Alzheimer’s disease (AD) is a chronic condition in which inflammation has been shown to contribute to neurodegeneration. Current thinking suggests that deposition of β-amyloid in the brain promotes inflammation resulting in neuronal damage/death. Alternatively, our data suggest that chronic inflammation observed in late-onset sporadic AD may be stimulated by infection with the obligate, intracellular bacterium, Chlamydia pneumoniae. Our results indicate that C pneumoniae is found in high frequency in glial cells in areas of neuropathology within the brains of patients with AD. Based on our evidence, nervous system infection with C pneumoniae should be considered a risk factor for sporadic AD.

(Key words: Alzheimer’s disease, Chlamydia pneumoniae, intracellular bacterium, chronic infection, neuroinflammation)

Alzheimer’s disease (AD) is one of the most severe dementing illnesses that increases with the increasing age of the population. Symptoms include progressive memory loss, decreased cognition, problems with spacial and perceptual recognition, and impairment of daily living. Great strides have been made in our understanding of the pathologic process associated with AD and in recognizing that inflammation appears to play a critical role in AD pathogenesis. The pathologic entities associated with AD include intraneuronal neurofibrillary tangles (NFTs) composed of paired helical filaments of the tau protein, a microtubule associated protein, and extracellular neuritic senile plaques (NSPs) composed principally of amyloid peptides termed β-amyloid (Aβ) deposited in β-pleated sheet conformations. Current thought by many studying AD supports the concept that Aβ deposition appears to be critical in the neuronal degeneration observed in patients with AD. However, controversy still surrounds the issue of whether Aβ deposition is causative for AD or whether Aβ deposition is the end result of a process stimulated by other factors.

In the early-onset, familial form of AD (FAD), mutations in the amyloid precursor protein gene (βAPP) are associated with increased Aβ deposition and early onset of symptoms. β-Amyloid is derived from the amyloid precursor protein (APP) following protease digestion of this protein near its C-terminus. The function of the APP has not been totally elucidated, though the protein is a type I transmembrane protein whose N-terminal sequence is extracellular and C-terminal sequence is intracellular. Mutations in the genes encoding two other transmembrane proteins, presenilin-1 (PS-1) and presenilin-2 (PS-2) also lead to increased Aβ deposition in FAD patients. Mutations in PS-1 and PS-2, in conjunction with those in βAPP, appear to account for most early-onset FAD cases.

Interestingly, and quite importantly, FAD accounts only for a small percent ( ~5%) of total AD cases. Late-onset AD is much more prevalent (~95%) than FAD, and while the latter is almost certainly genetically based, the former is not. One risk factor identified for late-onset disease is the expression of the apolipoprotein E (apoE) ε4 allele. The apoE protein functions in the transport of cholesterol and other hydrophobic molecules as it is a protein constituent of low-density lipoprotein and chylomicrons. The apoE typically is found as one of three isoforms corresponding to apoE ε2, ε3, or ε4. Most of the population demonstrates allelic variations of ε2 and ε3, with ε4 being the least expressed. Not all patients expressing ε4 have AD; however, its presence increases risk for the disease several-fold and is associated with earlier onset/more rapid progression in FAD.

Infectious agents considered
The neuropathologic characteristic of both early- and late-onset AD is similar, but the processes and causes initiating that pathology remain to be elucidated for late-onset disease. In this regard, neurologic disease can be caused by microorganisms, and several groups have attempted to establish a causal relationship between viral infection and late-onset sporadic AD, but no etiologic link has been unequivocally demonstrated. Among the viruses investigated have been measles virus, lentiviruses, adenoviruses, and others, but all have been dismissed as potential agents associated with late-onset AD. Herpes simplex virus type 1 (HSV-1) infection has been identified as a risk factor for development of AD in people expressing the apoE ε4 allele, though it is not clear how and under what circumstances this virus might interact with the allele or its gene product to produce or promote disease. In similar approaches to those investigating viral associations with AD, various bacterial species have been investigated and...
Alzheimer’s disease and Chlamydia pneumoniae

Recent evidence has demonstrated that infectious agents may well be associated with primary or secondary events in the development of late-onset sporadic AD. Obviously, we are far from understanding the interplay between genetics and the environment with regard to their roles in disease onset and progression. In this regard, infection as a causative factor or as a cofactor in numerous chronic diseases including those of the nervous system is an evolving concept. Our laboratory is focused specifically on defining how infection plays a role in late-onset AD. We recently demonstrated an association of the bacterium Chlamydia pneumoniae with late-onset sporadic AD.19 Presence of the organism was assessed in postmortem brain samples from patients with and without AD. We screened nucleic acids prepared from those samples for bacterial DNA sequences, and such PCR analyses showed that areas with typical AD-related neuropathologic processes were positive in 17 of 19 patients with AD. Similar analyses of identical brain regions in 18 of 19 control patients were PCR negative.

