptake by monocytes circulating in the small vessels of the lungs [49, 50]. Importantly, we have provided evidence demonstrating *C. pneumoniae*-infected glial cells, perivascular macrophages, and monocytes surrounding blood vessels in the AD brain [14,47,51]. Persistent infection of monocytes with *C. pneumoniae* has been demonstrated [50], and human brain microvascular endothelial cells (HBMECs) have been implicated in the entry of *C. pneumoniae* through an *in vitro* model of the blood-brain barrier [51]. HBMECs increased expression of the surface adhesion molecules ICAM-1 and VCAM-1 following *C. pneumoniae* infection *in vitro*. Increased expression of these surface adhesion molecules on the endothelial cells and the integrins on the monocytes resulted in a 3-fold increase in transmigration of monocytes through the *in vitro* barrier relative to the transmigration of uninfected monocytes through an uninfected endothelial barrier. These findings suggest that one result of *C. pneumoniae* infection of brain microvascular endothelia and/or peripheral monocytes is the enhanced entry of *C. pneumoniae* into the CNS from the blood.

In related studies, the roles of cadherin, catenin, and occludin in maintaining the endothelial junctional integrity following infection was examined [52]. Perturbations of the cytoskeleton can directly affect the endothelial junctional complex assembly, and infection may be involved with this function. Infection of HBMECs with *C. pneumoniae* resulted in the up-regulation of expression of several molecules involved in maintenance of the junctional assembly complex, including N–cadherin, and β-catenin. In contrast, infection resulted in the down-regulation of the tight junctional protein, occludin, but with recovery of occludin expression at 72 hr post-infection. These data suggest that a compensatory response occurred following infection; the junctional complex maintained the barrier integrity during the down-regulation of tight junctional proteins, at which time barrier permeability increased. As occludin expression returned to control levels at 72 hr post-infection, permeability changes were reversed. Thus, while the overall change was transient, there was an increasing likelihood for transmigration of monocytes through the HBMEC barrier [52]. Given the cumulative evidence of entry into the brain through the olfactory system as well as across the blood-brain barrier, a "double-hit" process may operate with the outcome of neuroinflammation and eventual neurodegeneration.

The chronic nature of infections by *C. pneumoniae*, and the initiation over time of significant immunopathology from those infections, presumably would result in cellular pathology and death in affected brain areas. Infected cells in the CNS could release organism into the extracellular fluid in the brain, as well as into the CSF. While we have not performed a controlled study investigating this possibility, others recently have reported a study designed to determine the presence of *C. pneumoniae* DNA in the CSF of AD patients, vascular dementia patients, and in control patients [53]. In this study, the prevalence of *C. pneumoniae* in the LOAD patient group as determined by PCR of CSF was 43.9% ($N = 57$ patients), and this was significantly higher than the prevalence in the vascular dementia group (9.5%, $N = 21$ patients) and in the control group (0.6%, $N = 47$ patients). The authors reported that the presence of *C. pneumoniae* DNA in the CSF of LOAD patients significantly increased the occurrence of the disease (odds ratio 7.21).

C. PNEUMONIAE, NEUROINFLAMMATION, AND LOAD

Chlamydia-induced disease is usually a result of immunopathogenesis, i.e., chronic inflammation characterized by the presence of activated monocytes and macrophages in infected tissues [54]. Further, chlamydial infection promotes secretion of many proinflammatory cytokines [54]. Strong inflammatory responses are engendered by chlamydial lipopolysaccharide (LPS), heat shock proteins, and outer membrane proteins. Potentially, LPS alone could account for numerous aspects of LOAD pathology as investigations by others have shown that *E. coli* LPS, when injected at low dose directly into the brains of rats, results in inflammation characterized by increased cytokine production and microglial activation [55]. Comparable damage to that found in LOAD was observed in the rat temporal lobe, as induction of the amyloid-β protein precursor was apparent, suggesting that products of infection produced by an organism, or by the host in response to it, may stimulate inflammation leading to LOAD-related neurodegeneration.

In the LOAD brain, inflammation is thought to arise as a result of Aβ deposition and has been advanced as the primary mechanism in LOAD pathogenesis [56]. Clinical trials investigating the effects of non-steroidal anti-inflammatory drugs in older populations also implicate inflammation as a factor in LOAD, since some trials have shown that such drugs can delay onset of LOAD [57]; however, these drugs appear to be ineffective as a therapeutic for the disease. The resident cells in the brain responsible for inflammation are typically microglia and to a lesser extent astroglia. Both of these are activated in the LOAD brain and often are identified in and around amyloid plaques [58]. Microglia and astroglia respond to insult by producing proinflammatory cytokines and reactive oxygen species. Identification of *C. pneumoniae* in the CNS in both cell types suggests that infection-initiated inflammation may be involved in LOAD neuropathology [14,15].

