What is the evidence for a relationship between *Chlamydia pneumoniae* and late-onset Alzheimer's disease?

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- PCR assays from two groups indicated that *C. pneumoniae* DNA is present in a high proportion of brain tissue samples from late-onset Alzheimer's disease (AD) patients but not in those from non-AD controls.
- Immunohistochemical analyses from two laboratories support this observation, and electron and immunoelectron microscopy have been used to visualize chlamydial organisms in the AD brain; however, some laboratories have been unable to repeat the PCR and immunohistochemical results.
- Other experimental data support a relationship between the pathobiology of *C. pneumoniae* and possession of the *APOE* ε4 allele type, which is a known risk factor for late-onset AD.

Selected Examples of Possible Associations Between Bacteria and Chronic Diseases T1

Disease	Bacterium	Reference*
peptic ulcer	*Helicobacter pylori*	*J Am Med Assoc*. 1995;274:1064-1066
cervical carcinoma	*Chlamydia trachomatis*	*J Am Med Assoc*. 2001;285:57-61
Parkinson's disease	*Nocardia asteroides*	*Infect Immun*. 1991;59:181-191
atherosclerosis	*Chlamydia pneumoniae*	*Proc Natl Acad Sci*. 1995;92:6911-6914 *FEMS Microbiol Lett*. 2001;197:1-9 (review)
temporomandibular joint dysfunction	*Chlamydia trachomatis*	*J Oral Maxillofac Surg*. 1999;57:683-688
schizophrenia	*Toxoplasma gondii*	*Clin Infect Dis*. 2001;32:842-844
rheumatoid arthritis	*Proteus mirabilis*	*Microbes Infect*. 2000;2:1489-1496

**References given represent only examples in some cases.*

Beginning with the demonstration of *Helicobacter pylori* as an etiologic agent for ulcers, the notion that microbial pathogens can play an initiating and/or exacerbatory role in chronic disease has gained increasing credence. Indeed, studies have suggested associations between *Chlamydia trachomatis* and cervical cancer,[1] *Nocardia asteroides* and Parkinson's disease,[2] and *Chlamydia pneumoniae* and atherosclerosis,[3] among many others [T1]. While most such associations are controversial, these lines of research remain under active investigation. In this article, evidence for and against the association between late-onset Alzheimer's disease (AD) and persistent infection with *C. pneumoniae* will be reviewed.

AD is a progressive neurodegenerative disease associated with atrophy/ death of neurons in affected brain regions. The disease occurs in two forms: an early-onset form which is primarily genetically determined, and a far more common late-onset form which is not genetically determined. Incidence of the latter increases with increasing age, and AD is considered to be the most significant single cause of senile dementia.[4] The disease usually manifests itself in its early stages by minor loss of

memory and progresses to major cognitive dysfunction, which can include language difficulties, loss of orientation, and some behavioral disorders.[5] The rate of progression along the cognitive dysfunction pathway varies widely among affected individuals and can be as long as two decades or more. Death in late-onset AD patients occurs not from the disease itself, but rather from secondary causes.[5]

The etiology underlying AD neuropathogenesis is poorly understood. The protein designated *tau* is a normal cytoskeletal component. Evidence suggests that its deposition in neurofibrillary tangles (NFT) results from aberrant post-translational modification or from unusual forms of the protein made by alternate splicing of its mRNA.[6] These aberrant forms of *tau* which form the NFT are thought to interfere with normal cytoskeletal formation in neurons, thereby contributing significantly to loss of those cells in the AD brain. Neuritic senile plaques (NSP) are comprised of β-amyloid (Aβ); deposition of this 39-42 amino acid peptide appears to be required for neuronal degeneration in AD,[7] although the mechanism by which this occurs is not fully understood. Aβ is derived from a region near the *C-terminus* of the amyloid precursor protein, which is encoded by the *βAPP* gene on chromosome 21. The normal function(s) of this large protein are unknown, but it is expressed in both neuronal and non-neuronal tissues.[7]

My laboratory became interested in a possible relationship between bacterial infection and late-onset AD following discussions with Brian Balin, PhD, who described unsuccessful efforts to identify bacteria and viruses associated with the disease. After several false starts (ie, screening for organisms we did not find in AD brain tissue), a collaborative effort among my group, the Balin group, the JA Whittum-Hudson group, and others did identify *C. pneumoniae* in the late-onset AD brain. In hindsight, this organism probably should have been our initial focus, since many of its characteristics were appropriate to the studies we had undertaken.

