

Emerging roles of pathogens in Alzheimer disease

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Chronic spirochetal infection can cause slowly progressive dementia, cortical atrophy and amyloid deposition in the atrophic form of general paresis. There is a significant association between Alzheimer disease (AD) and various types of spirochete (including the periodontal pathogen *Treponemas* and *Borrelia burgdorferi*), and other pathogens such as *Chlamydothyla pneumoniae* and herpes simplex virus type-1 (HSV-1). Exposure of mammalian neuronal and glial cells and organotypic cultures to spirochetes reproduces the biological and pathological hallmarks of AD. Senile-plaque-like beta amyloid (A β) deposits are also observed in mice following inhalation of *C. pneumoniae* in vivo, and A β accumulation and phosphorylation of tau is induced in neurons by HSV-1 in vitro and in vivo. Specific bacterial ligands, and bacterial and viral DNA and RNA all increase the expression of proinflammatory molecules, which activates the innate and adaptive immune systems. Evasion of pathogens from destruction by the host immune reactions leads to persistent infection, chronic inflammation, neuronal destruction and A β deposition. A β has been shown to be a pore-forming antimicrobial peptide, indicating that A β accumulation might be a response to infection. Global attention and action is needed to support this emerging field of research because dementia might be prevented by combined antibiotic, antiviral and anti-inflammatory therapy.

Alzheimer disease (AD) is characterised by a slowly progressive decline of memory and cognition. Alzheimer described the characteristic cortical senile plaques and neurofibrillary tangles in the brain of a 51-year-old woman with presenile dementia (Refs 1, 2). Because the presenile form, with onset before the age of 65, is identical to the most common form of senile dementia, today, the term AD is used for the

designation of both presenile and senile cases (Refs 3, 4).

Senile plaques were first described by Blocq and Marinesco (Ref. 5). Redlich (Ref. 6) first observed senile plaques in the brains of two patients with senile dementia. Recently, particularly from the use of the Gallyas silver technique (Ref. 7), neuropil threads or curly fibres were recognised as further characteristic

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lesions of AD. Granulovacuolar degeneration is another typical alteration of neurons (Ref. 8). Other important features include neuronal and synaptic loss (Ref. 9), Hirano bodies, reactive astrocytosis and microgliosis.

AD is a form of amyloidosis. The amyloid substance aggregated in the brain is a small, ~4 kDa amyloid beta peptide ($A\beta$). It is released by β - and γ -secretase cleavage from a larger 120 kDa transmembrane amyloid precursor protein (APP) (Refs 10, 11) and was purified and partially sequenced by Glenner and Wong (Ref. 12). APP exhibits features of a glycosylated cell-surface receptor and was shown to be a proteoglycan core protein (Ref. 13). APP is phylogenetically highly conserved and constitutively expressed by various cells other than neurons, including immune cells (Ref. 14). Human platelets, peripheral lymphocytes and leukocytes produce the major isoforms of APP, and after activation, they secrete amyloidogenic $A\beta$ (Refs 15, 16, 17, 18).

$A\beta_{1-42}$ has a higher ability to aggregate than the shorter $A\beta_{1-40}$ (Refs 19, 20). $A\beta$ exists in soluble nontoxic monomers, strongly toxic soluble oligomers and in the form of less toxic insoluble fibrils. The soluble oligomers of $A\beta_{1-42}$ are the most toxic (Refs 21, 22, 23, 24, 25, 26). They form annular or pore-like structures that are indistinguishable from a class of pore-forming bacterial toxins (Refs 24, 25, 27), which cause rapid calcium influx through the targeted cell membranes (Refs 28, 29, 30). Recent *in vitro* and *in vivo* studies showed that $A\beta$ is an antimicrobial peptide (AMP) that targets bacterial membranes (Ref. 31). AMPs have proinflammatory activities and have a role in innate immune responses (Ref. 31).

Neurofibrillary tangles and neuropil threads contain paired helical filaments (PHFs) (Refs 32, 33). The major component of PHFs is the microtubule-associated protein tau, which is in a pathological hyperphosphorylated state that abolishes microtubule assembly (Refs 34, 35). Sequestration of peptidyl-prolyl *cis/trans* isomerase NIMA interacting 1 (PIN1) is one theory that explains the formation of these pathological fibrillar lesions (Ref. 36).

Various hypotheses have been proposed to explain the pathogenesis of AD (Refs 37, 38, 39, 40, 41, 42, 43). A significant proportion of early-onset AD is inherited as an autosomal dominant

trait (Ref. 44). Missense mutations of the APP gene located on chromosome 21 (Refs 45, 46) are responsible for 5% of all early-onset familial AD cases (Ref. 44). Presenilin-1 (*PS1*) gene mutations are most frequent in early-onset familial AD (Refs 37, 47). More than 80 different *PS1* missense mutations or amino acid deletions have been identified (Refs 37, 47, 48, 49, 50). Presenilin-2 (*PS2*) mutations are responsible for another subset of early-onset familial AD (Refs 47, 51). As originally shown by Hardy (Ref. 39), mutations in these pathogenic genes alter the processing of APP and result in an increase in amyloidogenic $A\beta_{1-42}$ and $A\beta_{1-43}$ (Refs 50, 52, 53, 54, 55, 56, 57, 58, 59). The epsilon 4 allele of apolipoprotein E (*APOE* ϵ 4) is an important risk factor for late-onset AD, which also correlates with increased $A\beta$ burden (Ref. 60). Finally, there is an association between AD and polymorphisms of various other genes, which include a growing number of genes, implicated in immune defence mechanisms (Refs 61, 62).

The relation between the two major biological markers of AD, $A\beta$ (Refs 38, 41) and hyperphosphorylated tau (Refs 34, 35), is not clear. Soluble $A\beta$ and tau strongly interact (Ref. 63), and APP is expressed in neurofibrillary tangles (Ref. 64), suggesting that these apparently different pathologies are linked.

Alterations of various neurotransmitters, neuropeptides and hormones are reported to occur in AD (Refs 65, 66, 67, 68). The cholinergic hypothesis is based on the alteration of acetylcholine synthesis, transport and release (Refs 69, 70). Oxidative damage to proteins, lipids and nucleic acids (Refs 71, 72, 73, 74) and mitochondrial dysfunction (Ref. 75) are also significant contributors to the pathogenesis of AD. The role of various metals, including aluminium (Refs 76, 77) and iron (Ref. 78) was proposed several decades ago. Direct modulation of APP processing by metal ions, including Ca^{2+} , Zn^{2+} , Fe^{2+}/Fe^{3+} and Al^{3+} , suggests that disrupted metal homeostasis also leads to increased APP levels (Ref. 79). The calcium homeostasis hypothesis indicates that sustained deregulation of cytosolic calcium represents the common final pathway for neuronal death in AD (Ref. 80). Dysregulation of ubiquitylation or glycosylation processes (Refs 81, 82) has been shown in AD. Vascular lesions, including cerebral hypoperfusion and disturbed brain

microcirculation, are also important factors (Refs 83, 84, 85, 86, 87, 88, 89, 90, 91). Factors representing a risk for atherosclerosis (Refs 92, 93), are also risk factors for AD. Early involvement of the olfactory system in AD (Ref. 94) led to the 'olfactory hypothesis', which suggests that putative pathogenic agents might access the brain by the olfactory pathways (Refs 95, 96, 97). Deregulation of various signalling pathways, apoptosis, craniocerebral trauma, exercise, environmental and nutritional factors, among others, are also implicated in the pathogenesis of AD.

The critical role of chronic inflammation and the importance of interleukin (IL)-1 signalling in AD is now widely recognised (Refs 98, 99, 100). A series of inflammatory mediators, including cytokines, chemokines, proteases, adhesion molecules, free radicals, pentraxins, prostaglandins, anaphylatoxins and activated complement proteins, is present at the site of cortical lesions in AD (Refs 101, 102, 103). The membrane attack complex (MAC, C5b-9) is also associated with plaques, tangles and neuropil threads (Refs 100, 104). Use of nonsteroidal anti-inflammatory drugs reduces the risk of AD (Refs 105, 106, 107, 108).

Nearly a century ago, Fischer, Alzheimer and their colleagues (Refs 2, 109) discussed the possibility that microorganisms could have a role in the formation of senile plaques. That a slow-acting unconventional infectious agent, acquired at an early age and requiring decades to become active, might be involved in AD was considered by several authors (Refs 110, 111). A growing number of recent observations indicate that infectious agents are involved in the pathogenesis of AD. Here, I review historical and recent observations on infectious agents related to AD and analyse the significance of the association and causal relationship.

