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Evidence for an Infectious Etiology in Alzheimer's Disease

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1. Introduction

The possibility of an infectious etiology of several chronic diseases, including Alzheimer's Disease, has long been debated. More than a century ago Alois Alzheimer studied neurological infection with *Treponema pallidum*, the causative agent of syphilis, a spirochete later associated with dementia (Noguchi and Moore, 1913). There are many chronic diseases for which there is strong evidence of an infectious etiology (see table 1), and numerous chronic diseases for which there is suspicion of infection as the etiologic agent for that disease (see table 2) (Tables 1 and 2 are adapted from a colloquium sponsored by the American Academy of Microbiology from June 2004). Koch's postulates, that 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 most chronic diseases of microbial etiology, particularly those occurring late in life. Furthermore, they do not hold true for those of possible viral etiology, or for those that are multi-factorial in origin. In diseases of relatively old age, microbes acting earlier in life might operate by a "hit-and-hide" mechanism, or could over time be present at an extremely low level, so that searches for the organism might not reveal the culprit until long after damage has been initiated. In viral diseases and for those involving unique organisms such as obligate intracellular bacteria (eg, Chlamydia), the postulates requiring isolation and growth in pure culture cannot be met completely as these organisms reproduce only within living cells. In multi-factorial diseases, a causative organism might not be readily apparent, as other factors may be more prominent. However, absence of evidence is not proof of absence; in several cases when overwhelming experimental evidence was obtained, the pathogen concept had to be accepted even though it had met with great opposition initially. Two examples, among many, are the involvement of viruses in certain types of cancer such as human papillomavirus in cervical cancer, and of the bacterium *Helicobacter pylori* in stomach ulcers. Alzheimer's Disease (AD) is a neurodegenerative disease that is considered to be the single most significant cause of dementia in the elderly (Keefover, 1996). There are two major categories of AD, familial and sporadic late-onset. The familial form of AD accounts for approximately 5% of total cases and usually presents in individuals in their 40's and 50's. This form of disease is caused by rare mutations in genes associated with β -amyloid

production and processing resulting in β -amyloid deposition into senile plaques. The genes code for transmembrane proteins including β -amyloid precursor protein, presenilin 1 and presenilin 2 (Scheuner et al., 1996). In contrast, the sporadic late-onset form of AD accounts for ~95% of total cases, displays similar pathological accumulations such as amyloid and tau as occurs in familial disease, but does not exhibit mutations in the genes of familial disease. However, at least one genetic risk factor, the APOE ϵ 4 genotype, has been linked with

INFECTION	CHRONIC DISEASE(S)
Human T-cell Lymphotropic virus type I	Adult T cell leukemia
	Tropical spastic paraparesis
Human papilloma virus (HPV)	Cervical carcinoma
	Larynginal papilloma
Epstein-Barr virus (EBV)	Burkitt's lymphoma in Africa
	Nasopharyngeal carcinoma
	Hodgkin's disease
Hepatitis B virus (HBV)	Hepatocellular carcinoma, chronic hepatitis
Hepatitis C virus (HCV)	
HBV and delta virus	
HBV	Polyarteritis nodosa
HCV	Mixed cryoglobulinemia
Measles	Sub acute sclerosing panencephalitis
Kaposi's sarcoma-associated herpes virus	Lymphoma
	Kaposi's sarcoma
Parvovirus B19	Anemia; arthritis
Rubella	Post-rubella arthritis syndrome
	Congenital rubella syndrome
Prions	Creutzfeld Jacob disease
	Kuru
<i>Helicobacter pylori</i>	Gastric lymphoma
	Peptic ulcer disease (PUD)
Histoplasmosis	Chronic pericarditis
Syphilis	Tertiary & neurosyphilis
<i>Borellia burgdorferi</i>	Lyme disease
Group A <i>Streptococcus</i>	Post-streptococcal glomerulonephritis
<i>Chlamydia trachomatis</i>	Reiter's syndrome & reactive arthritis
<i>Tropheryma whipplei</i>	Whipple's disease
<i>Mycobacterium leprae</i>	Leprosy
<i>Mycobacterium tuberculosis</i>	Tuberculosis
<i>Campylobacter jejuni</i>	Guillan-Barre syndrome
<i>Chlamydia trachomatis</i>	Pelvic inflammatory disease
Osteomyelitis	Squamous cell carcinoma
<i>Escherichia coli</i> O157:H7	Hemolytic-uremic syndrome
Cytomegalovirus	Post-transplant accelerated atherosclerosis

Table 1. Chronic diseases for which there is strong evidence of an infectious etiology

DISEASE	SUSPECTED AGENT(S), IF ANY
Primary biliary cirrhosis	<i>Helicobacter pylori</i> , retrovirus
Mesothelioma	Simian virus 40
Multiple sclerosis	Epstein-Barr Virus
Tics and Obsessive Compulsive Disorder	Group A <i>Streptococcus agalactiae</i>
Obsessive compulsive disorder	Group A <i>Streptococcus agalactiae</i>
Crohn's disease	<i>Mycobacterium paratuberculosis</i>
Alzheimer's Disease	<i>Chlamydia pneumoniae</i>
Diabetes	Enteroviruses
Sjogren's disease	<i>H. pylori</i>
Sarcoidosis	<i>Mycobacterium</i> species
Atherosclerosis	<i>Chlamydia pneumoniae</i> , CMV
Bell's palsy	Herpes Simplex Virus
Schizophrenia	Intrauterine exposure to Influenza
ALS	Prions
Chronic fatigue	HTLV-1; EBV
Prostate cancer	BK virus

Table 2. Chronic diseases for which there is suspicion of an infectious etiology

late-onset disease (Roses, 1996). For late onset AD, there appears to be interplay between genetic risk(s) and environmental insult, but the exact etiology has yet to be clearly delineated.

Sporadic late-onset AD is thought to arise from a multi-factorial interplay between genetic and environmental factors. Speculation as to which environmental factors may have a great impact on the pathogenesis of this disease has led to studies of infectious disease. This is a rational approach as different types of infections have been associated with dementing illnesses, including infection with *Treponema pallidum*, mentioned previously, as well as other infections such as measles virus (Frings et al., 2002) and HIV (Zhou et al., 2010). Early studies of infection directly related to AD attempted to correlate viral infection with late-onset disease (Pogo, et al. 1987). The viruses considered were: Herpes Simplex 1 and 2, cytomegalovirus, measles virus, poliovirus, adenoviruses, hepatitis B virus, and the influenza A and B viruses. No association with disease was established for these viruses. More recent studies have found evidence for direct brain infection in AD with HSV1 (Itzhaki et al., 1997), *Borrelia burgdorferi* (Miklossy, 1993), and *Chlamydia pneumoniae* (Balin et al., 1998; Gerard et al., 2006). There are some reports indicating that systemic infections may correlate with increased incidence of AD and infection with *Helicobacter pylori*, the agent of gastric ulcers, and *Porphyromonas gingivalis*, an agent of periodontitis, have been studied in late-onset disease (Honjo et al., 2009; Kim et al., 2007). Given these reports and the need to identify and understand causative factors for sporadic late-onset AD, further investigations are required to determine the mechanisms by which these different infections might initiate and participate in the pathogenesis of AD.