Electron/immuno-electron microscopic studies of tissues from affected AD brain regions identified chlamydial elementary and reticulate bodies, but similar analyses of non-AD brains were negative for the bacterium. Immunohistochemical analyses of AD brains, but not those of controls, identified C pneumoniae within pericytes, microglia, and astroglia. Further immunolabeling studies confirmed the organisms’ intracellular presence primarily in regions of neuropathologic processes in the AD brain, and RT-PCR assays using RNA from affected areas of AD-affected brains demonstrated that transcripts from two C pneumoniae genes were present. The viability of the organism in frozen tissues also was demonstrated as C pneumoniae was cultured from two different AD-affected brains. Taken together, our data suggest that this organism plays some direct or indirect role in the pathogenesis of AD.

Our assessment of apoE genotype for our patient populations is consistent with its being a risk factor for AD, especially in combination with chlamydial infection of the central nervous system. Presence of at least one e4 allele in most AD patients PCR-positive for C pneumoniae may suggest that this gene product allows or promotes CNS infection, a contention that seems consistent with results from the HSV-1 report.13 More study will be required to assess this possibility, but we note that the two AD patients studied in our initial report who were PCR negative for C pneumoniae were e3 homozygotes, and they had the least severe neuropathologic condition of our patients with AD. The patients with the most severe neuropathology had chlamydial DNA in the temporal lobe, prefrontal cortex, and cerebellum; three of these latter four patients had an e4 allele.

Immunohistochemical staining showed chlamydia-infected cells associated with blood vessels in the brain, suggesting that dissemination of the organism from the site of primary infection involves the vasculature. Recent studies in our laboratory on the means of dissemination of C pneumoniae through the blood-brain barrier (BBB) support the hypothesis that infected monocytes can traffic the organism through the BBB.20 In this regard, the organism has been identified in circulating mononuclear cells in a rabbit model of infection.21 Blood-borne transport of C pneumoniae EBs, however, must also be considered as a means by which circulatory dissemination to the brain occurs. The mechanism by which EBs, or infected cells, pass the BBB is being addressed currently as sufficient evidence suggests that this barrier is compromised in patients with AD.22

Although no causal relationship has been established between acute or chronic C pneumoniae infection and the development of late-onset AD, our results demonstrated that microglia and astroglia in regions of neuropathologic lesions harbored this organism in a high proportion of patients with AD.19 Ultrastructural analysis demonstrated that the organism exhibited properties previously characterized for C pneumoniae with elementary bodies (EBs) and reticulate bodies (RBs) ranging from 0.2 μm to 1.2 μm in diameter easily discernable. In addition, and more important, we confirmed that the observed organisms were C pneumoniae using a highly specific, commercially available anti-MOMP monoclonal Ab (mAb) that targets a major outer membrane protein (MOMP) of the organism. A second mAb, which specifically immunolabels the membrane-bound lipopolysaccharide (LPS) of the genus Chlamydiae, was shown to immunolabel cells in AD-affected brains as well.

Double immunolabeling of glial cells with glial-specific antibodies such as glial fibrillary acidic protein (GFAP) and inducible nitric oxide synthase (iNOS) in conjunction with antibodies to C pneumoniae enabled us to identify the particular infected cell type found in regions demonstrating classic AD neuropathologic processes (for example, neuritic senile plaques). This unique demonstration of infection by a respiratory pathogen in the Alzheimer’s brain has been confirmed recently by two independent investigations for which data were presented at the World Congress on Alzheimer’s Disease 200024 and at the recent International Chlamydia Meeting in Helsinki, Finland.25,26 These studies found C pneumoniae...
infection in brains of patients with AD. In these reports, PCR and immunohistochemistry demonstrated a high percentage, 85% and 92%, respectively, of AD-affected tissues showing evidence of *C pneumoniae*. This is consistent with our initial findings, which showed that 90% of AD-affected tissues were *C pneumoniae* positive.