Chlamydia pneumoniae

C. pneumoniae is an obligate intracellular bacterial parasite of eukaryotic cells.

Selected Published Associations Between *C. pneumoniae* and Chronic Diseases

T2

Disease	Reference*
Alzheimers disease	*Med Microbiol Immunol.* 1998;187:23-42
multiple sclerosis	*Neurology* 1998;50:571-572
COPD	*Ann Med*. 1998;30:27-37
inflammatory arthritis	*Ann Rheum Dis*. 1994;53:100-105 *Arthritis Rheum.* 1999;42:1889-1893
myocarditis	*Scand J Rheumatol*. 1993;22:43-44
temporal arteritis	*Arthritis Rheum*. 2000;43:1543-1551

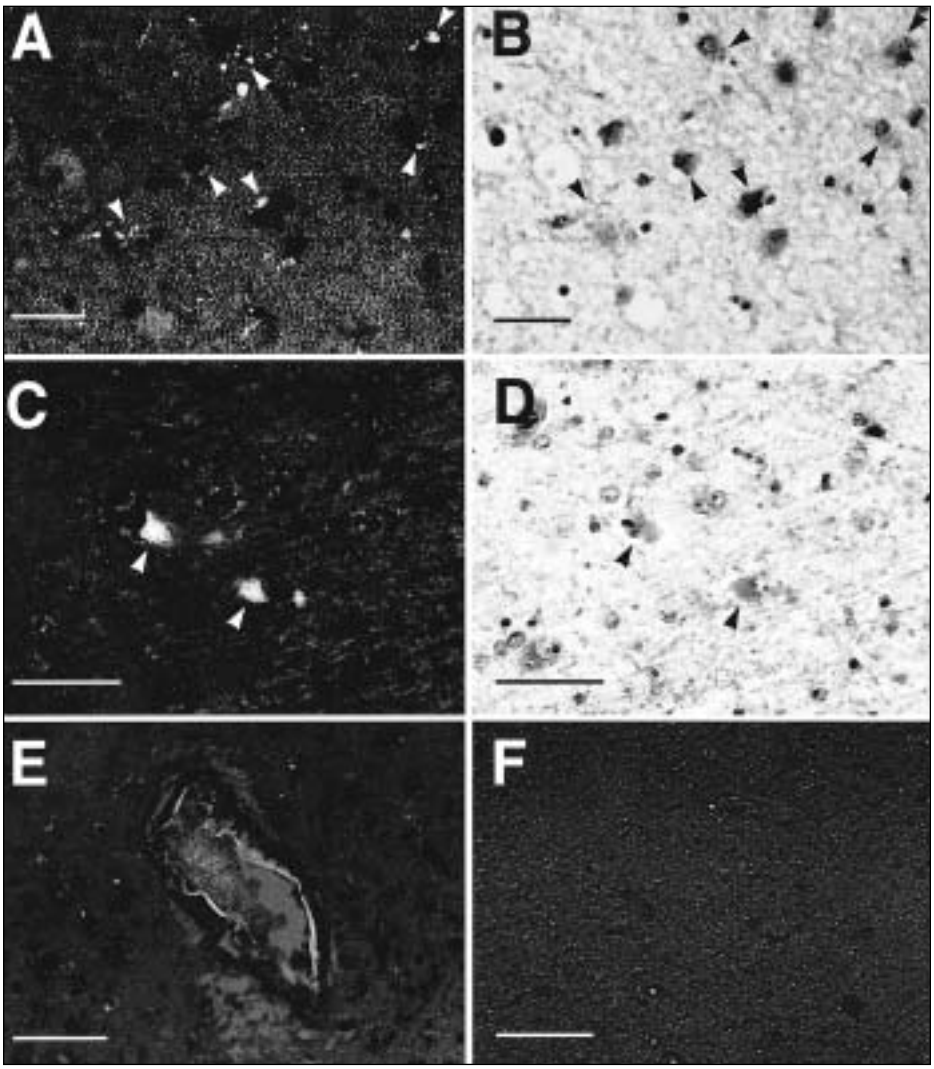
**References cited are examples only.*

Acquisition of all *Chlamydia* species is via mucosal surfaces. *C. pneumoniae* is a respiratory pathogen, and thus its initial sites of infection are the oral and nasal mucosa.[8] This organism is a significant agent in community-acquired pneumonia,[8] but studies have implicated *C. pneumoniae* in more severe pulmonary pathologies, including chronic obstructive pulmonary disease (COPD).[9] Infection with *C. pneumoniae* also has been associated with unexpected clinical entities, including arthritis[10] and atherosclerosis [T2].[3,11] In relation to the latter, in vitro studies show that *C. pneumoniae* infection induces foam cell formation and accumulation of cholesteryl esters within host cells,[12] providing biochemical evidence of a role for the organism in atherogenesis. Epidemiologic studies indicate that *C. pneumoniae* is ubiquitous. Prevalence of infection with the organism is high in all populations studied and appears to increase with increasing age.[8,13] In one study, males 60 years and older showed a prevalence rate of 70%.[13] In crowded living conditions, childhood infection with *C. pneumoniae* is more common than in less densely populated regions.[13] Studies from Scandinavia indicate that epidemics of *C. pneumoniae* occur every 5-7 years, and that most individuals incur several infections over a lifetime.[8,13]