Interestingly, when one considers factors that may drive the accumulation of amyloid and tau in AD, infectious triggers are some of the most significant and logical choices. In particular, the organisms likely to be involved in AD are those that can evade host defenses, gain entry to specific selectively vulnerable regions of the brain, and establish chronic/persistent and/or latent infection. Upon considering the other risk factors

associated with AD, infection may be the central hub connecting these factors. Currently, evidence from research on *Chlamydia pneumoniae*, Herpes Simplex Virus 1, and *Borrelia burgdorferi* in the AD brain, links numerous risk factors in the pathogenesis of AD with infection. Linkage has been recognized for risk factors such as ApoE ϵ 4 expression, chronic neuroinflammation, autoimmune mechanisms, oxidative and mitochondrial damage, cardiovascular factors, diabetes with insulin resistance, trauma to the blood brain barrier, and selectively vulnerable brain insult (Itzhaki et al., 2004; Miklossy, 2008). Thus, infection actually may be the overarching “unifying hypothesis” for sporadic late-onset AD, rather than other more mainstream hypotheses.

2. Alzheimer's Disease and Herpes Simplex Virus 1

Studies have implicated Herpes Simplex Virus type 1 (HSV1) as a potential etiologic agent for AD (Itzhaki and Wozniak, 2008). HSV1 is ubiquitous and produces latent infection in neurons, initially in the trigeminal ganglia of the peripheral nervous system. Reactivation and transport along axons allows access to the central nervous system (CNS), with latency and periodic reactivation possible in the CNS. While productive infection in the CNS may be mild and asymptomatic, it can also result in encephalitis. In fact, HSV1 is the leading cause of sporadic acute encephalitis in people. Interestingly, regions in the brain that are affected in herpes simplex encephalitis, namely the frontal and temporal cortices and the hippocampus, are also the areas associated with Alzheimer pathology (Ball, 1982). The chronic nature of this infection, with periodic reactivation of productive infection, may contribute to the gradual accumulation of pathology associated with AD.

Epidemiologic studies linking HSV1 with AD are difficult because the virus is ubiquitous and a large percentage of the population is exposed to this virus. However, in support of this connection, HSV1 DNA has been detected in a high proportion of elderly individuals, both AD patients and non AD patients, relative to younger individuals (Jamieson et al., 1991). Studies have also detected HSV1 antibodies in the cerebral spinal fluid (CSF) of elderly, but not younger, individuals (Wozniak et al., 2005), and elevated anti-HSV1 IgM antibodies in CSF of AD patients (Letenneur et al., 2008). These data indicate that reactivation of HSV1 occurs in the CNS and implies that reactivation is more likely to occur in older individuals.

While HSV1 is present in the brains of many elderly people, only a subset of these individuals will develop AD. Thus, it is reasonable to expect that host factors also play a role in determining one's risk of developing AD. For example, a recent study has shown that HSV1-infected individuals who are carriers of the APOE ϵ 4 allele are at greater risk of developing AD than those carriers who are not infected with HSV1 (Itzhaki et al., 1997). Consistent with the idea that APOE might influence susceptibility to CNS infection by HSV1 are several studies by Burgos et al. using transgenic mice expressing either the human APOE ϵ 4 allele or the APOE ϵ 3 allele (Burgos et al., 2003). These investigators show that entry into the brain and viral load in the brain of HSV1-infected mice is greater in animals expressing the APOE ϵ 4 allele than in those expressing the APOE ϵ 3 allele.

Mechanistically, the role that HSV1 plays in the etiology of AD requires direct and/or indirect insult leading to the pathology associated with the disease. Herpesvirus DNA has been detected in plaques in AD brains, suggesting that HSV1 may be involved in AD disease progression (Wozniak et al., 2009b). Further, HSV1 has been shown to associate with the amyloid precursor protein (app) during anterograde transport of viral capsids to the cell

surface (Cheng et al., 2011). This results in an abnormal distribution of APP in infected cells, which might influence cleavage of this protein and the generation of β -amyloid. APP levels also have been reported to decrease in cultured neuronal cells infected with HSV1, while several secreted and intracellular fragments of APP, including the neurotoxic β -amyloid peptides, 1-40 and 1-42, have been detected upon infection with HSV1 (De Chiara et al., 2010; Shipley et al., 2005; Wozniak et al., 2007). The decrease in levels of APP is likely the result of HSV1-induced down-regulation of host protein synthesis, as well as an increase in abnormal processing of APP in HSV1-infected cells. At least some of the altered APP processing observed in HSV1-infected human neuroblastoma cells is due to cleavage by host proteases, including β -secretase (also known as β -site APP cleaving enzyme 1, or BACE1), γ -secretase and caspase-3-like enzymes (De Chiara et al., 2010). In addition to the detection of novel fragments of APP in HSV1-infected cells, increased levels of BACE1 and nicastrin, a component of the γ -secretase complex, have been detected upon HSV1 infection of human neuronal cells (Wozniak et al., 2007). Furthermore, HSV1 produces an envelope glycoprotein, gpB, with homology to a segment of β -amyloid. Peptides synthesized from this region form fibrils *in vitro* that resemble β -amyloid and are able to seed the formation of neurotoxic amyloid plaques (Cribbs et al., 2000). Therefore, reactivation of HSV1 in the brain of AD patients can potentially contribute to the formation of β -amyloid and senile plaques, which in turn would exacerbate the disease process.

HSV1 can also be linked to the second major pathological feature of AD, neurofibrillary tangles (NFT), that form as a consequence of hyperphosphorylation of tau proteins. Infection of neuronal cells by HSV1 results in hyperphosphorylation of tau, resulting in neuronal damage and loss of viability (Wozniak et al., 2009a; Zambrano et al., 2008). In addition, cleavage of tau by caspase 3 has been demonstrated in HSV1-infected cells, an event which accelerates the aggregation of tau (Lerchundi et al., 2011). HSV1 also encodes an enzyme homologous to protein kinase A (PKA), one of the enzymes involved in phosphorylation of tau; this viral protein is functionally similar to PKA and could potentially phosphorylate tau and contribute to NFT formation (Benetti and Roizman, 2004). Thus, there is evidence that supports the hypothesis that HSV1 infection can contribute to both tau-mediated and β -amyloid-mediated neurodegenerative pathologies associated with AD.