**Chlamydia pneumoniae**

What is unique about *C pneumoniae* that makes it an intriguing suspect in AD pathogenesis? *Chlamydia pneumoniae* is an obligate intracellular bacterium that is a respiratory pathogen, initially infecting the oral and nasal mucosa. This organism is a significant agent in acute respiratory tract infections, including pneumonia, sinusitis, and bronchitis, as well as in more severe and chronic pulmonary pathologic conditions, including sarcoidosis and chronic obstructive pulmonary disease. Infection with *C pneumoniae* has been implicated in unexpected clinical manifestations, including meningencephalitis, multiple sclerosis, and atherosclerosis.

A causal relationship between infection with *C pneumoniae* and these diseases has not been demonstrated, but a significant correlation exists between serum anti-*C pneumoniae* Ab titers and coronary artery disease; the bacterium has been identified by electron microscopy and other methods in atheromatous plaques. Because a relationship may exist among atherosclerosis, ApoE ε4 expression, and late-onset AD, and because of the ubiquity of the bacterium in older adults, a relationship between infection with *C pneumoniae* and late-onset sporadic AD is reasonable.

*Chlamydia pneumoniae* exhibits two distinct morphologies during its developmental cycle: the EB and the RB. Host cells are infected by the small, metabolically inactive EB. EBs are approximately 0.2 μm to 1.2 μm in diameter, are often pear-shaped with a clear periplasmic space, and have condensed nuclear material. The elementary body is thought to bind the prospective host cell via a glycosaminoglycan moiety in conjunction with an unidentified protein thought to be the gene product of ompA (MOMP). Once bound, the bacterium is endocytosed into a membrane-bound vesicle where it will alter its morphology to the larger, vegetative reticulate body. The RBs will begin DNA, RNA, and protein synthesis before division into two daughter organisms via binary fission. Each RB undergoes seven to eight mitotic divisions before finally reverting to the EB phenotype. These newly formed EBs escape the host cell following cell lysis or via exocytosis. Alternatively, the organisms may remain viable in the host cells over extended periods through a process by which lysosomal degradation apparently is evaded. In these chronic infections, the chlamydia organisms show aberrant morphology while maintaining their metabolic activity and viability.

The unique persistence of *C pneumoniae* in the brains of patients with AD presents a number of possibilities regarding the organism’s disposition. Because we were the first to report *C pneumoniae* infection in the AD-affected brain, we could not predict whether this organism would exhibit unique characteristics when compared with *C pneumoniae* from other clinical conditions such as arthritis and atherosclerosis. Sporadic AD affects predominantly aged individuals, and because we do not know precisely when these individuals are initially infected, there is the possibility of either a chronic or an acute infection in each patient. Interestingly, chlamydia involved in persistent infections have been characterized by altered morphologic and biochemical features.

Medications (for example, antibiotics and anti-inflammatory drugs) prescribed to older individuals could affect the phenotypic and persistent nature of the organism, especially in the brains of patients with AD. The proinflammatory response following infection may result in damage to *C pneumoniae* as a result of evoked bacteriostatic events such as free radical damage in the tissues and/or nutrient starvation of the organisms. In this regard, typical and atypical forms of *C pneumoniae* were observed in the AD-affected brain. The main phenotypical differences were exhibited in the overall shape and size of the organisms, but immunoreactivity to the organism in the brain was similar to that of *C pneumoniae* laboratory strains.

**Modality of infection**

The brain tissues examined contained both intracellular and extracellular EBs and RBs with numerous organisms captured in a state of binary fission. These observations indicate the possibility that *C pneumoniae* bacteria can form a latent infection, and quite possibly promote an active chronic infectious state that results in the activation of astroglias and microglia in the AD-affected brain. Multiple EBs and RBs were found not only within the glial/neuronal tissue, but also within macrophages and presumptive pericytes. This infectious disposition may be indicative of multiple modalities of infection.

First, there is the possibility that the organism could spread hematogenously either in the infectious EB state or within monocytes/macrophages and invade the endothelial and/or perivascular cells. Stasis due to vessel damage, ischemia, or presence of atherosclerotic plaques within the lumen of the cerebral vessels could allow the organism to traverse the BBB.