Analogies between AD and the atrophic form of general paresis

Historical observations show that the clinical and pathological hallmarks defining AD are similar to those occurring in the atrophic form of general paresis, a chronic bacterial infection (Refs 112, 113, 114, 115, 116, 117, 118). In 1913, Noguchi and Moore (Ref. 119) provided conclusive evidence that spirochetes are responsible for slowly progressive dementia, cortical atrophy and local amyloidosis.

General paresis of the insane, parietic dementia or dementia paralytica is a chronic meningoencephalitis caused by the direct invasion of brain parenchyma by *Treponema pallidum*. Two forms are distinguished: the infiltrative and the atrophic form (Refs 114, 117). In the infiltrative form, mood disorders and psychosis predominate, and lymphoplasmocytic meningoencephalitis is the characteristic pathology (Refs 117, 118). The atrophic form is characterised by slowly progressive dementia and cortical atrophy, which is accentuated in the frontotemporal regions (Refs 114, 115). Spirochetes form masses, plaques or colonies (Fig. 1) and disseminate as individual filaments, which are restricted to the cerebral cortex (Fig. 1) (Refs 114, 115). These spirochetal masses and individual spirochetes are morphologically identical to senile plaques (Fig. 1) and neuropil threads (Fig. 1). Pacheco e Silva (Refs 114, 115), reported that the number of spirochetes and spirochetal 'plaques', which are numerous in the hippocampus and frontal cortex, increases in parallel with the severity of cortical atrophy (Refs 114, 115). Lymphoplasmocytic infiltrates are absent. Severe neuron loss is accompanied by reactive microgliosis and astrogliosis and by accumulation of 'paralytic iron' (Ref. 120). The occurrence of neurofibrillary tangles is also documented in general paresis (Refs 113, 118, 121, 122), and the local amyloid (Ref. 123), as in AD, consists of A β (Ref. 124).

Analogies between AD and other age-related chronic inflammatory disorders

Pathogens can produce slowly progressive chronic diseases. Following the pioneering work of Warren and Marshall (Ref. 125), it is today established that *Helicobacter pylori* causes stomach ulcers. Infectious agents are also linked to atherosclerosis, cardio- and cerebrovascular disorders (Refs 126, 127, 128, 129, 130, 131, 132, 133, 134), chronic lung diseases (Refs 135, 136, 137), inflammatory bowel diseases and neuropsychiatric disorders (Refs 138, 139, 140, 141, 142, 143).

Chlamydophila (Chlamydia) pneumoniae (Refs 126, 127), *H. pylori* (Refs 128, 129) and several periodontal pathogens, including invasive oral spirochetes (Refs 130, 131) and herpes viruses, have been found in human atherosclerotic lesions. Some of them also enhanced atherosclerosis in experimental animals

(Refs 131, 132). These pathogens were also reported to be associated with AD (Refs 144, 145, 146, 147, 148, 149, 150, 151).

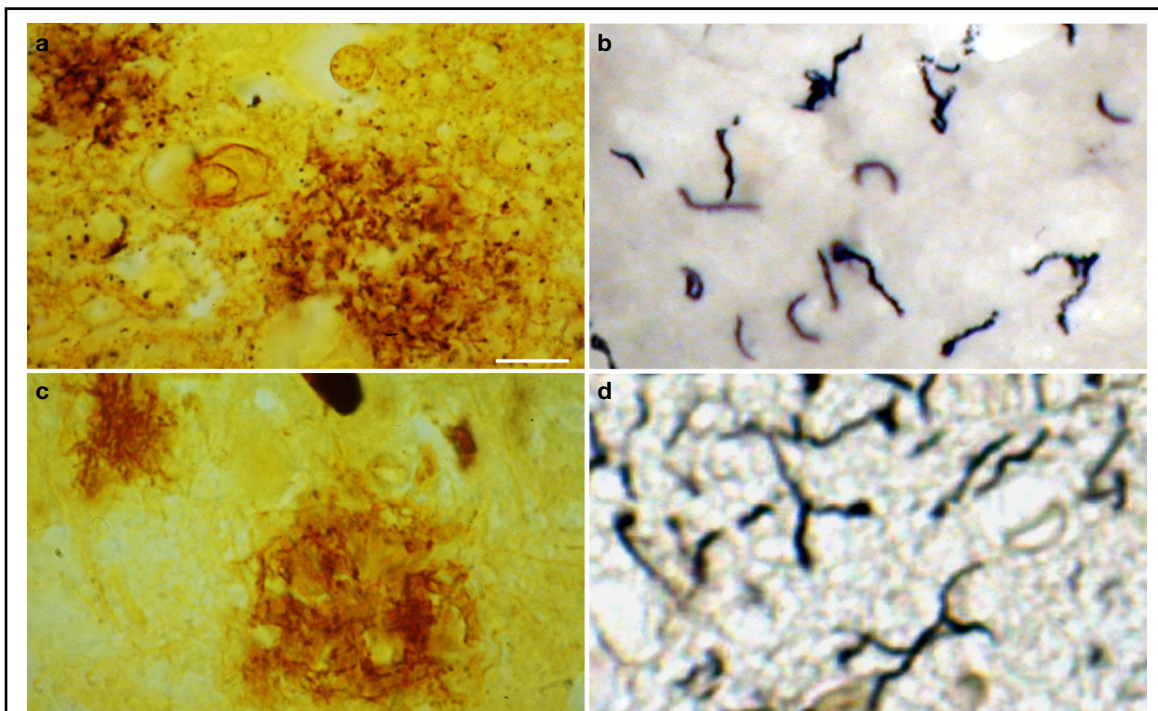
Epidemiological studies have confirmed that several of these chronic inflammatory disorders are associated with AD (Refs 152, 153, 154, 155). In addition, they are all linked to periodontal polybacterial disorders, which are primarily caused by Gram-negative bacteria (Refs 156, 157, 158, 159). Spirochetes and herpes viruses are predominant periodontal pathogens (Refs 160, 161, 162, 163), and *C. pneumoniae* is a major upper respiratory tract pathogen. An infectious origin might give a comprehensive explanation of the common cellular and molecular mechanisms, inflammatory processes and common inflammatory gene polymorphisms involved in

these chronic inflammatory disorders and AD (Refs 61, 164, 165).

Evidence for the association of pathogens with AD

Spirochetes

Spirochetes are Gram-negative, helical bacteria, which possess endoflagella, taxonomically distinguishing them from other bacteria. There are over 200 different spirochetal species or phylotypes (Ref. 166). Spirochetes are causative agents of important human diseases such as syphilis, pinta, yaws, bejel, Lyme disease, Vincent angina, relapsing fever, leptospirosis, ulcerative gingivitis and various periodontal disorders (Ref. 167). The major *Borrelia* species causing Lyme disease are *B. burgdorferi*



Distribution of spirochetes in the atrophic form of general paresis and in the frontal cortex of an Alzheimer disease patient with Lyme neuroborreliosis

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Figure 1. Distribution of spirochetes in the atrophic form of general paresis and in the frontal cortex of an Alzheimer disease (AD) patient with Lyme neuroborreliosis. Spirochetes form plaque-like masses (a,c) and disseminate as individual filaments (b,d) in general paresis (a,b) and AD (c,d), which are identical to senile plaques and curly fibres. (a,c) Bosma Steiner silver technique for the visualisation of spirochetes. (b) *T. pallidum*-specific polyclonal antibody. (d) Gallyas silver technique. Scale bars: 50 µm (a), 40 µm (c), 10 µm (b,d). All images were obtained from previously published studies: a (Ref. 124); b (Ref. 175); c, d (Ref. 149).

(Ref. 170), *B. afzelii*, *B. garinii* and *B. valaisiana*. Relapsing fever is caused by *B. recurrentis*. Oral spirochetes are predominant periodontal pathogens that are highly prevalent in the population and comprise diverse *Treponema* species (Refs 166, 171). Several are invasive (Refs 172, 173): they include *T. denticola*, *T. socranskii*, *T. pectinovorum*, *T. amylovorum*, *T. lecithinolyticum*, *T. maltophilum*, *T. medium* and *T. putidum* (Refs 166, 171, 174). *T. vincentii* causes Vincent angina, a necrotising fusospirochetal disease (Ref. 167).