Autophagy is a process involving clearance of abnormal proteins and cellular organelles by lysosomal proteases. Disruption of this process has been implicated in neurodegenerative disorders such as AD (Chu, 2006). Autophagosomes are known to accumulate in dystrophic neurites of damaged neurons in AD, and impairment of the autophagic pathway could account for the intracellular deposits of β -amyloid seen in this disease (Nixon, 2007). Furthermore, β -amyloid and enzymes responsible for processing of APP have been recognized in autophagosomes (Yu et al., 2004), which could enhance cleavage of APP into toxic fragments. While the accumulation of autophagic vacuoles could be the result of increased induction of autophagy, Boland et al. suggest that the AD pathology attributed to disruption of autophagy is due to impaired clearance of autophagosomes by lysosomal cathepsins (Boland et al., 2008). A recent study by Santana et al. presents evidence linking the accumulation of β -amyloid and disruption of autophagy with HSV1 infection (Santana et al., 2011). These investigators demonstrate that infection of neuroblastoma cells with HSV1 results in an accumulation of intracellular β -amyloid 1-40 and 1-42 peptides in autophagosomes. Furthermore, the data indicate that autophagosomes do not fuse with lysosomes in HSV1-infected cells, and β -amyloid co-localizes with a marker for

autophagosomal membranes (LC3) but not with a marker for lysosomes (CD63). While Santana et al did not observe any significant change in secretases involved in APP processing in HSV1-infected cells, as observed in other studies (De Chiara et al., 2010; Wozniak et al., 2007), their results indicate a mechanism whereby infection with HSV1 might contribute to the intracellular accumulation of β -amyloid by inhibiting the autophagocytic pathway.

Additional evidence that autophagy may be a key feature in AD pathogenesis with HSV1 infection involves another cellular process that has been implicated in AD pathology. This process is activation of protein kinase R (PKR) and subsequent phosphorylation of elongation initiation factor 2 α (eIF2 α), which ultimately results in inhibition of protein synthesis (Peel, 2004). PKR can be activated by the presence of dsRNA, which many viruses, including HSV1, generate during their replication cycle; this activation of PKR is a cellular defense mechanism to protect against these infections. HSV1, however, expresses a protein, US11, that binds dsRNA and prevents activation of PKR (Cassady and Gross, 2002). HSV1 also expresses a protein called infected cell polyprotein 34.5 (ICP34.5) that activates a cellular protein to dephosphorylate eIF2 α (He et al., 1997), thus allowing protein synthesis to occur. While these observations seem to contradict how the PKR pathway is affected in AD, it has been shown that HSV1 inhibition of PKR and eIF2 α phosphorylation disrupts autophagy (Talloczy, et al. 2002). Thus, the deleterious effects of disrupting autophagy might overshadow any advantage to inhibiting the PKR pathway especially since disruption of autophagy occurring in HSV1-infected cells has been associated with an accumulation of β -amyloid, a key ingredient in AD pathogenesis.

3. Alzheimer's Disease and Spirochetes

Spirochetes are gram negative bacteria. These microorganisms have life cycles both external to and within the human host. Once they have established an infection within the human host, they can spread throughout the periphery and enter the central nervous system. Infection by these bacteria can cause many human diseases and disorders. For example, *Treponema pallidum* is known to cause syphilis which, in later stages of the disease, can have neurologic components (Miklossy, 2008). Additionally, the genus *Borrelia* includes spirochetes capable of causing many human diseases. In the Northern hemisphere, *Borrelia burgdorferi* sensu lato cause Lyme disease. The organism is transmitted from tick saliva during a bite. The infection begins in the human host as a localized acute infection that then spreads systemically. Once the bacteria have entered the human host, changes occur within the bacteria to allow evasion of the human immune system (Rupprecht et al., 2008). MacDonald has described life cycle/phases of *Borrelia* within the human host which have different morphologic appearances, such as corkscrew, cysts, and granular forms (MacDonald, 2006). Other changes occur in the expression of surface proteins on the *Borrelia* which prevent complete elimination of the organism and help to establish a more chronic infectious state. Further, *Borrelia* can infect the immune cells such as monocytes allowing further dissemination via a blood route. *Borrelia* cross the blood brain barrier into the central nervous system where they cause Lyme neuroborreliosis (Rupprecht et al., 2008). An *in vitro* model has shown that *Borrelia* can cross the blood brain barrier by affecting calcium signaling in endothelial cells of the blood brain barrier (Grab et al., 2009).

In 1987, MacDonald and Miranda described a well documented case of Alzheimer's dementia that had symptoms of a tertiary stage *Borrelia* neurospirochetosis. The damage

from the infection occurred in the frontal cortex where the damage from AD is located. They suggested that there may be a link between chronic infection due to *Borrelia* and AD (MacDonald and Miranda, 1987). Miklossy in 1993 presented a study of 27 autopsy cases of which 14 had an AD diagnosis and the other 13 cases were non AD age matched cases. They used sterile post-mortem brain biopsy material. All 14 AD cases were positive for spirochetes. Further, from these 14 AD cases they were able to isolate motile coiled spirochetes from the sterile CSF and blood samples. One of the 14 cases had both a Lyme disease and an AD diagnosis. In this case, *Borrelia burgdorferi* immunoreactivity was found in senile plaques and neurons (Miklossy, 1993; Miklossy et al., 2004). The report by MacDonald and Miranda and the studies by Miklossy et al have established a link between tertiary neurospirochetosis and AD pathology.

To examine whether *Borrelia* could induce the amyloid and tau pathologies seen in AD, *in vitro* studies were performed. Miklossy et al exposed mammalian neuronal, astrocytic and microglial cells in culture to *Borrelia burgdorferi* for 2-8 weeks. They also infected mixtures of primary rat cells from the telencephalon. They were able to show by Western blot analysis that there were increased levels of beta amyloid precursor protein and hyperphosphorylated tau in extracts of the cells that had been exposed to the bacteria or to the bacterial product lipopolysaccharide when compared to uninfected or untreated cell cultures. Furthermore, they were able to show formation of plaque- like structures when they infected cells with *Borrelia*. The amyloid deposits that formed were extracellular and reacted with antibodies to β -amyloid and stained with thioflavin S. They analyzed these aggregates with Synchrotron InfraRed MicroSpectroscopy to examine the secondary structure of the proteins and were able to determine that the amyloid was similar to the beta sheet structure seen in AD senile plaques. Additionally, the neuronal cells showed morphological changes similar to the neurofibrillary tangles observed in AD. These "tangle-like" structures were immunoreactive with anti-*Borrelia* antibodies. The spirochetes and bacterial lipopolysaccharide alone induced the AD-like pathology (Miklossy et al., 2006).

In addition to stimulating the production and aggregation of amyloid, bacterial products, such as lipopolysaccharide, can cause an inflammatory response (Miklossy et al., 2006). In this regard, the bacterial lipopolysaccharide and the induced amyloid plaque formation could induce neuroinflammation similar to that seen in AD. This self perpetuating cycle of production of amyloid and inflammation leading to more amyloid may start in the early stages of a neuroborreliosis, which then perpetuates and exacerbates as it becomes more chronic. Thus, a chronic inflammatory state could be initiated by infection with *Borrelia* which could explain, in part, the neuroinflammation observed in AD.

4. Alzheimer's Disease and *Chlamydia pneumoniae*

Chlamydia pneumoniae is an atypical bacterium classified as an obligate intracellular pathogen that most commonly infects the human respiratory tract (Grayston et al., 1990). First classified in 1989 (Grayston et al., 1990), the organism has been determined to be ubiquitous in the human population (Leinonen, 1993) and infects mucosal epithelial cells in the nasal passages and the pulmonary tract (Hahn et al., 2002). *Chlamydia pneumoniae* often will spread to the systemic circulation following infection of monocytes in lung tissues (Moazed et al., 1998). The organism exhibits a distinctive biphasic life cycle similar to that of other chlamydia. In this regard, there is an infectious elementary body form as well as an actively metabolizing reticulate body form of the organism. The organism typically attaches

to host cells and is endocytosed into a vacuole in which the elementary bodies convert into reticulate bodies that will replicate by binary fission. After 48 to 72 hrs of infection, the organism reorganizes into the elementary body form and is released from the host cell either following host cell lysis or exocytosis.