Second, if there is an active infection, chronic or acute, cells of the monocyte/macrophage lineage may scavenge the organisms, enter the bloodstream, and spread these pathogens to other susceptible regions including the brain. This mechanism of spread is being addressed currently in our laboratory using an in vitro model of the BBB.

Third, we are analyzing the likelihood that *C pneumoniae* could gain entry to the CNS by anterograde and retrograde transport to the olfactory forebrain regions. Deficits in the olfactory system have been documented for patients with AD. Because *C pneumoniae* is harbored in the respiratory tract and has a predilection for infecting epithelial cells, the olfactory neuroepithelia in the nasal passages are a like-
ly target for infection. Following entry into these epithelia, potential damage and/or cell death may occur in the main olfactory bulb and olfactory cortex, thereby setting the stage for further retrograde neuronal damage. As some of the earliest AD pathology is purported to occur in the cortical olfactory and entorhinal cortices, the amygdala, and the hippocampus, infection with C pneumoniae could account for this neuropathologic processes.

**Inflammation and chlamydial infections**

In vivo sites of chlamydial infection demonstrate chronic inflammation characterized by activated monocytes and macrophages. Immunopathogenesis resulting from inflammation is the hallmark for chlamydia-induced disease. Chlamydial infection may elicit the inflammatory response via up-regulated cytokine production in infected or neighboring cells. Ingredients for this elicitation can include direct infection, lipopolysaccharide stimulation (LPS found on the outer surface of C pneumoniae), and/or production of heat shock proteins, such as Hsp60. Proinflammatory cytokines (IL-1β, TNFα, IL-6) and TH1-associated cytokines such as IFNγ and IL-12 have been identified at sites of chlamydial infection. In systemic chronic disease such as observed with reactive arthritis, TH1/TH2 CD4+ cells and macrophages can all be observed at sites of inflammation. Because of the feedback mechanisms involved in cytokine production and their influence on chlamydial growth and persistence, a persistent state of chlamydial infection can be maintained following infection.

**Neuroinflammation in Alzheimer’s disease and chlamydial infections**

Aside from the issue of whether chlamydial infection of the brain is directly responsible for AD-related pathologic processes, this infection may explain some characteristics of late-onset sporadic AD. Inflammation is common in the AD brain in areas of neuropathology, and this inflammation, currently thought to result from Aβ deposition, has been advanced as a pathogenic mechanism in the disease. Indeed, inflammation has been implicated as an important factor in a number of diseases, and one study indicated that administration of nonsteroidal anti-inflammatory drugs could be beneficial in treating AD. Because chlamydial infection engenders a strong inflammatory response, infection by C pneumoniae, in part, may be responsible for the inflammation observed in the AD-affected brain. Importantly, our results demonstrate the frequent infection of microglia and astroglia with C pneumoniae in the AD-affected brain. Intriguingly, an animal model, in which low-dose infusion of LPS has been used, revealed remarkable parallels with AD inflammation including APP induction, increased cytokine production with microglial reactivity, and temporal lobe pathology.

Microglia are the resident tissue macrophages of the brain, and once activated they, like astroglia, are a source of inflammatory cytokines, including IL-1β, TNFα, and IL-6. Our results indicate that chlamydia-infected glial cells are concentrated in areas of AD pathology, regions that usually show inflammation, suggesting a relationship between that pathology and producers of relevant cytokines. Uncannily, there is superimposition of the inflammatory response elicited by C pneumoniae to that observed in AD.

We would like to know whether chlamydia-infected microglia, astroglia, and/or pericytes are involved in aberrant production/deposition of Aβ via alteration of βAPP, PS-1, or PS-2 expression, or modification of processing of the βAPP gene product. We are exploring these questions with appropriate C pneumoniae-infected cell lines and have been able to demonstrate that different cell types (for example, monocytes, astrocytes, endothelia, and epithelia) respond to infection with C pneumoniae in different ways.

Monocytes and endothelial cells, in particular, appear to increase processing of βAPP into both the 1-40 and 1-42 β peptide forms. In addition, monocytes are activated by C pneumoniae and produce proinflammatory cytokines such as IL-1β, TNFα, and IL-6, all previously shown to be produced and involved in brain damage in AD. Furthermore, reactive oxygen species such as superoxide and nitric oxide are produced by these infected monocytes. In the AD brain, presumably the monocytes/microglial cells are contributing to neuronal damage through activation of similar pathways.