Because spirochetes are strongly neurotropic (Ref. 167), it was expected that several types of spirochetes, in an analogous way to *T. pallidum*, might cause dementia, plaque- and tangle-like lesions, A β deposition and consequently might be involved in the pathogenesis of AD. To detect all types of spirochete, neutral techniques need to be used (Ref. 146). Using dark-field microscopy, spirochetes were detected in the cerebrospinal fluid (CSF), in the blood and in the brain in 14 definite AD cases tested (Table 1). Spirochetes were not found in 13 age-matched controls without any AD-type cortical changes (Ref. 146). Silver-stained helically shaped spirochetes were also detected by electron microscopy. Spirochetes were isolated from the cerebral cortex in these 14 AD cases (Table 1), and in three of them, they were cultivated from the brain in a selective medium for *B. burgdorferi* (Ref. 146). Spirochetes were detected and isolated from the brains of eight additional AD cases derived from another laboratory and in the blood of five living patients with clinically diagnosed AD-type dementia (Ref. 186; Table 1). Four healthy controls did not show spirochetes. Taxonomical analyses showed that the helically shaped microorganisms belong to the order *Spirochaetales* (Ref. 187). To ensure the consistency of these results, the detection of spirochetes was also performed using various other techniques, including histochemistry, dark-field microscopy, atomic force microscopy, electron microscopy and immunoelectron microscopy, immunohistochemistry using spirochete and bacterial peptidoglycan (PGN)-specific antibodies (Refs 146, 147, 149, 186, 187, 176, 175, 188, 189), and detection of specific and nonspecific bacterial DNA (Refs 149, 176). PGN is the building block of the cell wall of Gram-negative and Gram-positive bacteria; however,

mycoplasma and chlamydiae lack detectable PGN (Refs 190, 191). The morphology of spirochetes detected by spirochete- or PGN-specific antibodies is identical (Fig. 2; compare also Fig. 7 G and H of Ref. 175). PGN-immunoreactive spirochetes were detected in 32 definite AD cases and in 12 cases with mild or moderate AD-type cortical changes.

Other authors found no evidence of spirochetes in the brains of seven AD cases by dark-field or electron microscopy (Ref. 192). However, spirochetes were observed in the blood of one of 22 living patients with AD-type dementia (Table 1). The spirochete observed by these authors corresponded to the vegetative, regularly spiral form. They suggested that it could correspond to an oral spirochete. Whether the atypical, pleomorphic spirochetal forms, which commonly occur in infected tissues, blood and CSF (Refs 167, 175, 177, 193), were considered by the authors is not clear.

Periodontal pathogen *Treponemas*

Using molecular and immunological techniques, six of seven periodontal *Treponema* species, namely *T. socranskii*, *T. pectinovorum*, *T. denticola*, *T. medium*, *T. amylovorum* and *T. maltophilum*, were identified in the brains of AD patients using species-specific polymerase chain reaction (PCR; Table 1). At least one oral *Treponema* species was present in 14 of 16 AD brains, compared with 4 out of 18 controls (Ref. 147). *T. pectinovorum* and *T. socranskii* antigens were observed in 15 of 16 AD brains and in 7 of 18 controls. In the hippocampus and in the frontal cortex. Six different *Treponema* species were detected in one AD patient, five species in four, four or three species each in one AD case, and one species in seven AD brains. Two *Treponema* species were observed in one control and one species in the other three positive controls. These results reinforce previous observations (Ref. 146) and indicate that these periodontal pathogen spirochetes, in an identical way to *T. pallidum* and *B. burgdorferi*, have the ability to invade the brain, persist in the brain, and cause dementia, cortical atrophy and amyloid deposition.

Borrelia burgdorferi

The causative agent of Lyme disease is transmitted by the bite of infected ticks (Ref. 170). Neurological complications occur in about 15% of affected

Table 1. Detection of spirochetes in AD

	Material	Number	Method	AD	Control	Culture	Serology	Refs
All types of spirochete	Brain	27	DF, HC, IHC BI, CSF EM, AFM	14/14	0/13 ^a	14/14	ND (BI 4/5)	146,187
	Brain	12	DF	8/8	0/4 ^a	8/8	ND	186
	Blood	5	DF, Cult	5/5		ND	ND	186
	Brain	24	DNA-DAPI	20/20	0/4 ^a	ND	ND	176
			10 ^b		10/10 ^b			176
	Brain	54	IHC	32/32	0/10 ^a 12/12 ^c	ND	ND	188,189
						ND	ND	
	Brain	7	DF, EM	0/7		ND	ND	192
	Blood	28		1/22	0/6	ND	ND	192
	Total brain	102		64/71	0/31^d	$P = 4.8 \times 10^{-18}$ OR = 274 ^d 95% CI = 32–11 345		
Periodontal pathogen spirochetes	Brain	34	PCR, IHC	15/16	6/18	ND	ND	147
	Total brain	34		15/16	6/18	$P = 3.6 \times 10^{-4}$ OR = 30 CI = 2.8–1364		
<i>Borrelia burgdorferi</i>	Brain	2	IHC, Cult	1/1	0/1	+	ND	178
	Brain	1	DF, IHC, Cult	1/1		+	ND	199
	Brain	10	EM, IHC, Wbl	0/6	0/4	–	ND	185
	Brain	27 ^b	Cult, IHC, EM BI, CSF, ISH, Serol 16SrRNA	3/14 ^b	0/13 ^b	3/14	2/14	146,149
	Brain	1	IHC, Serol	1/1		ND	1/1	146
	Brain	10	PCR, Cult	0/10		–	ND	180
	Brain	30	PCR	0/15	0/15	ND	ND	181
	Brain	34 ^b	PCR, seq	5/16 ^b	1/18 ^b	ND	ND	147
	Brain	1	PCR	1/1		ND	1/1	179
	Brain	11	PCR, ISH	7/10	0/1	ND	1/1	183, 184
Total brain	127		19/75	1/52	$P = 2.9 \times 10^{-4}$ OR = 17 CI = 2–732			
All studies detecting spirochetes	Brain	214		102/143	6/71	$P = 1.5 \times 10^{-19}$ OR = 26 CI = 10–80		
	Brain, blood, CSF	247		108/170	6/77 ^d	$P = 1.1 \times 10^{-17}$ OR = 20 CI = 8–60		

Data reviewed in the literature with respect to the detection of all types of spirochetes using neutral techniques and the specific detection of periodontal pathogen Treponemas and Bb. Results of the statistical analysis are given for each group and for all studies together. AD indicates number of AD cases positive for spirochetes/number of AD cases analysed; Control indicates number of control cases positive for spirochetes/number of control cases analysed. AD, Alzheimer disease; BI, blood; CSF, cerebrospinal fluid; EM, electron microscopy; AFM, atomic force microscopy; DF, dark-field microscopy; HC, histochemistry (Warthin and Starry, Bosma-Steiner silver stain for spirochetes); IHC, immunohistochemistry; ISH, in situ hybridisation; PCR, polymerase chain reaction; Bb, *Borrelia burgdorferi*; Wbl, Western blot; *P* is exact value of the significance calculated by Fischer test; OR, odds ratio; CI, 95% confidence interval; +, positive; –, negative; ND, not determined; seq, sequencing.

^aControls without any AD-type changes.

^bCases from previous studies, which were subtracted when the total number of cases of studies was considered.

^cCases with mild or moderate AD-type cortical changes.

^dWhere the number of positive controls was zero, in order to calculate the OR and 95% CI, one positive case was added to the control group.