Some investigations have demonstrated that under certain host cell conditions such as nutrient deprivation, a persistent form of *Chlamydia pneumoniae* develops (Byrne et al., 2001). These organisms exhibit aberrant phenotypes as well as unusual transcriptional characteristics and are thought to contribute to the chronicity of disease recognized in numerous chlamydia-associated conditions (Hogan et al., 2004) including both respiratory and non-respiratory diseases such as chronic obstructive pulmonary disease and atherosclerosis, respectively (Rosenfeld et al., 2000).

The initial report of an association of *Chlamydia pneumoniae* in AD demonstrated that 90% of sporadic late-onset AD brains contained DNA from the organism as determined by polymerase chain reaction (Balin et al., 1998); in contrast, 5% of control brains were positive. These data were corroborated using other tests including: immunohistochemistry, *in vitro* culturing, electron microscopy, immunoelectron microscopy, and reverse transcriptase polymerase chain reaction. All tests utilized brain tissues from regions of the brain typically affected in AD, including those of the pre-frontal cortex, entorhinal cortex, hippocampus, and the parietal cortex. The cerebellum was analyzed also as an internal control since this region is far less affected in this disease. PCR analysis demonstrated that 17 of 19 AD brains were positive for *Chlamydia pneumoniae* in regions with distinct neuropathology, whereas only 4 brains were positive for the organism in the cerebellum. The organism was shown to be present in perivascular macrophages, microglia, and astroglia. In later studies (Gerard et al., 2006), the organism also was shown to infect approximately 20% of neurons in the AD brain. Further analysis of the brain samples indicated that 64% of the polymerase chain reaction-positive samples contained at least one allele for the apoE ϵ 4 isoform which is consistent with earlier findings of the APOE ϵ 4 allele conferring risk for developing sporadic late-onset AD (Roses, 1996).

Interestingly, in a separate and non-brain related study of reactive arthritis, 68% of individuals who demonstrated infection in synovial tissues with *Chlamydia pneumoniae* were carriers of at least one APOE ϵ 4 allele (Gerard et al., 1999). As these percentages were consistent with what had been determined by Roses for risk in AD, further analysis of the relationship of apoE with infection with *Chlamydia pneumoniae* as it would apply to AD was undertaken. Analysis of late-onset disease brains with *in situ* hybridization for *Chlamydia pneumoniae* that were ϵ 4-containing as compared to those that were ϵ 2 or ϵ 3 indicated that more cells were positive for the organism in the ϵ 4 brains than those of the other two (Gerard et al., 2005). Real time polymerase chain reaction revealed that the ϵ 4 brains contained significantly higher bacterial loads than did the ϵ 2 or ϵ 3 brains. These data are consistent with previous findings that the ϵ 4 positive individuals have both a higher risk of developing AD, and a higher likelihood of exhibiting a faster progression of cognitive dysfunction (Roses, 1996). Mechanistically, apoE appears to bind to the *Chlamydia pneumoniae* elementary body and to enhance the attachment of the organism to the host cell (Gerard et al., 2008). The apoE and *Chlamydia pneumoniae* complex is thought to utilize the low density lipoprotein receptor protein for uptake. This receptor is the normal receptor for the apoE glycoprotein. Thus, the apoE ϵ 4 isoform appears to interact with *Chlamydia pneumoniae* to promote infection, and in this way, may contribute as a risk factor to the development of infection-related AD.

4.1 Analysis of *Chlamydia pneumoniae* cultured from the brain

Culturing of *Chlamydia pneumoniae* from the late-onset AD brain has been performed from multiple AD brain samples (Balin et al., 1998; Dreses-Werringloer et al., 2009), two of which were obtained from different geographic regions in North America. Organisms from these two brains were detectable after passaging in HEp-2 cells (Dreses-Werringloer et al., 2009). Using PCR assays for *Chlamydia pneumoniae*-specific genes Cpn0695, Cpn1046, and tyrP, both isolates were demonstrated to be *Chlamydia pneumoniae*. The omp1 gene from each isolate was sequenced from DNA prepared from several brain tissue samples shown to be PCR-positive for *Chlamydia pneumoniae*. This sequencing revealed that the chlamydial populations from the two brains were genetically diverse. In addition, the brain isolates carried different numbers of copies of the tyrP gene indicating that the brain isolates were more closely related to respiratory strains of *Chlamydia pneumoniae* than to vascular or atheroma strains.

4.2 Entry of *Chlamydia pneumoniae* into the brain

Chlamydia pneumoniae typically infects through the respiratory tract. This route of entry allows Chlamydia access to the brain through the olfactory system since olfactory neuroepithelial cells in the nasal passages can be infected with this pathogen (Little et al., 2004). The olfactory pathway has been shown to be affected early in AD (Kovacs et al., 2001), and this may be the single most vulnerable site for which a respiratory pathogen, like *Chlamydia pneumoniae*, can gain access to the brain. Evaluation of the olfactory bulbs from late-onset AD using PCR and reverse transcriptase PCR techniques has revealed *Chlamydia pneumoniae*-specific sequences at this site (Balin et al., 1998). Since olfactory bulbs contain some of the earliest pathology occurring in the AD brain, even prior to pathology observed in the entorhinal cortex, the suggestion has been made that olfaction is actually damaged with alterations in the sense of smell as a preclinical event prior to incipient AD (Kovacs et al., 2001). As damage progresses from the olfactory bulbs into the entorhinal cortex, layers II and III demonstrate neurofibrillary tangles (Braak and Braak, 1997). Neural projections arise from these layers to pass through the perforant pathway to innervate the hippocampal formation. Our studies have shown that *Chlamydia pneumoniae* was also present in the AD entorhinal cortex, hippocampus, and other areas of the temporal cortex (Balin et al., 1998; Gerard et al., 2006; Hammond et al., 2010), thus implicating *Chlamydia pneumoniae* infection of the olfactory pathway in the early pathological changes observed in AD, ie, damage to the mesial temporal cortex. For some time, pathogen entry into the brain following infection of the olfactory path has been well-recognized (Flexner and Clark, 1912; Morales et al., 1988). Whether there is direct damage to this pathway leading to changes in the sense of smell and pathology in the brain proper or whether the path is just a conduit for deeper brain infection must be addressed for each individual pathogen. As stated above, we have observed the presence of *Chlamydia pneumoniae* in this pathway and brain regions connected directly to this pathway (Balin et al., 1998; Hammond et al., 2010). However, we have not correlated infection with changes in the sense of smell at this time, although this appears reasonable and will be tested in the future. Furthermore, our animal model studies, in which the normal BALB/c mouse has been inoculated intranasally, have demonstrated the organism in the olfactory neuroepithelia, olfactory bulb, and in deeper brain structures, as well as concordant amyloid pathology (Little et al., 2004), suggesting that the infection induces pathological change consistent with what is observed in the AD brain.