Infected microglia, astroglia, and perivascular macrophages [14,15] as well as neurons [15], were observed in our studies in areas of amyloid deposition. Activation of microglia and astroglia [59] in response to the presence of infected, activated monocytes could promote increased production of a variety of cytokines and chemokines [60]. In a recent *in vitro* study [61], several proinflammatory molecules including MCP-1, IL-6, and TNFα were significantly higher in supernatant fluids of *C. pneumoniae*-infected murine microglial cells compared with controls. Infected murine astrocytes displayed higher levels of MCP-1 and IL-6 compared to controls. Neurons exposed to conditioned supernatant from infected murine microglial cells displayed a significant increase in cell death compared with mock infected supernatants; addition of neutralizing antibodies to IL-6 and TNFα to the conditioned supernatant reduced neuronal cell death by $\sim$50%. These data suggest that *C. pneumoniae* infection plays a role in neuroinflammation by stimulating a strong pro-inflammatory response that results in neurodegeneration.

REPLICATION STUDIES

The results of our initial studies and the implications of bacterial infection in the genesis of LOAD led other groups to attempt identification of *C. pneumoniae* in various tissue and other samples from patients with LOAD. Reports that appeared shortly after our initial study provided mixed results. Two reports presented at the European Chlamydia Research Meeting in 2000 and published only as abstracts identified *C. pneumoniae* in brain samples from LOAD patients [62,63]. One of these used replicative PCR assays to show that $\sim$85% of frozen AD brain samples were PCR-positive for *C. pneumoniae* DNA, while controls were uniformly negative [62]. The other report used immunohistochemical analyses to show that *C. pneumoniae* antigens were present in 11 of 12 LOAD brain tissue samples, but not in samples from non-demented controls [63]. In contrast, other groups have had little success in finding an association between *C. pneumoniae* infection and LOAD [64–67]; many different techniques were used in these studies, with no other study using identical methodology to our own. In a recent review of the literature from other areas in which *C. pneumoniae* is implicated as a factor in disease genesis (e.g., atherosclerosis), significant discrepancies in analytical methods used among laboratories, and the variable data resulting from them, was pointed out [68]. Furthermore, and quite intriguingly, one study on the presence of *C. pneumoniae* infection in atherosclerotic arteries from various vascular regions including the brain demonstrated that 7 of 9 ($\sim$78%) patients were PCR positive for *C. pneumoniae* in brain samples [69]. All of these patients suffered from severe atherosclerosis, but none were diagnosed with LOAD at the time of death. As atherosclerosis is now considered a risk factor for the development of LOAD [17], an interesting possibility is that the risk is incurred due to infection as well as to changes in cholesterol. At the time of writing, we do not understand all the reasons for variable results from screening studies targeting *C. pneumoniae* in LOAD patient samples. However, it is clear that multiple reasons for these discrepancies are present, including sampling error, enormously variable methodologies, and absence of standardized techniques.

In light of these discrepancies, we recently replicated our initial findings of an association of *C. pneumoniae* in LOAD using new tissues from LOAD and non-LOAD control brains [15]. PCR analysis in multiple assays targeting the *C. pneumoniae* Cpn1046 and Cpn0695 genes revealed that tissues from 20 of 25 LOAD brains, and from 3 of 27 non-LOAD control brains, were PCR-positive [15]. The organism was cultured from some of the LOAD brains (see above), and various chlamydial transcripts from several additional brains, demonstrated the viability and metabolic activity of the organisms in those samples. Immunohistochemical analyses revealed that astrocytes, microglia, and $\sim$20% of neurons were infected by *C. pneumoniae*. The finding of a large proportion of neu-

rons PCR-positive for *C. pneumoniae* in this later study was unique [15]. Similar to the finding of the initial study [14], infected cells were located in close proximity to both neuritic senile plaques and neurofibrillary tangle-containing neurons in the brain. These observations have implications for a direct effect of *C. pneumoniae* on neuronal cell injury/death, as well as on the potential for *C. pneumoniae* to act as perpetrator/initiator of granulovacuolar degeneration in the LOAD brain.

ANTIBIOTIC TREATMENT STUDIES

If infection by *C. pneumoniae* is involved in the genesis of LOAD, then antimicrobial treatment might be a therapeutic approach. A clinical trial has been reported that used a combination approach for treatment of LOAD [70]; doxycycline and rifampin were given daily for 3 months to patients with probable LOAD and mild/moderate dementia. The primary outcome was a change in Standardized AD Assessment Scale cognitive subscale (SADAScog) at 6 months. Secondary outcomes included changes in the SADAScog at 12 months and analysis of dysfunctional behavior, depression, and functional status. Results showed less decline in SADAScog score at 6 months in the antibiotic group compared to the placebo group ($p = 0.034$); the SADAScog score at 12 months in the antibiotic and placebo groups was not significantly different ($p = 0.079$). However, the antibiotic group showed significantly less dysfunctional behavior at 3 months ($p = 0.028$), and at 12 months the antibiotic group showed reduced decline in mini-mental status scores ($p = 0.032$). No correlations to change in criteria for *C. pneumoniae* infection were apparent as determined by analysis of serum antibody titers and PCR of blood samples. As with antibiotic trials to assess efficacy in obviating aspects of atherogenesis and cardiovascular disease, the outcome of the LOAD-related antibiotic trial indicated no meaningful efficacy in amelioration of relevant pathogenesis. In hindsight, and perhaps not surprisingly, these failures have been understood to mean that simple, straightforward antibiotic treatment of complex disease entities, which are advanced at the time of treatment initiation, is not a viable strategy. However, it remains to be determined whether an antibiotic/anti-inflammatory regimen in at-risk patients or following early diagnosis could be a rational approach.