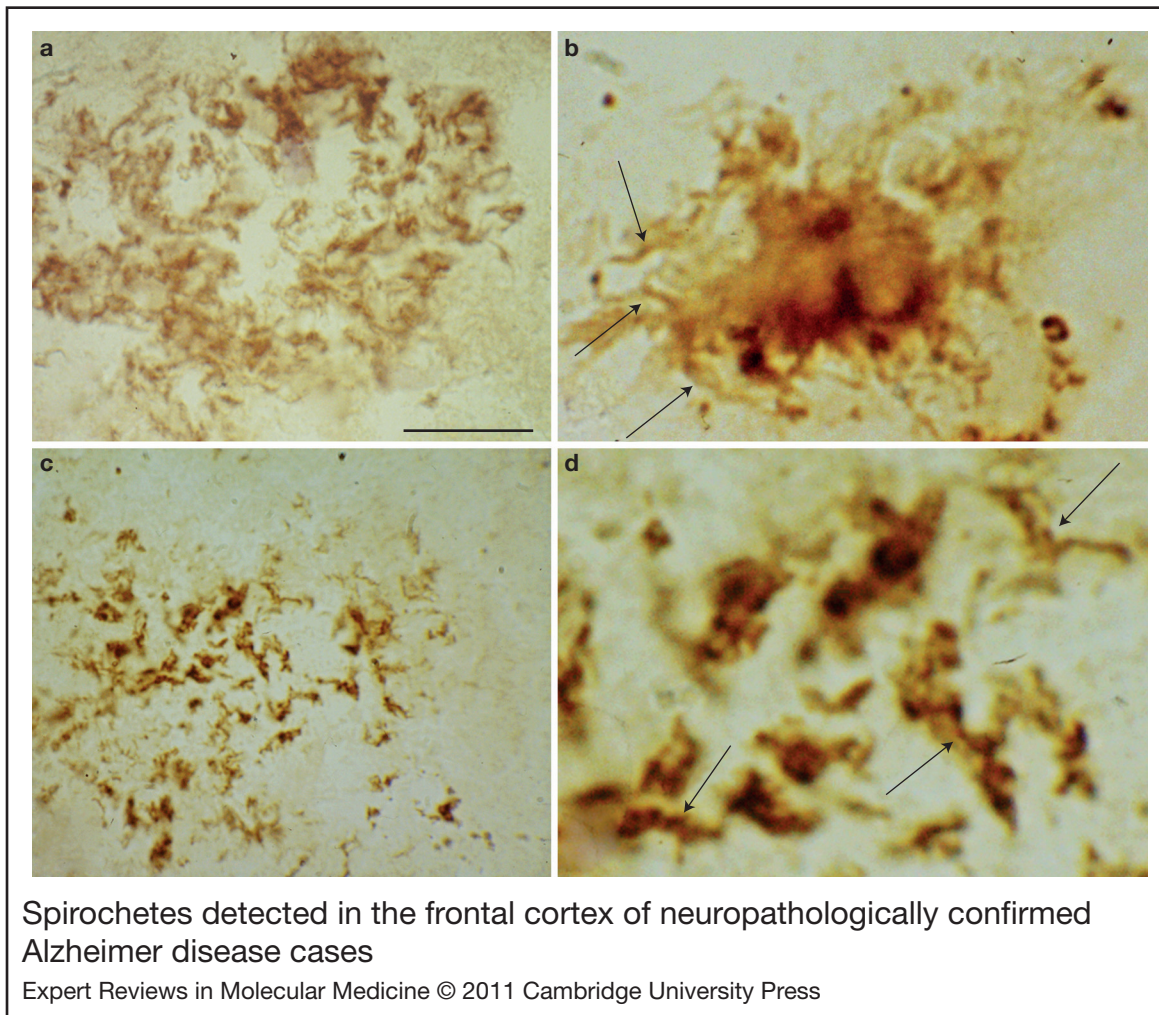


Figure 2. Spirochetes detected in the frontal cortex of neuropathologically confirmed Alzheimer disease cases. Spirochetes in an immature senile plaque detected by a cocktail of antibodies against *Borrelia burgdorferi* (a), in a mature plaque detected by in situ hybridisation (b, arrows) using *Borrelia*-specific probes, and in an amorphous plaque detected by antibacterial peptidoglycan antibody (c). (d) The central part of panel c at higher magnification. Arrows indicate helical spirochetes with the same morphology as observed in b. Scale bars: 80 µm (a), 30 µm (b), 80 µm (c), 20 µm (d). Images are from previously published studies: a,b (Ref. 149); c,d (Ref. 188).

individuals. Dementia and subacute presenile dementia both occur in Lyme disease (Refs 146, 149, 194, 195, 196, 197, 198). *B. burgdorferi* was first detected in the brains of two AD patients (Table 1) by MacDonald and Miranda (Ref. 178) and MacDonald (Ref. 199). This spirochete was detected in the cerebral cortex by dark-field microscopy and with a specific antibody against *B. burgdorferi*. This species was also detected and cultivated from the brains of three definite AD cases in an initial series of 14 AD cases (Ref. 146). Molecular characterisation, using 16S

rRNA gene sequence analysis, identified these spirochetes as *B. burgdorferi sensu stricto* (s.s.) (Ref. 149). Electron microscopy analysis confirmed that these spirochetes possess 10–15 endoflagella typical of *Borrelia* species. Two of the three AD patients had a positive CSF serology for *B. burgdorferi*, and the 31 kDa outer surface protein A (OspA) band, which is highly specific for *B. burgdorferi*, was detected by western blot in these three AD cases (Ref. 149). The pathological changes found in the brain were identical to those occurring in the atrophic

form of general paresis (Ref. 149). The cortical distribution of spirochetes in masses or colonies was identical to those of senile plaques, and the morphology of individual spirochetes was identical to those of curly fibres (Ref. 149). *B. burgdorferi* antigens colocalised with A β in cortical plaques and in leptomeningeal and cortical arteries containing amyloid deposits. Neurofibrillary tangles were also immunoreactive for *B. burgdorferi*. *OspA* and flagellin genes were detected in AD-type lesions using in situ hybridisation (ISH) (Fig. 2b). In additional AD patients with concurrent Lyme neuroborreliosis, *B. burgdorferi*-specific antigens (Ref. 146) and DNA (Ref. 179) were observed. Using species-specific PCR, *B. burgdorferi* DNA was detected in 5 of 16 AD patients tested and in 1 of 18 controls, all of which also had oral *Treponema* spirochetes (Ref. 147). Finally, *B. burgdorferi*-specific DNA was detected by both ISH and PCR in the hippocampus in 7 of 10 pathologically confirmed AD cases (Refs 183, 184) (Table 1).

Pappolla and colleagues (Ref. 185) failed to detect *B. burgdorferi* in six AD patients. They stated that they could not exclude other spirochetes not detected by their methods. In all other studies where *B. burgdorferi* was not detected, evidence is lacking on whether the analysed AD patients had Lyme neuroborreliosis or not (Refs 180, 181) (Table 1). Similarly, the analysis of the serology of *B. burgdorferi* alone, as a result of the low incidence of Lyme dementia compared with AD, might give false-negative results (Refs 180, 182). To demonstrate the role of *B. burgdorferi*, AD patients with Lyme neuroborreliosis should be analysed.

Taken together, these observations show that various authors detected and cultivated various types of spirochetes from the brains of AD patients. Coinfection with several types of spirochetes occurs.

Chlamydomytila pneumoniae

Several authors reported the existence of an association between *C. pneumoniae*, an obligate intracellular respiratory pathogen, and AD (Refs 144, 200, 201, 202, 203) (Table 2). *C. pneumoniae*-specific DNA was detected in the brains in 90% of sporadic AD patients and in 5% of controls (Ref. 144) (Table 2). Two mRNAs, encoding KDO transferase and a 376 kDa protein, specific to *C. pneumoniae*, were also

identified in frozen AD brain tissue by reverse transcriptase-PCR (RT-PCR).

Immunohistochemical analyses of AD brains showed *C. pneumoniae* in microglia, perivascular macrophages and astrocytes, and in about 20% of neurons (Refs 144, 200, 201, 203, 207, 211). They were commonly found in brain regions showing the characteristic neuropathology of AD (Refs 144, 200, 201). The presence of *C. pneumoniae* in the brains of AD patients was also confirmed by immunoelectron microscopy using specific monoclonal antibody against the outer membrane protein of *C. pneumoniae*. Electron and immunoelectron microscopy identified both chlamydial elementary bodies and reticulate bodies (RBs) (Refs 212, 144). That the replicative RB form was also detected in glial cells, neurons and pericytes indicates that a viable and transcriptionally active form of the microorganism is present in these cells (Refs 201, 213, 214, 215). Pleomorphic forms of *C. pneumoniae* were also observed (Ref. 213). *C. pneumoniae*-specific DNA was detected in the CSF in a significantly higher number of cases in AD patients (43.9%) than in controls (10.6%) (Ref. 202). *C. pneumoniae* was cultured from various brain samples of AD patients originating from different geographic regions of North America (Refs 144, 201, 215) and was also isolated from the CSF (Ref. 202). Tor-1 and Phi-1 isolates were characterised by PCR assays targeting *C. pneumoniae*-specific genes *Cpn0695*, *Cpn1046* and *tyrP*. Two groups, using paraffin-embedded brain samples, failed to detect *C. pneumoniae* in AD or in controls by PCR (Refs 216, 217). In another study, *C. pneumoniae* was detected in 2 of 15 AD cases and in 1 of 5 controls (Ref. 204).