The other likely pathway by which *Chlamydia pneumoniae* can enter the brain is through the blood brain barrier. *Chlamydia pneumoniae* can be engulfed by monocytes that circulate within the lung vasculature following inhalation into the lungs (Boman et al., 1998). Following uptake of the organism into monocytes, the monocytes can traffic the organism throughout the circulation for potential penetration into the brain, much like what is observed for HIV infection in HIV-dementia cases (Roberts et al., 2010). In the AD brain, *Chlamydia pneumoniae* was revealed in glial cells, perivascular macrophages, and monocytes within and around blood vessels (Balin et al., 1998; MacIntyre et al., 2003), suggesting that indeed the organism can enter the AD brain by this mechanism. We have obtained experimental evidence for this occurrence using an *in vitro* model of the blood-brain barrier. This model analyzed the transmigration of *Chlamydia pneumoniae*-infected monocytes through an intact monolayer of infected human brain microvascular endothelial cells (MacIntyre et al., 2003). Up-regulation of ICAM-1 and VCAM-1 on the endothelial cells, and up-regulation of integrin molecules on the monocyte surface were detected. This would allow enhanced binding of monocytes to the endothelial cell monolayers and could account for the observed 3-fold increase in transmigrated cells. Further support for this occurrence followed analysis of the junctional molecules maintaining the adherens and tight junctional complexes between the endothelial cells (MacIntyre et al., 2002). Transient up-regulation of expression was observed for N-cadherin and β -catenin, two proteins involved in the adherens junctional assembly complex. In contrast, down-regulation occurred for occludin, a tight-junctional protein, with recovery of expression by 72 hr post-infection. These data suggest that a compensatory response to infection was evident in the endothelial cells, and that transient opening of the tight junctions between endothelial cells would allow transmigration of infected monocytes through the barrier.

Consequences of monocyte infection and subsequent entry into the brain can result in further damage and spread throughout the central nervous system. The chronic nature of *Chlamydia pneumoniae* infections could lead to significant immunopathology resulting in neuronal cell damage and death. Evidence for spread in the human nervous system also has been reported by others who evaluated whether *Chlamydia pneumoniae* DNA was present in the cerebrospinal fluid of individuals diagnosed with AD and vascular dementia as compared to control, non-demented individuals (Paradowski et al., 2007). This investigation used polymerase chain reaction techniques to determine that the prevalence of the organism in the AD brains was 43.9% (N = 57 patients). This prevalence was much higher than that for vascular dementia which was 9.5% (N = 21 patients) and for controls which was 0.6% (N = 47 patients). From these data, the odds ratio for persons having *Chlamydia pneumoniae* in their cerebrospinal fluid and also having AD was 7.21, thus indicating a significant association of this infection with AD. In an unrelated report, the presence of *Chlamydia pneumoniae* was examined in atherosclerotic arteries from various vascular regions including the brain (Rassu et al., 2001). Seven of 9 (78%) patients were PCR-positive in brain samples. Interestingly, none of these patients were diagnosed with late-onset AD at the time of death, but all had severe atherosclerosis. Atherosclerosis is considered a risk factor for the development of late-onset AD, although the risk has more often been attributed to cholesterol processing abnormalities than to infection (de la Torre, 2006). Ironically, this conclusion fails to consider that *Chlamydia pneumoniae* infection in blood vessels has been shown to result in the development of foam cells containing abnormal accumulations of cholesterol (Kalayoglu and Byrne, 1998).

4.3 Association of neuroinflammation with *Chlamydia pneumoniae*

Neuroinflammation has been well-documented in the AD brain and is thought to arise as a result of glial cell exposure to toxic forms of β -amyloid (Lue et al., 1996). While this eventuality is likely, there is a gap in our understanding of how, why, and when the toxic forms of β -amyloid arise in disease pathogenesis. In this regard, infection with *Chlamydia pneumoniae* may be a trigger or stimulus for neuroinflammation that actually precedes the processing of β -amyloid into toxic entities. Immunopathogenesis as a result of chronic inflammation is a hallmark of infection with *Chlamydia pneumoniae* and typically involves inflammatory cells such as monocytes and macrophages (Rasmussen et al., 1997). Components of chlamydia such as heat shock protein 60, outer membrane proteins, and lipopolysaccharide elicit strong inflammatory responses in tissues. The inflammatory response is usually pro-inflammatory with the production of interleukins IL-1 β , IL-6, IL-12, tumor necrosis factor- α , and reactive oxygen species. These inflammatory molecules have been found in the AD brain and are thought to promote nerve cell damage (Lue et al., 1996). The cell types in the brain involved with secreting these inflammatory molecules are microglia, astroglia, and perivascular macrophages. All of these cell types in the AD brain have been shown to be infected with *Chlamydia pneumoniae* (Balin et al., 1998; Gerard et al., 2006; Hammond et al., 2010). In addition, approximately 20% of neurons in the hippocampal formation also have been shown to be infected (Gerard et al., 2006). Intriguingly, all of these infected cells were found in areas of amyloid deposition. The relationship of these infected cells to pathology has been investigated with *in vitro* studies in which *Chlamydia pneumoniae*-infected murine microglial cells were shown to secrete several pro-inflammatory cytokines including MCP-1, IL-6, and tumor necrosis factor- α . Neurons exposed to the supernatants containing these cytokines exhibited an increase in cell death as compared to those exposed to mock infected supernatants (Boelen et al., 2009). Thus, the pro-inflammatory response to *Chlamydia pneumoniae* infections may result in neurodegeneration in the immediate environment and in the neuropathology such as amyloid deposition characteristic of Alzheimer's Disease.

Neuronal cell death in AD may occur through several mechanisms that lead to the characteristic amyloid and tau pathologies. There is increasing evidence to suggest that dysregulation of apoptosis and autophagy may be the interconnecting link in the abnormal cellular processing that occurs in AD. The initiation of the apoptotic process and mitochondrial dysfunction which may play a central role in neurodegeneration, have been observed in AD brains (Pereira et al., 2004). Autophagy has been linked to Alzheimer's pathogenesis through its merger with the endosomal-lysosomal system, which also has been shown to play a role in aberrant amyloid processing. Contents of an autophagosome are degraded as a result of the autophagosome fusing with the lysosome. Research has demonstrated that the activity of the lysosomal system is enhanced in patients with AD (Nixon et al., 2000). The lysosomal system is also related to the endosomal pathway since early endosomes that are formed fuse with late endosomes or lysosomes. Neurons from AD brains have been found to exhibit an increase in the number of enlarged early endosomes. This is significant in the development of AD because early endosomes sequester proteins such as apolipoprotein E and app, and studies have demonstrated that A β is formed in early endosomes (Nixon et al., 2000). From these observations, the data suggest that aberrant autophagy induction may result in an accumulation of autophagic vacuoles in the AD brain containing β -amyloid. With regards to infection, apoptosis and autophagy are common pathways by which infected cells, incapable of eliminating the infectious agent, undergo cell