Initial Studies of *C. pneumoniae* in the Alzheimer's Brain

We developed polymerase chain reaction (PCR) assays targeting chromosomal DNA sequences of *C. pneumoniae*[10] and used them to assess DNA preparations from frozen brain autopsy tissues from 19 late-onset AD and an equal number of non-AD control patients. As described in the initial publication,[14] samples from brain areas showing characteristic neuropathology were PCR-positive for the organism in 17/19 AD patients in repeated assays; only one sample from an age-matched, non-AD control patient was positive. Electron microscopy (EM) to visualize the organism in AD brain samples revealed that areas of the hippocampus, temporal cortex, and/or other regions of AD brains with NFT and NSP contained objects that resembled *C. pneumoniae*, as well as chlamydial inclusions. We could identify no similar structures in non-AD control brains in parallel analyses. Immunoelectron microscopy (IEM) using a monoclonal antibody (mAb) targeting the major outer membrane protein (MOMP) of *C. pneumoniae* identified the objects resembling chlamydiae as authentic *C. pneumoniae*. Parallel examination of brain tissue sections from PCR-negative patients showed no labeling (ref. 14 for extensive controls and discussion).

For immunostaining of tissues from affected AD brain regions and congruent regions from non-AD brains, we used two mAb: a genus-specific mAb targeting the lipopolysaccharide (LPS) of *Chlamydiae* and the *C. pneumoniae*-specific anti-MOMP mAb. In sections from AD brains, we found staining in perivascular regions of blood vessels in the neuropil and in microglia- and astroglia-like cells not adjacent to such vessels; no labeling was seen with either mAb in brain sections from non-AD patients. We confirmed these cell types