Other bacteria

Propionibacterium acnes, an atypical anaerobic bacterium, was identified in biopsy specimens of the frontal cortex in three of four AD patients and in one of five controls with cerebral tumour. The *P. acnes* positive control was an elderly patient with cardiovascular risk factors and glioblastoma (Ref. 205). *P. acnes* was identified by microbiological methods and by gas chromatography. The bacterium was cultivated from frontal cortical biopsy specimens in Schaedler blood agar, at 35°C, under anaerobic conditions (Refs 205, 206). *P. acnes* has long been considered to be a commensal bacillus of the

Table 2. Detection of *Chlamydomypha pneumoniae* and other bacteria in AD

	Material	Number	Method	AD	Control	Refs
<i>C. pneumoniae</i>	Brain	38	PCR, EM, IHC, RT-PCR, Cult	17/19	1/19	144
	Brain	25	PCR, IHC	0/25		216
	Brain	20	PCR, IHC	0/20		217
	Brain	20	PCR, Cult	2/15 ^a	1/5 ^a	204
	Brain	21	PCR, ISH	21/21	0/1	200
	Brain	52	PCR, Cult, RT-PCR	20/25	3/27	201
	CSF	104	PCR, Cult	25/57	5/47	202
	Total brain	177	$P = 4.5 \times 10^{-7}$ OR = 8.7 CI = 3.1–29.5	60/125	5/52	
	Brain and CSF	281	$P = 9.8 \times 10^{-11}$ OR = 7.8 CI = 3.7–17.8	85/182	10/99	
<i>Propionibacterium acnes</i>	Brain	3	Cult	3/4	1/5	219, 220
<i>H. pylori</i>	Stomach	80	HC	44/50	14/30	225

AD, number of AD cases with positive detection/number of AD cases analysed; Control, number of control cases with positive detection/number of control cases analysed; CSF, cerebrospinal fluid; PCR, polymerase chain reaction; RT-PCR, reverse transcriptase-PCR; EM, electron microscopy; IHC, immunohistochemistry; Cult, culture. *P*, exact value of significance following Fischer test; OR, odds ratio; CI, 95% confidence interval values. ^aPositive in at least one of several samples.

skin. Recent observations showed that *P. acnes*, the causative agent of acne vulgaris, is implicated in various infections, including brain abscesses, endocarditis, endophthalmitis and osteomyelitis (Refs 208, 209). *P. acnes* was also shown to be a predominant periodontal pathogen (Ref. 218). By haematogenous dissemination, it can reach and infect various organs, including the brain. Stabilisation of the clinical symptoms and memory improvement were observed in two AD cases treated with *P. acnes*-sensitive cephalosporine combined with enalapril and oestrogen (Ref. 206). The author pointed to microangiopathy as the underlying pathology (Refs 219, 220). Because the number of AD cases analysed is low, further studies should be encouraged to determine any association of *P. acnes* with AD.

Actinomyces have also been suggested to be involved in AD, with an incidence four times

higher than in other pathological conditions (Ref. 221). Ultrastructural analysis revealed that the fibronectin-immunopositive fibrillary lesions in senile plaques, which were negative for neuronal, glial and macrophage markers, are compatible with filamentous microorganisms and might correspond to *Actinomyces* (Ref. 221). It is noteworthy that *Actinobacillus actinomycetemcomitans* is a frequent periodontal pathogen (Ref. 222) and that *Nocardia asteroides* was reported to cause Parkinson-like symptoms in experimental animals (Ref. 223).

Finally, the causative agent of stomach ulcers, *H. pylori* (Ref. 125), has also been suggested to be associated with AD (Refs 224, 225, 226). Serum IgG and IgA antibodies against *H. pylori* occurred in a higher percentage in the group of 30 AD patients compared with 30 controls (Ref. 224). The difference is statistically significant as determined by post-hoc

comparison (Ref. 210). An almost twofold higher prevalence of gastric *H. pylori* infection was observed in clinically diagnosed AD patients and in patients with mild cognitive decline (Refs 225, 227) than in controls. *H. pylori*-specific IgG antibody levels were also significantly higher in the blood and CSF of AD patients than in controls (Ref. 226). Additional studies would be of interest in detecting whether *H. pylori* is present in the brain and to analyse the possibility of a causal relationship between *H. pylori* and AD.

Herpes simplex virus type-1 (HSV-1) and other viruses

HSV-1 is a common neurotropic virus that infects around 70% of the population after the age of 50 (Refs 150, 228, 229, 230). HSV-1 DNA was detected (Table 2) in brain samples using ISH in

some elderly patients with dementia (Ref. 231). Three other studies failed to detect HSV-1 by ISH in AD or in control brains (Refs 232, 233, 234, 235). Increasing numbers of recent observations have detected HSV-1 DNA in the brain in AD (Refs 150, 151, 228, 229, 236, 237, 238). Using PCR, Jamieson et al. (Ref. 150) observed HSV-1 DNA in the brains of a high proportion of elderly subjects, with or without AD, which was absent or less frequent in young controls (Ref. 228). Several authors showed that HSV-1 is a significant risk factor (Table 3) when present in AD patients who are carriers of *APOE* ε4 (Refs 236, 237, 238), but this is not supported by Beffert et al. (Refs 239, 240). In situ PCR showed that HSV-1 DNA is localised to senile plaques (Ref. 243). Ninety per cent of senile plaques in AD and 80% in normal ageing subjects contained viral DNA (Ref. 241).

Table 3. Detection of HSV-1 in AD

Material	Number	Method	AD	Control	P value	Refs
Brain	6	ISH	2/3	1/3	0.423	231
Brain	5	ISH	0/3	0/2		232
Brain	23	ISH	0/18	0/5		233
Brain	13	ISH	0/8	0/5		234
Brain	6	ISH	0/4	0/2		235
Brain	14	PCR	8/8	6/6		150
Brain	36 + 10	PCR	14/21	9/15 (0/5 middle aged; 0/5 young)		228
Brain	155	PCR	73/98	41/57	0.485	229
Brain	90	PCR	36/46	28/44	0.295 (<i>P</i> < 0.0001, <i>APOE</i> ε4)	236
Brain	69	PCR	14/46	5/23	0.295 (<i>P</i> < 0.0047, <i>APOE</i> ε4)	237
Brain	109	PCR	45/61	30/48	(<i>P</i> < 0.0001, <i>APOE</i> ε4)	238
Brain	110	PCR	54/74	26/36	0.82	239, 240
Total brain	556		210/344	118/212	P = 0.2152 OR = 1.25 CI = 0.9–1.8	

Number of Alzheimer cases with positive detection/number of Alzheimer cases analysed; Control, number of control cases with positive detection/number of control cases analysed. *P*, value of significance following Fischer test; OR, odds ratio; CI, 95% confidence interval values; +, positive; -, negative; AD, Alzheimer disease; CSF, cerebrospinal fluid; HSV-1, herpes simplex virus type-1; IHC, immunohistochemistry; ISH, in situ hybridisation; PCR, polymerase chain reaction; *APOE* ε4: epsilon 4 allele of apolipoprotein E.

Anti-HSV-1 antibodies as detected in the CSF by enzyme-linked immunosorbent assay (ELISA) were also significantly higher in AD patients than in younger controls, but without a significant difference between the AD and age-matched control groups (Ref. 242). Increased titres of HSV-1 IgGs, which characterise past infection, were observed in AD cases and age-matched controls, without a significant difference between the two groups (Refs 243, 244). In a large prospective study, in addition to HSV-1 IgG, the presence of IgM, which characterises active primary infection or reactivation of the infection, was also assessed in the sera of 512 elderly patients, initially free of dementia. During 14 years of follow-up, 77 AD cases were diagnosed. In contrast to IgG, IgM-positive subjects showed a significantly higher risk of developing AD, indicating that reactivation of HSV-1 seropositivity is correlated with AD (Ref. 245).

Other herpes viruses were also detected in the brain using PCR: human herpes virus 6 (HHV6) types A and B, herpes simplex virus type-2 (HSV-2) and cytomegalovirus (CMV). HHV6, HSV-2 and CMV were observed in 70%, 13% and 36% of AD patients and in 40%, 20% and 35% of controls, respectively. The differences between the groups were not statistically significant (Ref. 246).

In addition to CMV, a possible association between HLA-BW15 and AD has also been reported (Ref. 247). A recent study revealed that elderly subjects with high levels of antibody against CMV develop more severe cognitive decline over 4 years (Ref. 248) compared with controls.

The adenovirus early region 1A (E1A) gene and its expression using ISH and immunohistochemistry were analysed in five AD cases and in two controls. Reactive microglial cells in both AD (5/5) and control brain tissue (2/2) showed positive hybridisation and immunoreactive expression of adenovirus E1A, indicating a monocyte- or microglia-mediated entry of adenovirus into the central nervous system (CNS) (Ref. 249).