death. *Chlamydia pneumoniae* has been shown to inhibit apoptosis in neuronal cells and monocytes thereby prolonging cell viability (Appelt et al., 2008), and previous work from other laboratories has demonstrated that chlamydiae-infected host cells are resistant to proapoptotic stimuli (Fischer et al., 2001). Inhibition of apoptotic activity may be important in the earlier stages of infection. This anti-apoptotic activity may block cytochrome c release from the mitochondrial membrane and subsequent activation of caspases that would promote apoptosis. *Chlamydia pneumoniae* infection has been shown to modulate the pro-apoptotic cytoplasmic proteins, such as caspase-3 and cytochrome c, as well as the anti-apoptotic mitochondrial protein Bcl-2 and the anti-apoptotic nuclear protein NF- κ B (Fischer et al., 2001). Interestingly, intracellular pathogens, such as Chlamydia, have been shown to alter the apoptosis pathway and interfere with the autophagy pathway to ensure survival of the host cell (Al-Younes et al., 2004). We have demonstrated that infection with *Chlamydia pneumoniae* results in changes in gene expression in the apoptotic and autophagic pathways (unpublished observations) consistent with previous work by others, suggesting that infection may be altering these pathways in AD.

4.4 Approaches to prove causation of AD by infection with *Chlamydia pneumoniae*

4.4.1 Clinical trial to treat for CNS infection with *Chlamydia pneumoniae*

Investigations to prove that chronic infection with *Chlamydia pneumoniae* can be causative for late-onset AD have used clinical trial approaches and animal modeling. Anti-microbial treatment may be feasible in combating infection-initiated AD. One reported clinical trial has used an antibiotic combination approach for treatment of late-onset disease (Loeb et al., 2004). Patients with probable late-onset disease and/or mild to moderate dementia were treated for 3 months with doxycycline and rifampin. Primary and secondary outcomes were assessed. Primary outcome was any change in the Standardized AD Assessment Scale cognitive subscale (SADAScog) at 6 months and secondary outcomes were any change in SADAScog at 12 months along with analysis of dysfunctional behavior, depression, and functional status. There was significantly less decline at 6 months in the antibiotic group as compared to placebo for SADAScog ($p = .034$), whereas the same score at 12 months was not significantly different. However, the antibiotic group showed significantly less dysfunctional behavior ($p = .028$), and less decline in mini-mental status scores ($p = .032$). There was no correlation that could be determined for change in *Chlamydia pneumoniae* infection based on serum antibody titers in blood or by PCR of blood samples. Although the correlation to change in infection was not apparent based on these limited measures, there was some limited improvement in patient status. Future studies must include better measures of Chlamydial infection in the CNS (ie, tests of cerebrospinal fluid) to better understand how antibiotics may be affecting change in CNS infection, in addition to possibly using delivery adjuvants with antibiotics to obtain better concentration effects in the CNS.

4.4.2 Experimental animal models to study AD

As mentioned previously, AD has an early onset form that is primarily driven by autosomal dominant genetic alterations in genes encoding app, as well as the loci encoding presenilins-1 and 2. Genetically modified mouse models have taken advantage of these genes to induce enhanced β -amyloid production and subsequent deposition of β -amyloid (Wisniewski and Sigurdsson, 2010). One important issue that cannot be addressed using these model systems is how to target the early initiating events in sporadic late-onset AD and not just the

“tombstone lesions that are the result of a long chain of pathological processes” (Wisniewski and Sigurdsson, 2010). Transgenic animals serve as models for early onset AD, which accounts for ~5 % of all reported cases.

Animal models that mimic the sporadic late-onset form of AD have been hampered by the lack of understanding of the primary factors that promote the deposition of β -amyloid. Currently, models that experimentally induce AD-like pathology use bacterial toxins such as streptozotocin (Labak et al., 2010), chronic stress (Alkadhi et al., 2010), or colchicine to chemically induce damage (Kumar et al., 2007) to the CNS to initiate pathology. Several infectious agents, including *Chlamydia pneumoniae*, have been proposed to play a causal role in AD. Animal models based on this infection as well as on infections with other organisms such as Herpes Simplex Virus-1 and *Borrelia burgdorferi* (Itzhaki et al., 2004) are being pursued, but at this time are limited. The paucity of experimental animal systems that model sporadic late-onset AD leaves the scientific community with few options to address key questions related to the initiation/progression of late-onset disease.

4.4.3 Experimental induction of progressive AD-like pathology following infection with *Chlamydia pneumoniae*

The identification of *Chlamydia pneumoniae* in AD brain tissue (Balin et al., 1998) was a stimulus to investigate the potential role *Chlamydia pneumoniae* plays in the induction and progression of late-onset disease. In addition to utilizing cell culture systems to investigate changes in particular cell populations, we have established a mouse model to investigate induction of AD-like pathology following infection with *Chlamydia pneumoniae* (Little et al., 2004). In this experimental system, BALB/c mice were infected with *Chlamydia pneumoniae* isolated from human AD brain autopsy tissue. The isolate of *Chlamydia pneumoniae*, 96-41, was propagated in HEp-2 cells and then introduced into 3 month old BALB/c mice via intranasal inoculation; brain tissue was analyzed at monthly time points following infection. In mice infected with *Chlamydia pneumoniae*, β -amyloid deposits were identified as early as two months post-infection, with the greatest number of deposits identified at three months post-infection. The number and size of amyloid deposits increased over time, thus the development of AD-like pathology was progressive.

The experimental induction of mouse derived β -amyloid deposits in inbred BALB/c mice (not genetically modified) at 5 and 6 months of age (2 and 3 months post-infection) indicates that infection can trigger the production and deposition of β -amyloid in the mouse brain. In transgenic mouse models used to study AD, 6 months of age is very early to observe substantial amyloid deposits, yet we observed substantial pathology 2 months after introduction of the infectious agent into non-transgenic animals. *Chlamydia pneumoniae* is a respiratory pathogen and was introduced into mice via an intranasal inoculation. This is the natural route of infection and the organism is responsible for an acute respiratory illness in mice. The respiratory infection precedes dissemination to other organ systems (Little et al., 2005) and age is an important factor in the host's ability to control the dissemination, with even greater spread with the advent of immunosenescence.

The first study to utilize a human AD-brain isolate of *Chlamydia pneumoniae* to induce AD-like pathology in non-transgenic mice (Little et al., 2004) was designed to address Koch's postulates. The first postulate requires that the infectious organism be isolated from autopsy brain tissue of an affected individual. In this particular case, the first postulate is satisfied, but for other cases of the disease this issue is still debated (Itzhaki et al., 2004). To

satisfy Koch's second postulate, the pathogen must be isolated from a diseased organism and grown in pure culture. *Chlamydia pneumoniae* was isolated, post mortem, from human AD-brain tissue and grown in culture. Third, the organism was introduced into a mouse, and induced pathology consistent with AD, while uninfected mice did not display the same pathology. Fourth, the organism was identified in the tissues of affected mice, but was not re-isolated from the tissue. Thus, Koch's postulates were used as a general guide, and although difficult to use in their purest sense when addressing any intracellular infection, our findings support the hypothesis that *Chlamydia pneumoniae* infection can induce β -amyloid deposition and contribute directly to pathogenesis.