Intriguingly, our earlier study showed that the infected cells in the AD-affected brains were microglia, astroglia, perivascular pericytes, and macrophages. These findings and those of our in vitro studies suggest that the glial cells and blood monocytes that traffic into the brain are primarily infected with C pneumoniae. This scenario begs the question of how these infected and activated cells contribute to early and/or late inflammatory states in the AD brain, and how this infection may contribute directly to nerve cell damage, β-amyloid accumulation, and blood vessel problems.

Our in vitro studies using a BBB model clearly show that infected human brain microvascular endothelia facilitate the entry of monocytes through the endothelial monolayer following up-regulation of surface receptors such as ICAM and VCAM, and protein change at the junctional complexes. In addition, the infected endothelial cells process βAPP into the fibrillogenic 1-42 β peptide. How this may relate to congoophilic angiopathy (another commonly observed pathologic entity in the AD brain) is unknown. These data are exciting because of the accumulating evidence in the cardiovascular arena that suggests a similar mechanism for C pneumoniae trafficking through peripheral blood vessels in the process of atherosclerosis. The overlap with our studies and those demonstrating peripheral vessel damage may provide a greater understanding of how C pneumoniae infection is involved in stroke, cerebrovascular disease, and AD.

Given the recent corroborating reports on the localization of C pneumoniae in the AD-affected brain, we are compelled to continue our efforts in
uncovering and further defining how *C. pneumoniae* is involved in causing these neurodegenerative processes. The fundamental question that must be answered is whether this organism could be a causative agent or an opportunistic pathogen that finds its way into damaged tissues. In either case, the organism’s presence in AD can affect aspects of symptomatology and progression that have their roots in the inflammation and blood vessel damage currently thought to play significant roles in this disease. These studies also have great implications for helping to define the more global issue of pathogen entry into the CNS. Our preliminary data suggest an intimate relationship with the infection and neurodegeneration, a relationship based on chronic, persistent infection and the insidious entry of the organism into the CNS.

**General emergence of infectious disease**

A great body of evidence has arisen in recent years on the emergence and/or reemergence of infectious diseases. Currently, many studies are focusing on developing new experimental approaches designed around infection and chronic disease connections. Organisms that were once thought to be minimal threats to our health must be reevaluated because of our expanding knowledge of their increasing presence in chronic disease and in areas of the body never before thought to contain these entities. There is great value in identifying infectious causes of chronic diseases as treatment regimens are either in place or can be explored to specifically eradicate the infectious agent, and presumably, the chronic disease.

Historically, scientists have explored the roles of infection in neurologic disease with a primary focus on acute infections and acute disease. With the advent of HIV and AIDS, chronic herpes infections, post-polio syndrome, Lyme neuroborreliosis, prions, and so forth, we have come to realize that nervous system infection is a complex and often chronic issue, one that is not easily studied or well understood. Current strategies for treatment apply to acute infections and short-term or direct consequences of infection. Microorganisms such as *C. pneumoniae* do not act in a conventional manner. They appear to establish subclinical and apparently asymptomatic infections, but the long-term effects can be irreversible and life-threatening by the time the patient exhibits clinical symptoms.

**Comments**

Although we have not yet established a causal relationship between CNS infection with *C. pneumoniae* and development of late-onset AD, the association between chlamydia-infected glial cells and areas of neuropathology indicates that investigation of such a relationship is required. In this regard, it will be important to examine specimens from patients with other neurodegenerative diseases (for example, Parkinson’s disease) to determine whether dissemination of the bacterium to the brain also occurs in these conditions, or whether it is confined to those with late-onset AD. In late-onset AD, involvement of the apoE-e4 gene product in *C. pneumoniae* infection of the CNS must also be addressed. Our studies clearly suggest that CNS infection by *C. pneumoniae* should be considered a risk factor for sporadic AD, with the hope that with continued study a definitive role for the organism in the pathogenesis process will be elucidated.

**Acknowledgments**

The authors thank Mr Christopher Scott Little, Ms Angela MacIntyre, and Ms Chris Hammond for their contributions to this work. Studies reported herein were supported in part by the Foundation for Research Into Diseases of Aging (FRIDA) and PHS/NIH grant AI44055.

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