Borna disease virus (BDV) was linked to affective disorders and schizophrenia (Refs 139, 142). A few attempts have been made to analyse the prevalence of BDV antibodies and BDV p40 gene coding sequences in AD (Refs 250, 251, 252), which did not show an association (Table 3). Following Borna-virus-induced

infection in APP(Tg2576) transgenic mice, a reduction of cortical and hippocampal A β deposits was observed (Ref. 253). One explanation is that the stimulation and activation of microglia might produce this effect.

The human immunodeficiency virus type-1 (HIV-1) (Ref. 254) is able to induce the biological hallmarks of AD; however, the virus is present in the brains of mostly young patients suffering from acquired immune deficiency syndrome (AIDS). The virus invades mostly macrophages and glial cells and the virus itself does not reproduce the pathological hallmarks of AD. However, by affecting immune defences, HIV-1 facilitates infection by various pathogens, including, among others, HSV-1, CMV, *C. pneumoniae* and spirochetes (Refs 255, 256, 257, 258).

Correlates of infection risk and AD

Based on the data available on the association of spirochetes, *C. pneumoniae* and HSV-1 with AD, contingency tables were used to analyse the strength of the association and the risk of infection in AD. In those studies where all types of spirochetes were detected using neutral techniques, spirochetes were observed in the brain in 90.1% (64/71) of AD cases and were absent in controls without any AD-type changes. This difference is significant (Table 1), and the association remains significant when cases where spirochetes were analysed in the blood are also included. The association between periodontal pathogen spirochetes and AD is also statistically significant. They were detected in the brain in 93.7% of AD cases and in 33.3% of controls. *B. burgdorferi* was about 13 times more frequent in AD cases than in controls, a statistically significant difference. It is noteworthy that *B. burgdorferi* was detected in all AD cases with a positive serology or where spirochetes were cultivated from the brain (Table 1). Taken together, in all studies where spirochetes or their specific species (periodontal *Treponema* spirochetes or *B. burgdorferi*) were detected in the brain, it can be concluded that the frequency of spirochetes is more than eight times higher in AD cases (90/131; 68.7%) than in controls (6/71; 8.41%). That spirochetes were cultivated from the brains of AD patients indicates that viable spirochetes are present in advanced stages of dementia. They can sustain persisting infection and inflammation and cause neuronal destruction (Ref. 148).

The frequency of *C. pneumoniae* was about five times higher in AD cases (60/125; 48%) than in controls (5/52; 9.6%). The difference remains significant when those cases where *C. pneumoniae* was detected in the CSF were also included (Table 1). That *C. pneumoniae* was cultivated from the brain (Refs 200, 213) and CSF (Ref. 202) and that replicative RBs were present in glial cells, neurons and pericytes in AD indicate that this microorganism is also present in a viable, active form in the brain in AD.

There is no significant difference between the frequency of HSV-1 detected in the brain in AD patients compared with age-matched control. However, a significant difference was observed between *APOE* ϵ 4-positive and -negative AD carriers, as reported by several authors (Refs 236, 237, 238, 259, 260), except for Beffert and colleagues (Refs 239, 240). A significant association of AD with ongoing or reactivated HSV-1 infection was reported (Ref. 245).

Evidence for a causal role of pathogens in AD

Additional studies have brought further evidence in favour of a probable causal relationship between spirochetes, *C. pneumoniae*, HSV-1 and AD. Exposure of primary mammalian neuronal and glial cells to spirochetes, namely to *B. burgdorferi*, which can be cultivated and propagated in pure culture, generated thioflavin-S-positive and $A\beta$ -immunoreactive amyloid plaques and tangle- and granulovacuolar-like formations in vitro (Refs 175, 261). In situ, in the spirochete-induced $A\beta$ plaques, synchrotron infrared microspectroscopy analysis revealed the presence of a β -pleated sheet conformation (Ref. 261). Spirochete-induced increases in $A\beta$, APP and phosphorylated tau were all detected by western blot in infected cell cultures (Ref. 261). This additional experimental evidence indicates that spirochetes are able to induce an AD-type host reaction and reproduce the defining pathological and biological hallmarks of AD (Refs 175, 261). Similar in vitro studies performed on CNS organotypic cultures, which aim to replace in vivo experiments, showed identical results (Ref. 261). Reference *B. burgdorferi* spirochetes (strain B31) and those cultivated from the AD brain (strains ADB1 and ADB2) invaded neurons and glial cells and induced nuclear

fragmentation in vitro, indicating that the spirochetes cultivated from the brains of AD patients are invasive and cause neuronal and glial damage and apoptosis (Refs 175, 261). Spirochetes occur in both extra- and intracellular locations. They invade neurons in vitro, in primary neuronal cultures (Refs 175, 261), in the cerebral cortex and in the trigeminal ganglia of AD patients (Refs 146, 147, 148, 149, 175, 183, 184, 261). Historical observations and illustrations showing that chronic spirochetal infection can reproduce the clinical, pathological and biological hallmarks of AD strongly support a causal relationship between spirochetal infection and AD (Refs 114, 115, 124). All these observations indicate that various types of spirochetes, including *B. burgdorferi* and several periodontal pathogen spirochetes, in an analogous way to *T. pallidum*, can cause dementia, cortical atrophy and the pathological and biological hallmarks of AD.

Astrocytes and microglia infected in vitro with *C. pneumoniae* display inclusions that are indistinguishable from those characteristic of active infection of the standard HEP-2 host cell line (Refs 214, 215). It was reported that chronic or persistent infection of CNS cells with *C. pneumoniae* can affect apoptosis in AD, in both a pro- and antiapoptotic manner (Refs 203, 214, 215). Furthermore, infection of BALB/c mice by intranasal inhalation of *C. pneumoniae* initiated $A\beta_{1-42}$ deposits in the brain that resembled senile plaques (Ref. 262). Antibiotic treatment following *C. pneumoniae* infection limited the number of induced amyloid plaques in vivo (Ref. 263).

The glycoprotein B (gB) of HSV-1 has a highly homologous sequence to a fragment of $A\beta$ (Ref. 264). Synthetic peptides derived from this region accelerate fibrillar aggregation of $A\beta$ in vitro. They can self-assemble into fibrils, which are ultrastructurally indistinguishable from $A\beta$ and are neurotoxic at a similar dose to $A\beta$. It was proposed that HSV-1 might act as a 'seed' for senile plaque formation (Ref. 265). It was also shown that HSV-1 is associated with APP during its anterograde transport, which might affect APP degradation and synaptic function (Ref. 265).

Exposure of cultured cells to HSV-1 results in increased intracellular $A\beta$ levels in neurons and glial cells as analysed by immunocytochemistry,

ELISA and western blot. Tau phosphorylation was also observed at a number of sites that are phosphorylated in AD (Refs 266, 267, 268). It was suggested that the association of viral DNA and senile plaques is very likely to be causal, because HSV-1 is able to increase the level of A β in neurons of infected mice in vivo (Ref. 266).

Evidence for underlying mechanisms
Sources and dissemination

Spirochetes, *C. pneumoniae* and HSV-1 are all able to invade the brain (Fig. 3) and generate latent and persistent chronic infection (Refs 144, 145, 146, 147, 148, 149, 228, 229, 269). The strong neurotropism of spirochetes is well known (Ref. 167). In addition to haematogenous dissemination, spirochetes can spread through the lymphatic system and along nerve fibre tracts (Ref. 167). Periodontal invasive spirochetes were detected in the trigeminal ganglia and along the trigeminal nerve (Ref. 147). They might also propagate along the fila olfactoria and tractus olfactorius, which

would enable them to reach the CSF, the septal and hippocampal regions in the earliest stages of the disease. This would be in harmony with the olfactory hypothesis (Refs 94, 95, 96) and the early involvement of the olfactory tract and bulb (Ref. 97).

Through infected circulating monocytes, *C. pneumoniae* can also spread by haematogenous dissemination and, by crossing the blood–brain barrier, infect the brain. *C. pneumoniae* is an upper respiratory tract pathogen and can reach the brain through the olfactory system (Ref. 95). Intranasal inhalation of *C. pneumoniae* initiated plaque-like A β_{1-42} deposits in the brain in BALB/c mice, and *C. pneumoniae*-specific DNA was detected by PCR in the olfactory bulb in AD (Ref. 262).