ANIMAL MODELS FOR C. PNEUMONIAE INFECTION

Previous models of AD have utilized transgenic mice over-expressing the well-characterized mutants of presenilin and gene products of amyloid-β protein precursor [71]. Over-expression of amyloid results in development of amyloid plaques in the brain, paralleling the pathology observed in familial AD. Experimental systems using transgenic mice, however, do not address the initiating events of LOAD, in which mutations of the amyloid-β protein precursor and presenilin are not present. Development of a non-transgenic animal model was undertaken to address how infection could play a role in the pathogenesis of LOAD independent of predisposing genetic factors [72]. This work utilized *C. pneumoniae* (AD brain isolate [14]) infection of naïve BALB/c mice to determine whether infection could promote damage in the brain similar to that identified in sporadic LOAD [22]. BALB/c mice were shown previously to be susceptible to a respiratory infection with *C. pneumoniae* (respiratory isolate, AR-39) and to maintain a persistent respiratory infection [73]. The more recent study tested the hypothesis that *C. pneumoniae* infection in BALB/c mice could initiate processes that result in the development of AD-like pathology in the brain [72].

Following intranasal infection, *C. pneumoniae* was identified in the olfactory epithelia (chlamydial antigens) and the olfactory bulbs (chlamydial antigens and typical morphology) by both light and electron microscopy [72]. Analysis of pathology in the brain revealed Aβ1–42 deposits that resembled amyloid plaques found in human AD. Activation of astrocytes as well as co-localization of some of these reactive astrocytes with the amyloid deposits suggested that a cellular inflammatory response was initiated. This response could be due to the presence of *C. pneumoniae* or directed against amyloid deposits or soluble amyloid induced by *C. pneumoniae* infection. Interestingly, these findings suggest that Aβ generation is a response to the infectious insult, and lend support to the hypothesis that Aβ possibly could act as a "bioflocculant" as suggested by others [74]. The induction of amyloid deposits in the brains of non-transgenic BALB/c mice supports the hypothesis that infection with *C. pneumoniae* is capable of accelerating or inducing AD-like pathology, and it may be a trigger in the pathogenesis of LOAD.

Further animal modeling has been initiated to determine if antibiotic intervention following intranasal infection could treat or limit the pathology induced fol-

lowing infection in the CNS [75]. Following intranasal infection with *C. pneumoniae* (respiratory isolate, AR-39), different groups of mice were treated with moxifloxacin hydrochloride (Avelox) at days 7–21, 28–42, 56–70, or 84–98 post-infection. The animals were sacrificed at 6 months post-infection and their brains analyzed for the presence of *C. pneumoniae*, Aβ1–42 deposition (plaques), and astrocyte (GFAP) cellular reactivity. Immunohistochemistry analysis revealed that the antigens of the organism could still be identified at 6 months post-infection in olfactory tissues and in the brain proper. Intriguingly, at the earliest time of antibiotic treatment (i.e., 7–21 days post infection), the number of Aβ1–42 reactive amyloid plaques was equivalent to the baseline level as observed in uninfected mice. However, in the infected mice in which the antibiotic treatment was delayed until 56 days post-infection, the number of amyloid plaques was 8–9 fold higher than baseline; this number of plaques was comparable to the number found in the brains of infected animals that received *no* antibiotics. These data suggest that early antibiotic intervention after infection is effective in limiting the number of amyloid plaques that arise as a result of infection, eventhough complete antigen eradication may not be achieved. Thus, while more studies of early antibiotic treatment are underway, these results suggest that early intervention strategies appear to be most effective in limiting amyloid deposition.

CONCLUSIONS

The studies briefly summarized in this review demonstrate that much information has been gathered to increase our understanding of the means by which *C. pneumoniae* might contribute to the neuropathogenesis of LOAD. We have demonstrated *C. pneumoniae* infection of the brain, using various techniques, in 80–90% of LOAD brain tissue samples, correlated *C. pneumoniae* infection in LOAD to the expression of *APOE* ε4, demonstrated that astroglia, microglia, neurons, endothelial cells, and monocytes in the LOAD brain may be infected with the organism, and defined two probable pathways of entry into the CNS. Further, we have correlated *C. pneumoniae*-stimulated inflammatory responses to the neuroinflammation of LOAD, and we have developed an animal model that demonstrates triggering of neuropathology consistent with AD pathology *in vivo* by *C. pneumoniae* infection. Taken together, this work provides a reasonable basis for continued investigation of detailed and specific molecular and cell biologic mechanisms that underlie infection with *C. pneumoniae* as at least a risk factor, and possibly as a principal etiologic event, in the genesis of LOAD.

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