[1] Double immunolabelling of AD brain tissues for identification of specific host cell types harboring *C. pneumoniae*, plus a control from an AD patient. Preparation of tissues and immunolabelling were as described in ref. 14. Panels A, B: tissue section from the temporal lobe of an AD patient double immunolabelled for the chlamydial lipopolysaccharide (LPS; white arrows, A) and glial fibrillary acidic protein (GFAP; black arrows, B). Labelled cells in Panel A were detected using an FITC-conjugated secondary antibody, while those in Panel B were identified with the DAB chromogen. The cells identified are astrocytes. Panels C, D: tissue section from the temporal cortex of an AD patient double immunolabelled for inducible nitric oxide synthase (iNOS; white arrows, C) and the chlamydial major outer membrane protein (MOMP; black arrows, D). Labelled cells in Panels C, D were detected as in Panels A, B. The cells identified are microglia. Panel E: immunolabelling of cells with the anti-iNOS antibody in the hippocampus of an AD patient. The cells identified are pericytes. Panel F: representative section from the temporal cortex of an AD patient showing minimal autofluorescence. Bars in Panels A-D = 50 µm. Bars in Panels E, F = 100 µm. Used with permission. See ref. 14 for more details.

as harboring *C. pneumoniae* via double immunolabeling, using the anti-chlamydial LPS mAb and an anti-glial fibrillary acidic protein mAb for astroglia; an anti-inducible nitric oxide synthase (iNOS) Ab in combination with the anti-MOMP mAb confirmed activated microglia as host cells [1]. The chlamydia-infected cells surrounding blood vessels in the AD brain were pericytes since they labeled with the anti-iNOS and anti-MOMP Ab as well as a mAb targeting CD68.[14]

The mAb designated PHF-1 (paired helical filaments of *tau*) identifies neuritic pathology in AD brain tissues. Staining of consecutive brain tissue sections with the PHF-1 and anti-MOMP mAb demonstrated the presence of *C. pneumoniae*-infected glial cells near PHF-*tau* protein deposition in neurites in the AD brain. Similarly performed immunolabeling of brain areas showing little or no neuropathology in AD brains showed essentially no cells harboring *C. pneumoniae*, and no infected cells were identified in any non-AD brain examined.[14] Thus, chlamydia-infected astroglia and microglia in the late-onset AD brain are concentrated in regions of characteristic neuropathology.

To determine whether the identified *C. pneumoniae* were viable, we cultured the organism from homogenates of AD and control brains using the human monocyte cell line THP-1 as host. Immunocytochemistry using the anti-MOMP mAb was done on THP-1 cells after culture with brain homogenates. Cells incubated with homogenates of temporal cortex from control brains were negative for *C. pneumoniae* using the anti-MOMP mAb. THP-1 cells from cultures incubated with homogenates from temporal cortex of AD patients were immunopositive using the same mAb. *C. pneumoniae* within infected cells also were visualized by transmission EM. Thus, viable *C. pneumoniae* were present in brain tissue from the late-onset AD patients studied, but not in congruent tissues from non-AD patients.[14]

APOE Allele Types and the Pathobiology of *C. pneumoniae*

The one well-accepted risk factor for development of late-onset AD is possession of the ε4 allele type at the apolipoprotein E (*APOE*) locus on chromosome 19.[15] Not all patients bearing this allele type develop AD, but its presence increases risk for disease development several-fold as well as promoting earlier onset and more rapid neurodegeneration. The *APOE* gene has five allele types, two of which (ε1, ε5) are rare, and three of which (ε2, ε3, ε4) are relatively common. The product of the *APOE* gene is therefore polymorphic and is important for cholesterol transport both in the central nervous system and the general circulation.[16, 17] Precisely how the product of the ε4 allele type promotes late-onset AD is not understood, although several hypotheses have been advanced including interac-