HSV-1 infection might also reach the brain through the olfactory system, by infection of cranial and peripheral nerves and their ganglia and through haematogenous dissemination (Refs 270, 271). The virus can reside in the brain

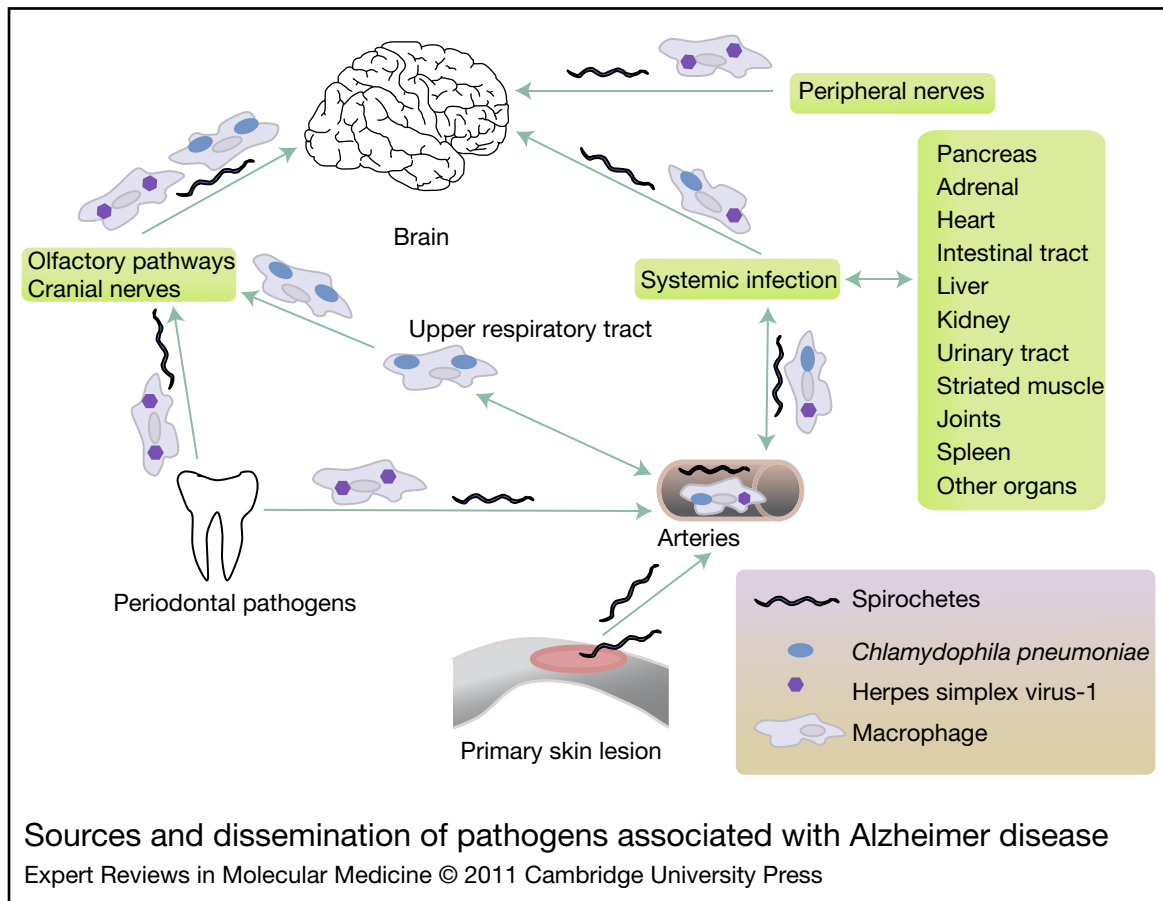


Figure 3. Sources and dissemination of pathogens associated with Alzheimer disease.

Emerging roles of pathogens in Alzheimer disease

in a latent form and be reactivated by peripheral infection, stress or immunosuppression (Ref. 268).

Neuroinflammation and TLR signalling

Persisting, poorly degradable bacterial remnants in mammalian tissues act as chronic inflammatory stimuli (Refs 272, 273, 274). Lipopolysaccharides (LPSs), PGN and various bacterial lipoproteins elicit a variety of proinflammatory responses and might represent an important source of inflammation in AD. Complex interactions between the innate and adaptive immune systems have a major role in infection (Ref. 275). The functions of innate immunity allow host cells to recognise most microorganisms, execute proinflammatory defences and start adaptive immune responses.

Bacteria attach to host cells through a variety of cell-surface components, including surface amyloid proteins, which interact with host proteases (Refs 276, 277, 278, 279, 280, 281, 282, 283). Proteolysis of the extracellular matrix allows bacteria to penetrate the basement membrane and invade host cells.

It is the innate immune system that provides the first line of defence against microorganisms. Pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs), recognise unique structures of invading microorganisms (Ref. 284). Patients with genetic defects related to signalling pathways activated by TLRs frequently suffer from severe recurrent infection (Refs 285, 286, 287, 288, 289). In the absence of TLRs, death from experimental sepsis is significantly enhanced (Ref. 287). Various bacteria, including spirochetes, activate TLR signalling and interact with CD14 (Refs 290, 291, 292). *T. pallidum* and *B. burgdorferi* or their synthetic membrane lipoproteins are major inflammatory mediators (Refs 293, 294) and activate both the classical and alternative complement pathways, which opsonise and kill pathogens without the need for antibody.

Complex interactions between pathogen-associated molecular patterns (PAMPs), PRRs and TLR signalling pathways have a major role in linking innate and adaptive immunity and maintaining pathogen-free host tissues (Ref. 275). TLRs activate two major signalling pathways (Fig. 4). The core pathway activated by most TLRs leads to the activation of transcription factor nuclear factor-kappa B (NF- κ B) and the mitogen-activated protein kinases

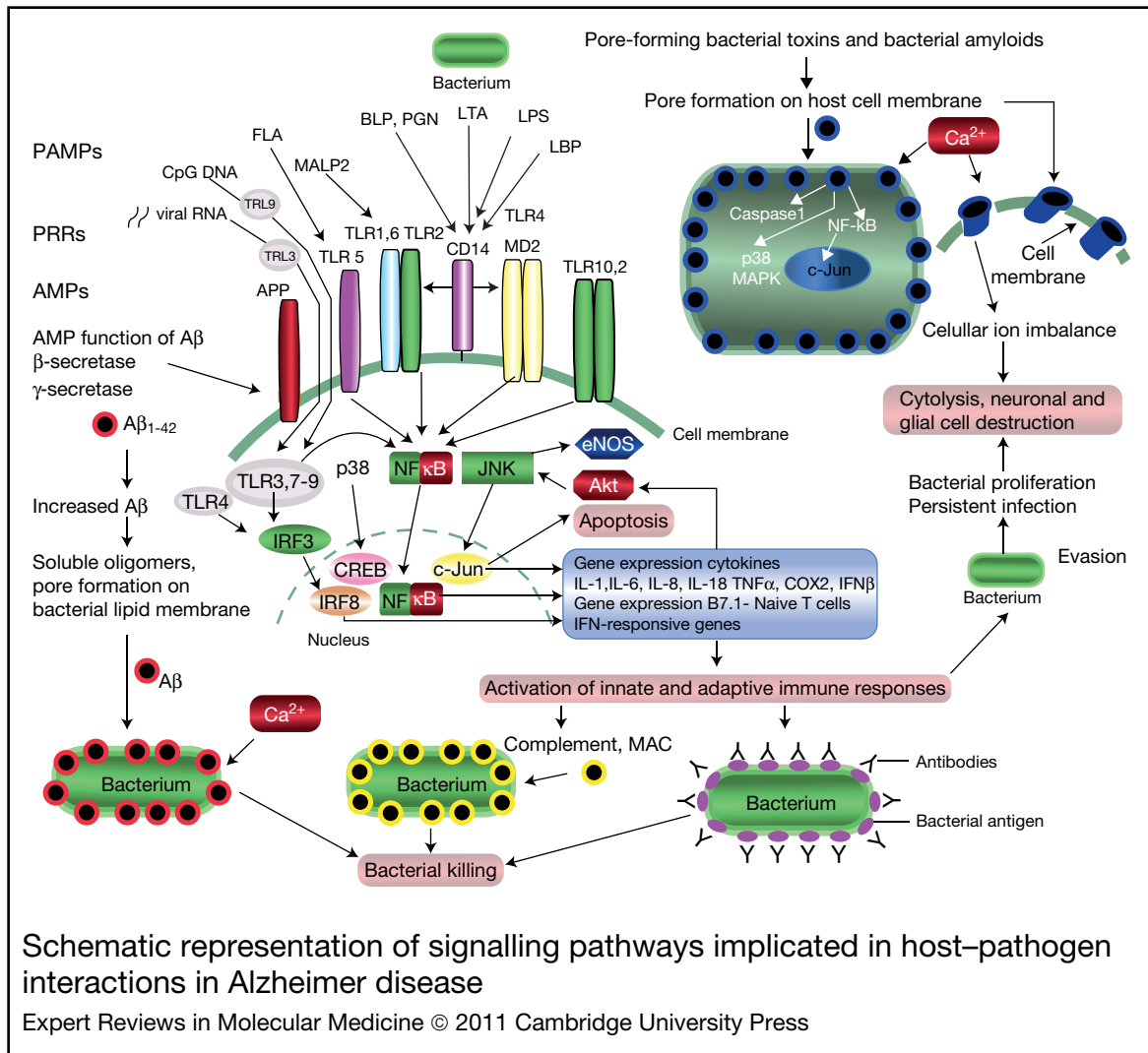
(MAPKs) p38 and Jun kinase (JNK). The second pathway is activated by TLR3 and TLR4 and results in the activation of both NF- κ B and interferon regulatory factor-3 (IRF3), allowing the induction of another set of inflammatory genes, including the antiviral interferon- β gene (*IFNB*).