All experimental animal models of human disease have limitations. In this experimental model, tau pathology was not noted, though due to the relatively short duration of the experiment, amyloid was the primary AD pathology expected to develop. In addition, no learning/memory deficits were measured following infection with *Chlamydia pneumoniae*. The pathology was noted in multiple regions of the brain, and subsequent experiments using the respiratory/laboratory isolate of *Chlamydia pneumoniae*, AR-39, have determined that a majority of the amyloid deposition co-localized in the same area as chlamydia antigen (unpublished observations). Substantial AD-like pathology was noted at three months post-infection, although to determine the full extent of pathology induced and to further characterize the progressive nature of infection with the human AD-brain isolate (96-41) of *Chlamydia pneumoniae*, additional time points still need to be analyzed and evaluated for amyloid and tau pathology. BALB/c mice have an average lifespan of 22-24 months and analysis of brain tissue at six, nine, twelve, and fifteen months post-infection (that is 18 months old) would help to determine the degree of AD-like pathology induced over the course of a persistent *Chlamydia pneumoniae* infection. Similar to any animal model for human disease this system has limitations, but this model of experimentally induced AD-like pathology in the brains of BALB/c mice is well-suited to address key early events in the initiation of pathology associated with sporadic late-onset disease.

4.4.4 Experimental induction of non-progressive AD-like pathology following infection with respiratory isolates of *Chlamydia pneumoniae*

One investigation designed to replicate the initial report of experimental induction of AD-like pathology in BALB/c mice did not identify substantial AD-like pathology following infection with the respiratory isolate/laboratory strain of *Chlamydia pneumoniae* (TWAR 2043) (Boelen et al., 2007). Boelen and co-workers infected BALB/c mice, via intranasal inoculation and examined brain tissue at one and three months post infection, based upon the assumption that TWAR 2043 and the human AD brain isolate 96-41 would both induce a progressive pathology following infection. The number of amyloid beta deposits was not given in this study, but the researchers indicated that *Chlamydia pneumoniae* was not detected in the CNS at 1 month or 3 months post-infection. In addition, both mock-infected and *Chlamydia pneumoniae*-infected mice displayed no difference in amyloid deposits. The clear difference noted in our study of number and size of deposits was notably different than in this report. The researchers noted that these discrepancies could be due to the fact that the TWAR 2043 *Chlamydia pneumoniae* strain used may have different virulence properties than the human AD-brain isolate, 96-41.

Given that TWAR 2043 and 96-41 display different phenotypes with respect to both the ability to establish a persistent infection and the subsequent induction of pathology within

the brains of BALB/c mice, we initiated experiments to address these issues using the respiratory isolate/laboratory strain of *Chlamydia pneumoniae* AR-39. In our laboratory, BALB/c mice were given a single intranasal inoculation, in an identical manner to our initial report with the respiratory isolate/laboratory strain. Brains were analyzed at 1, 2, 3, and 4 months post-infection by immunohistochemistry with antibodies specific for Chlamydia antigen and antibodies specific for β -amyloid 1-42. Similar to the initial report utilizing the 96-41 human AD-brain isolate, no substantial amyloid deposits were observed at 1 month post-infection and a limited degree of AD-like pathology was identified at 2 months post-infection with AR-39. In contrast to the study utilizing the 96-41 isolate of *Chlamydia pneumoniae*, at 3 months post-infection, AD-like pathology was comparable to that observed in uninfected mice (at all time points) or infected mice at 1 month post-infection. The degree of pathology had decreased between 2 and 3 months post-infection. In contrast to infection with the 96-41 isolate, at 3 and 4 months post-infection amyloid deposits in the brains of mice infected with AR-39 resembled that of uninfected controls. Identification and quantitative analysis of Chlamydia antigen burden indicated that peak Chlamydia antigen burden preceded peak amyloid deposition. The greatest Chlamydia antigen burden, in brains of infected BALB/c mice, was noted at 1 month post infection (a mean of 51 immunoreactive sites per mouse), and decreased at 2 months (45), 3 months (30), and 4 months (25) post-infection. Taken together, the burden of Chlamydia antigen and number of amyloid deposits suggests that *Chlamydia pneumoniae* infection serves as the primary stimulus for β -amyloid processing and deposition. Host immune responses that limit or reduce *Chlamydia pneumoniae* replication and antigen burden effectively decrease the primary stimulus for the production of β -amyloid in this experimental system. We propose that the difference in progressive versus non-progressive pathologic profiles of amyloid deposits are due to as yet uncharacterized differences between the human AD-brain adapted isolate (96-41) and respiratory isolates/laboratory strains (TWAR 2043 and AR-39). This implies that there are different virulence factors including tissue tropism for different strains of *Chlamydia pneumoniae*. The ability of the organism to persist in the central nervous system and elicit a chronic inflammatory response may be critical to the initiation of AD pathogenesis.

4.4.5 Experimental induction of progressive AD-like pathology following multiple inoculations with a respiratory isolate/laboratory strain of *Chlamydia pneumoniae*

Exposure to *Chlamydia pneumoniae* is a common event, and over the course of an individual's life, one may be infected multiple times (Leinonen, 1993). We assessed the potential consequences of multiple exposures and infections with *Chlamydia pneumoniae* using BALB/c mice that were intranasally inoculated, at monthly intervals, once (day 0), twice (days 0 and 30), or three times (days 0, 30 and 60) with the respiratory *Chlamydia* isolate/laboratory strain, AR-39. The brain tissue of experimentally infected and control mice was isolated at day 90, processed in an identical manner as previously described (Little et al., 2004) and analyzed by light microscopy following immunohistochemistry using amyloid-specific antibodies. The total number and size of amyloid deposits was quantified and compared with uninfected BALB/c mice as well as BALB/c mice receiving only a single inoculation with *Chlamydia pneumoniae*. BALB/c mice inoculated twice with *Chlamydia pneumoniae* strain AR-39 displayed 68 amyloid deposits/mouse. In contrast, the brains of BALB/c mice inoculated 3 times had 177 amyloid deposits/mouse. Mice receiving only a

single intranasal inoculation had fewer than 10 deposits per mouse, which was comparable to uninfected control mice. Based upon these findings, we concluded that the primary stimulus for the induction of amyloid deposition is the extent or continuous exposure of *Chlamydia pneumoniae* infection.