Published Attempts to Identify *C. pneumoniae* in the Alzheimer's Brain

T3

Study	Patient Samples	Source	Methods	Positives Identified	
Balin et al.[14]	19 AD, 19 control	Autopsy, frozen	PCR, ISH, culture, EM, IEM	PCR ISH**** Culture EM IEM*****	17/19 AD, 1/19 control 6/6 AD, 0/6 control (anti-MOMP, anti-LPS) 2/2 AD, 0/2 control 10/10 AD, 0/6 control 10/10 AD, 0/7 control
Nochlin et al.[23]	12 AD, 7 possible AD, 6 control	Autopsy, paraffin	PCR, ISH	PCR ISH	0/25 0/25 (anti-LPS)
Gieffers et al.[24]	20 AD	Autopsy, paraffin	PCR, ISH	PCR ISH	0/20 0/20
Ring & Lyons[25]	15 AD, 5 control	Autopsy, frozen	PCR, some culture	PCR Culture	2 weak AD, 1 weak control 0/3 (2 AD, 1 control)
Ossewarde et al.[26]	12 AD, 16 control*	Autopsy, paraffin	ISH***	ISH	11/12 AD*** 3/16 control
Mahony et al.[27]	21 AD, 10 control	Autopsy, frozen	PCR		18/21 AD** 0/10 control

* *Includes both non-demented patients and patients with other neurological (non-AD) diseases*

** *Positive in at least 1 of 10 repetitions of PCR reaction.*

*** *mAb targeting* C. pneumoniae *hsp60, MOMP, LPS used. Not all samples positive with each mAb.*

**** *mAb targeting the* C. pneumoniae *MOMP and LPS employed.*

***** *mAb targeting the* C. pneumoniae *MOMP employed.*

tion with the neuronal cytoskeleton via *tau* and promotion of Aβ formation.[18-20]

We reasoned that if the *APOE* ε4 gene product is associated with late-onset AD, and if *C. pneumoniae* is associated with the same disease, then some relationship might exist between ε4 and the bacterium. We therefore asked whether we could identify any connection between possession of *APOE* ε4 and pathogenesis by *C. pneumoniae* in some other, non-AD context. We chose to examine synovial infection with *C. pneumoniae* since the organism had been identified in the joints of some arthritis patients.[10] DNA prepared from synovial tissue biopsies of a large patient cohort (average age 43 yr) was screened by PCR for *C. trachomatis*, *C. pneumoniae*, and other bacteria. *APOE* genotype was defined for patients in each of 4 groups: those PCR-positive for *C. trachomatis* only, *C. pneumoniae* only, other bacteria only, or no bacteria. RT-PCR confirmed *APOE* expression in synovial tissue. Genotyping showed that patients PCR-negative in all assays, and those positive in the *C. trachomatis*-only and non-*Chlamydia* directed assays, showed distributions of the three main *APOE* allele types (ε2, ε3, ε4) which mirrored those of the general population.[21] In contrast, 68% of patients with synovial *C. pneumoniae* DNA had a copy of ε4,[22] a rate 4-5 times that of the general population. This observation strongly suggests a role for this gene product in the pathobiology of the organism, strengthening the association between *C. pneumoniae* and development of late-onset AD. The data may also suggest that possession of the *APOE* ε4 allele is a surrogate marker for risk of late-onset AD but a direct marker for the pathobiology of *C. pneumoniae*.

Related Studies from Other Laboratories

Several laboratories have attempted to replicate aspects of the studies outlined above, and these have provided variable results [T3]. The Seattle group was unsuccessful in identifying *C. pneumoniae* in paraffin embedded brain tissues from 12 confirmed and 13 suspected late-onset AD patients, using PCR and immunohistochemical analyses.[23] A similar study from a German group also could not identify the organism in any of 20 paraffin embedded AD brain samples, again using PCR and immunohistochemistry.[24] In a third study from California, multiple samples from each of 15 confirmed AD and 5 non-AD control brains were screened by PCR, and each sample was assayed independently several times.[25] Samples were prepared from frozen, rather than paraffin embedded, brain tissues, and culture of the organism from some samples was attempted. The latter analyses were negative for all samples studied, but a small number of weak positives were obtained in the PCR analyses. In all these studies, the authors concluded that the presence of *C. pneumoniae* in the AD brain could not be confirmed,[23,24] or that the association between organism and the late-onset AD brain was neither strong nor unique.[25]