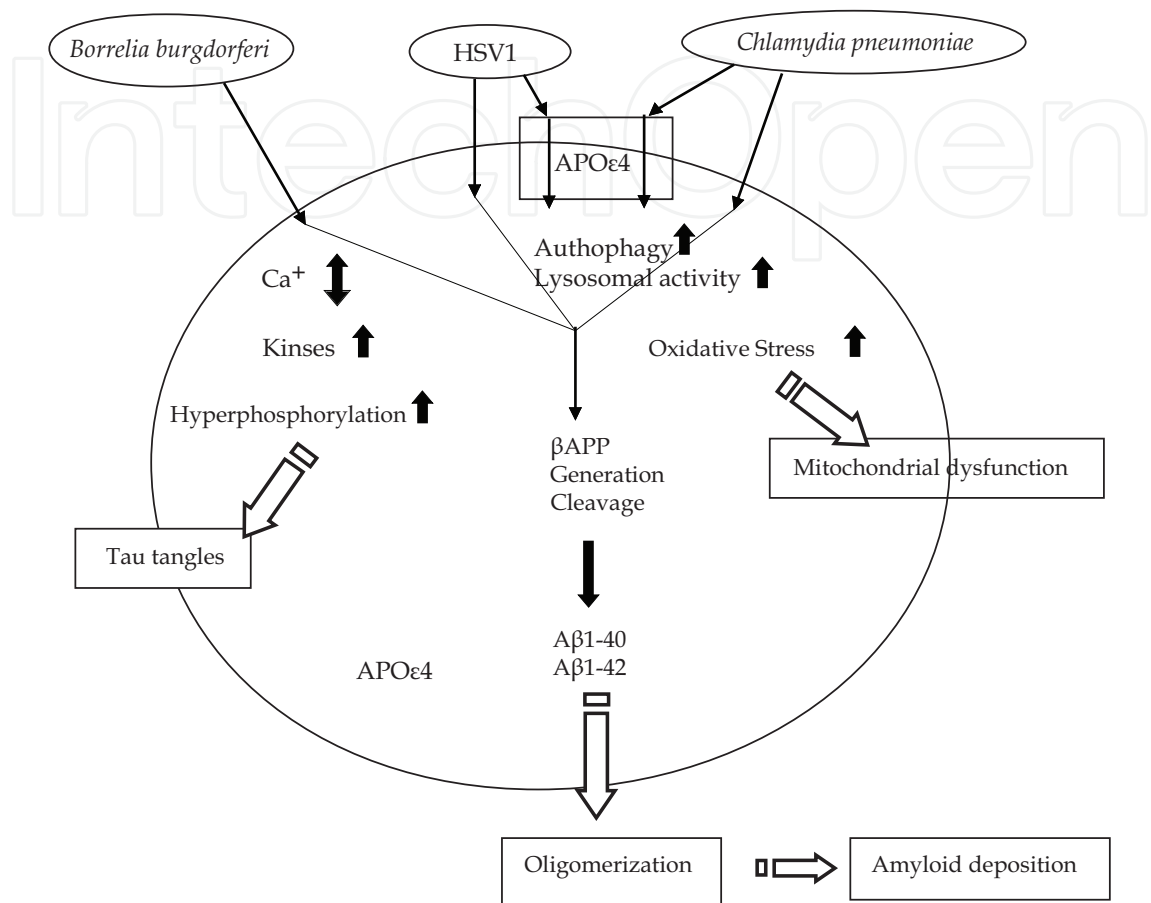


Fig. 1. The diagram illustrates a proposed process of AD pathology development following infectious insult. There are common cellular processes that appear to be activated by all three infectious agents (ie, Herpes, Borrelia, Chlamydia), although the particular activation pathways may differ between the different organisms. In any event, data have been obtained for all three infectious agents that support the contention that infection can initiate changes in the human brain resulting in AD pathology. Thus, specific infections may be primary factors in the pathogenesis of sporadic late-onset Alzheimer’s Disease

Based on the isolate of *Chlamydia pneumoniae* used to experimentally induce AD-like pathology in BALB/c mice we have observed progressive as well as non-progressive amyloid pathology. The human AD-brain isolate, 96-41, establishes a persistent infection and promotes chronic inflammation leading to a progressive accumulation of amyloid deposits (Little et al., 2004). Experimental evidence suggests that the respiratory isolates/laboratory strains are able to infect the CNS and induce substantial amyloid deposits (comparable to that of the human AD-brain isolate) at day 60 post infection, but fail to establish a persistent infection and do not promote amyloid deposition at later times. As the burden of *Chlamydia pneumoniae* antigen decreases the number of amyloid deposits also decreases. This model of experimentally induced AD-like pathology in the brains of BALB/c mice supports a role for infection in the

induction of AD-pathology, and will enable the Alzheimer's research community to address key early events in the initiation of pathology associated with sporadic late-onset AD.

5. Conclusion

New concepts of infectious disease are evolving with the realization that pathogens are key players in the development of progressive chronic diseases that originally were not thought to be infectious. Infection is known to be associated with numerous neurological diseases and its role in inducing pathologic effects has been well documented (Johnson, 1996). What has remained unclear, however, has been the role of infection as a causative agent or risk factor in the development of chronic neurodegenerative diseases, in particular, Alzheimer's Disease. In this regard, numerous studies over the past 20 years have investigated whether there is an association between various infectious agents and Alzheimer's Disease, the most prevalent neurodegenerative condition accounting for dementia in the elderly. Of the pathogens being considered in sporadic late-onset Alzheimer's Disease, Herpes Simplex Virus 1 (HSV-1) (Itzhaki et al., 1997; Itzhaki and Wozniak, 2008), *Borrelia* species (Miklossy, 1993), and *Chlamydia pneumoniae* (Balin et al., 1998; Gerard et al., 2006) have garnered significant attention. Work from other laboratories on systemic infectious disease (Kamer et al., 2008) has also led to further interest in the role that infection may play in contributing to the neurodegenerative process in older populations. Data from these investigations are intriguing, and have led to a renewed interest in investigating the role(s) of pathogens in the etiology of sporadic late-onset Alzheimer's Disease. Furthermore, there is renewed interest in challenging long-held hypotheses in the Alzheimer's research arena as investigations are uncovering more novel features of the amyloid protein, as well as the inflammatory response, associated with this disease. Manifestations of chronic disorders are more and more frequently being attributed to a consequence of chronic infection, and infections must be considered as significant contributors to the morbidity and mortality of Alzheimer's Disease.

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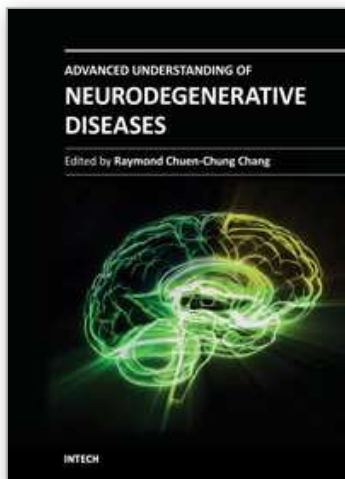
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Advanced Understanding of Neurodegenerative Diseases focuses on different types of diseases, including Alzheimer's disease, frontotemporal dementia, different tauopathies, Parkinson's disease, prion disease, motor neuron diseases such as multiple sclerosis and spinal muscular atrophy. This book provides a clear explanation of different neurodegenerative diseases with new concepts of understand the etiology, pathological mechanisms, drug screening methodology and new therapeutic interventions. Other chapters discuss how hormones and health food supplements affect disease progression of neurodegenerative diseases. From a more technical point of view, some chapters deal with the aggregation of prion proteins in prion diseases. An additional chapter to discuss application of stem cells. This book is suitable for different readers: college students can use it as a textbook; researchers in academic institutions and pharmaceutical companies can take it as updated research information; health care professionals can take it as a reference book, even patients' families, relatives and friends can take it as a good basis to understand neurodegenerative diseases.

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