Recovery of usable amplification template from paraffin embedded tissue is often unreliable, and this may explain in part the PCR-negative results from two of the studies cited above; indeed, this is the reason that only frozen brain tissue samples have been used in all our studies. However, it is difficult to understand why the paraffin embedded tissue samples used in the other studies were negative in immunohistochemical analyses since a Dutch group reported success in identifying *C. pneumoniae* by

this method in 11 of 12 paraffin embedded AD brain samples.[26] All control samples were negative for the organism in this study, including several from patients with neurologic diseases other than AD. In our own work, the negative immunohistochemical studies, and in the positive Dutch study, the same or similar mAb targeting the organisms' MOMP or LPS were employed. This suggests differences in technique as a limiting factor in obtaining positive results.

Yet another recent report from a Canadian group[27] provides insight into the mostly negative PCR data from the California study. This study used replicate PCR assays and probit regression analyses to show that DNA prepared from up to 85% of the frozen AD brain samples analyzed were PCR-positive for *C. pneumoniae* if enough replicates were performed; multiply assayed controls were always PCR-negative. Replicate PCR assays targeting *C. pneumoniae* have also been shown to be required for identification of this organism in peripheral blood mononuclear cells and nasopharyngeal samples.[28] Another study indicated that for certain PCR systems targeting the hepatitis C viral genome, replicate assays and probit regression analysis are useful.[29] These data indicate that *C. pneumoniae* load in the AD brain and elsewhere can be extremely low, and thus identification of chlamydial DNA in preparations from such materials requires not only careful template preparation but also repetitive analyses.

Additional Data Supporting an Association Between *C. pneumoniae* and Late-onset Alzheimer's Disease

In our original study, three non-AD control brain samples assessed by PCR for *C. pneumoniae* DNA were derived from patients with multiple sclerosis (MS)[14] and all were negative. Recently we extended PCR screening assays to frozen brain tissue samples from patients with Parkinson's disease (PD) and additional controls. DNA samples from temporal cortex and hippocampus were assayed from three non-AD/non-PD control individuals, three patients with PD, and two patients with both PD and AD. In multiple PCR assays targeting the *C. pneumoniae* 16S rRNA gene, the gene encoding MOMP, and other genes, each sample from controls was negative, as were all samples from the PD patients. However, samples from each patient with PD plus AD were PCR-positive for *C. pneumoniae*. Further, we have obtained frozen tissue samples from temporal cortex, hippocampus, and other brain regions from nine additional confirmed AD patients and several controls. Multiple PCR assays targeting three *C. pneumoniae* genes were negative for all controls but positive for 8 of the 9 AD patients. We have also begun analyses of mRNA production from the *βAPP, PS-1, PS-2*, and several other AD-relevant host genes in *C. pneumoniae*-infected and control astrocytoma cell lines (*APOE* ε4-bearing and -lacking). Our initial data suggest that infection with the organism alters host production of mRNA encoding these proteins in this cell type, especially in those cells bearing a copy of the *APOE* ε4 allele.

Conclusions

Several earlier studies attempted to define an etiologic relationship between infection with various viruses or bacteria and development of late-onset AD, but no such link has been demonstrated as yet. The observations reviewed here from our initial study and those from two confirming studies argue that *C. pneumoniae* is present and viable in the AD brain, and that the organism is present primarily in regions of characteristic neuropathology. While much work remains, including providing full explanation for the several studies unable to find the organism in AD brain samples, these results seem to be the first to demonstrate consistent proximity of a known bacterial pathogen to regions of standard AD-related neuropathology. It is important that the organism was identified only in AD brain materials and not in congruent brain samples from patients with MS or PD or from patients without neurologic disease. However, the number of samples assayed from patients with MS and PD in my laboratory and others so far is small. Further assessment of *C. pneumoniae* in brain from patients with other neurodegenerative diseases is required. It is also critically important for other laboratories to replicate the PCR, immunohistochemistry, EM, and IEM observations in larger AD and control patient populations.

Assuming that *C. pneumoniae* is indeed present in the late-onset AD brain but not those of normal individuals or patients with other neurologic diseases, such an association does not constitute a demonstration that the organism actually causes the disease. *C. pneumoniae* is a capable opportunistic pathogen, and it is possible, perhaps even likely, that it takes up residence in the AD brain in infected individuals only after the initiation of relevant neuropathology by some other means. All species of *Chlamydia* are known to cause inflammation at sites of their residence, and data from many studies indicate that treatment of AD patients with anti-inflammatory drugs is beneficial.[30] This observation suggests that the inflammation which characterizes the AD brain is an important component of neuropathogenesis. Thus, whether *C. pneumoniae* actually initiates the neurodegeneration process in late-onset AD or not, it seems probable that some portion of the AD-related inflammation is attributable to chlamydial infection.

We do not know at what point, by what means, or under what physiologic conditions the organism reaches the central nervous system in susceptible individuals. It seems unlikely that it does so solely as a result of normal aging processes since virtually none of our or others' age-matched control patients was positive for the organism. Moreover, the organism does not appear to be present in brain tissues from individuals with neurodegenerative diseases other than late-onset AD. Another issue of importance will be to determine whether *C. pneumoniae* infecting AD brain tissues represents a specific neurotropic strain. All these questions must be major foci of future research in this area. Moreover, given the demonstrated beneficial effect of anti-inflammatory drugs on late-onset AD patients, more study of these and related compounds may prove to be useful therapeutically for AD patients. Use of antibiotic and other anti-*Chlamydia pneumoniae* compounds in late-onset AD patients may also be useful, and trials to examine efficacy of such treatment regimens are now underway in a limited manner in some laboratories.

Acknowledgments

The work described and the writing of this article were supported by grants from the US National Institutes of Health (AR-42541 and AI-44055 to A.P.H.; EY-03324 to JA Whittum-Hudson.; AG-10160 to BJ Balin) and a grant from the US Veterans Administration Medical Research Service (to APH).

1. Anttila R, Saikku P, Koskela P, et al. Serotypes of *Chlamydia trachomatis* and risk for development of cervical squamous cell carcinoma. *J Am Med Assoc*. 2001;285:47-51.
2. Kohbata S, Beaman BL. L-dopa-responsive movement disorder caused by *Nocardia asteroides* localized in the brains of mice. *Infect Immun*. 1991;59:181-191.
3. Mahony JB, Coombes BK. *Chlamydia pneumoniae* and atherosclerosis: does the evidence support a causal or contributory role? *FEMS Microbiol Lett*. 2001;197:1-9.
4. Keefover RW. The clinical epidemiology of Alzheimer's disease. *Neurol Clin*. 1996;14:337-351.
5. Schellenberg GD. Genetic dissection of Alzheimer disease, a heterogeneous disorder. *Proc Natl Acad Sci* (USA). 1995;92:8552-8559.
6. Wang J-Z, Grundke-Iqbal I, Iqbal K. Glycosylation of microtubule-associated protein tau: an abnormal post-translational modification in Alzheimer's disease. *Nature Med*. 1996;2:871-875.
7. Selkoe DJ, Podlisny MD, et al. β-amyloid precursor protein of AD occurs at 110- to 135-kilodalton membrane-associated proteins in neural and nonneural tissues. *Proc Natl Acad Sci (USA)*. 1988;85:7341-7345.
8. Grayston JT. *Chlamydia pneumoniae*, strain TWAR pneumonia. *Annu Rev Med*. 1992;43:317-323.
9. Von Hertzen LC. *Chlamydia pneumniae* and its role in chronic obstructive pulmonary disease. *Ann Med*. 1998;30:27-37.
10. Schumacher HR, Gérard HC, Arayssi T, et al. Lower prevalence of *Chlamydia pneumoniae* DNA compared with *C. trachomatis* in synovial tissues of arthritis patients. *Arthritis Rheum*. 1999;42:1889-1893.
11. Kuo CC, Grayston JT, Campbell LA, et al. *Chlamydia pneumoniae* (TWAR) in coronary arteries of young adults (15-34 years old). *Proc Natl Acad Sci (USA)*. 1995;92:6911-6914.
12. Kalayoglu MV, Byrne GI. Induction of macrophage foam cell formation by *Chlamydia pneumoniae*. *J Infect Dis*. 1998;177:725-729.
13. Leinonen M. Pathogenetic mechanisms and epidemiology of *Chlamydia pneumoniae*. *Eur Heart J*. 1993;14:57-61.
14. Balin BJ, Gérard HC, Arking EJ, et al. Identification and localization of *Chlamydia pneumoniae* in the Alzheimer's brain. *Med Microbiol Immunol*. 1998;187:23-42.
15. Roses AD. Apolipoprotein E alleles as risk factor in Alzheimer's disease. *Annu Rev Med*. 1996;47:387-400.
16. Mahley RW. Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. *Science* 1988;240:622-630.
17. Strittmatter WJ. Apolipoprotein E and Alzheimer's disease: signal transduction mechanisms. *Biochem Soc Symp*. 2001;67:101-109.
18. Lovestone S, Anderton B, Betts J, et al. Apolipoprotein E gene and Alzheimer's disease: is *tau* the link? *Biochem Soc Symp*. 2001;67:111-120.
19. Ohm TG, Hamker U, Cedazo-Minguez A, et al. Apolipoprotein E and beta A4-amyloid: signals and effects. *Biochem Soc Symp*. 2001;67:121-129.
20. Huang Y, Liu XQ, Wyss-Coray T, et al. Apolipoprotein E fragments present in Alzheimer's disease brains induce neurofibrillary tangle-like intracellular inclusions in neurons. *Proc Natl Acad Sci (USA)*. 2001;98:8838-8843.
21. Ordovas JM, Litwack-Klein L, Wilson PWF, et al. ApoE isoform phenotyping methodology and population frequency with identification of apoE1 and apoE5. *J Lipid Res*. 1987;28:371-380.
22. Gérard HC, Wang GF, Balin BJ, et al. Frequency of apolipoprotein E (APOE) allele types in patients with *Chlamydia*-associated arthritis and other arthritides. *Microb Pathogen*. 1999;26:35-42.
23. Nochlin D, Shaw CM, Campbell LA, et al. Failure to detect *Chlamydia pneumoniae* in brain tissues of Alzheimers disease. *Neurology* 1999;53:1888.
24. Gieffers J, Reusche E, Solbach W, et al. Failure to detect *Chlamydia pneumoniae* in brain sections of Alzheimers disease patients. *J Clin Microbiol*. 2000;38:881-882.
25. Ring RH, Lyons JM. Failure to detect *Chlamydia pneumoniae* in the late-onset Alzheimers brain. *J Clin Microbiol*. 2000;38:2591-2594.
26. Ossewarde JM, Gielis-Proper SK, Meijer A, et al. *Chlamydia pneumoniae* antigens are present in the brains of Alzheimer patients but not the brains of patients with other dementias. In: *Chlamydia Research*, Saikku P (ed). Societa Editrice Esculapio, Bologna. 2000; p 284.
27. Mahony J, Woulfe J, Munoz D, et al. *Chlamydia pneumoniae* in the Alzheimers brain - is DNA detection hampered by low copy number? In: *Chlamydia Research*, Saikku P (ed). Societa Editrice Esculapio, Bologna. 2000; p 275.
28. Smiega M, Mahony JB, Goldsmith CH, et al. Replicate PCR testing and probit analysis for detection and quantitation of *Chlamydia pneumoniae* in clinical specimens. *J Clin Microbiol*, 2001;39:1796-1801.
29. Saldanha J. Assays for viral sequences and their value in validation of viral elimination. *Dev Biol Stand*, 1993;81:231-236.
30. Breitner JC. The role of anti-inflammatory drugs in the prevention and treatment of Alzheimer's disease. *Annu Rev Med*. 1996;47:401-411.