Activation of p38 MAPKs during bacterial infection was also shown to be crucial in the local production of cytokines such as IL-8 (Ref. 295) and in the development of effective immune responses in vivo (Ref. 296). Toxin-induced p38 MAPK activation requires pore formation and is inhibited by the relief of osmotic stress.

Pore-forming toxins are the most common class of bacterial protein toxins and are often important virulence factors (Ref. 297). Pore-forming bacterial toxins are typically oligomers of soluble, monomeric proteins or peptides, which form transmembrane channels. Channel formation in the membrane of targeted cells, which triggers cellular ion imbalance, is a widely used form of bacterial attack (Refs 298, 299). These pore-forming bacterial toxins generate calcium-dependent and lipid-mediated signalling on host cell surfaces, leading to a variety of events such as tyrosine phosphorylation (Ref. 300), actin rearrangement (Ref. 301), NF- κ B activation (Ref. 302) and regulation of gene expression through histone modification (Ref. 303).

Mammals also use bacterial porin-like proteins such as perforins as part of their innate immune defences (Ref. 304). A β ₁₋₄₂ was shown to be an AMP that belongs to the innate immune system. Toxic oligomers of A β ₁₋₄₂ bind to lipid bilayers of bacterial membranes and enveloped viruses, triggering Ca²⁺ influx and bacteriolysis or viral destruction. Deregulation of the balance between host cell destruction by pathogens and pathogen destruction by the host immune systems will influence the outcome of infections.

The innate and alternative pathways through the common membrane attack complex (MAC, C5b9) cause bacteriolysis (Refs 305, 306, 307). Cellular and humoral components of the immune system reactions are both associated with AD (Refs 98, 99, 100, 101, 102). The adaptive immune system kills pathogens through the formation of specific antibodies directed against invading microorganisms. Monocytes, macrophages and microglia



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Figure 4. Schematic representation of signalling pathways implicated in host-pathogen interactions in Alzheimer disease. Pattern recognition receptors (PRRs) recognise conserved structural components of microorganisms, called pathogen-associated molecular patterns (PAMPs) or ligands, which include peptidoglycan (PGN), lipoteichoic acid (LTA), flagellin (FLA), bacterial lipoprotein (BLP) and nucleic acid structures, such as bacterial CpG DNA or viral RNA, unique to bacteria and viruses. Lipopolysaccharide (LPS) is recognised following its binding to lipoprotein binding protein (LBP). Only Toll-like receptors (TLRs) have a cytoplasmic domain for signal transduction. Plasma-membrane-localised TLRs include TLR1, TLR2, TLR4, TLR5, TLR6 and TLR10, whereas endosomal TLRs include TLR3, TLR7, TLR8 and TLR9. TLR9 is responsible for the recognition of CpG islands of bacterial DNA. Complex interactions between pathogen-associated molecular patterns (PAMPs), pattern recognition receptors (PRRs) and TLR signalling pathways have a major role in linking innate and adaptive immunity and maintaining pathogen-free host tissues. Mammals use antimicrobial peptides (AMPs) as part of their innate immune defences to destroy invading pathogens. In contrast, in a similar way, pore-forming bacterial surface proteins are also able to destroy host cells. Evasion of pathogens from destruction by the host immune defences will result in chronic persistent infection and host cell destruction. APP, amyloid precursor protein; Aβ, amyloid beta; CD14, cluster of differentiation 14; NF-κB, nuclear factor-kappa B; MALP2, mycoplasma diacylated lipopeptide 2; p38 MAPK, mitogen-activated protein kinase; JNK, c-Jun kinase; MAC, membrane attack complex; MD2, lymphocyte antigen 96; Akt, serine/threonine protein kinase Akt1; IRF, interferon regulatory factor; IL, interleukin; TNF, tumour necrosis factor; COX2, cyclooxygenase 2; IFN, interferon, CREB, cAMP response element binding.

participate in both the innate and adaptive immune responses. When activated, they secrete chemokines and cytokines and express various proinflammatory molecules needed for the efficient removal of pathogens and damaged cells.

Neuroinflammatory responses that include activation of glia with marked elevation of cytokine expression, in particular elevation of IL-1, are observed in AD, in Down syndrome fetuses and children before plaque formation, and also in HIV infection (Refs 99, 308, 309). IL-1 directs both the in vivo (Ref. 310) and in vitro synthesis of APP (Ref. 311), thus favouring senile plaque formation in AD. IL-1 upregulation of ApoE promotes ApoE-mediated induction of APP (Ref. 312) and MAPK-p38-mediated tau phosphorylation, in part by upregulation of MAPK-p38 synthesis and activation and by favouring the formation of neurofibrillary tangles (Refs 313, 314, 315). IL-1 also influences neurotransmission because it increases the synthesis and activation of AChE (Ref. 316) and induces neuron loss by increasing caspase 1 (IL-1 β cleavage enzyme) activity (Refs 317, 318). Microglial activation by APP and release of secreted APP (sAPP) modulated by apolipoprotein E also have an important role in the degenerative process of AD (Refs 313, 319). Activation of microglia with TLR2, TLR4 and TLR9 ligands increases A β ingestion in vitro (Ref. 320). Furthermore, stimulation of the immune system through TLR9 in APP transgenic (Tg2576) mice reduces A β deposition (Ref. 321).

Evasion and establishment of latent and chronic infection

The ability of spirochetes and *C. pneumoniae* to evade destruction by host immune systems and establish chronic infection is well known. HSV-1 can also remain in a latent state and persist throughout life in human tissues. In the latent state, the viral genome is present, but no viral particles are produced. Viral gene expression during latency is limited to one locus – the gene encoding the latency-associated transcript (Ref. 151).

Pathogens use a broad range of strategies to overcome antigenic recognition, complement lysis and phagocytosis. Blockade of the complement cascade or acquisition of host-derived complement inhibitors results in their

evasion from complement lysis. This allows microbial survival and proliferation even in immune-competent hosts. Complement-resistant strains of *B. burgdorferi* possess five complement regulatory acquiring surface proteins, which bind host complement inhibitors (Refs 307, 322) and a CD59-like molecule (Refs 322, 323). A fragment of HSV-1 glycoprotein C (gC) shares sequence similarity with host complement receptor 1 (CR1), showing that viruses can also escape from attack by the MAC. Bacteria and viruses protect themselves from destruction by the host adaptive immune system. *B. burgdorferi* induces IL-12, a cytokine recently recognised to be critical for driving cellular responses towards the Th1 subset of T helper cells (Refs 293, 324). This shift retards antibody induction by Th2 cells against bacteria. These various ways of evasion allow bacteria to survive and proliferate in host tissues and sustain chronic infection.

Iron and nitric oxide

Bacterial cell wall components, including LPSs and PGNs, are highly resistant to degradation by mammalian enzymes and persist indefinitely in mammalian tissues. During chronic exposure, bacteria and bacterial debris accumulate in infected host tissues, sustaining chronic inflammation and slowly progressive cell damage (Refs 272, 273, 274, 325, 326). Bacteria, LPSs and PGNs have a variety of biological actions in mammals (Ref. 327). They not only are inflammatory cytokine inducers and activators of complement pathways, but they also affect vascular permeability (Ref. 328), induce nitric oxide and free radicals, inhibit DNA synthesis, and cause apoptosis and cellular damage (Ref. 329).

Macrophage regulation of immune surveillance involves iron depletion (Refs 330, 331, 332). Lactoferrin, which is similar in structure to transferrin, has a role in natural defence mechanisms in mammals and is upregulated in neurodegenerative disorders. Lactoferrin exerts its anti-inflammatory action by inhibiting hydroxyl radical formation. This antioxidant property prevents DNA damage (Ref. 329). By contrast, free iron abolishes the bactericidal effects of serum and strongly enhances infection and bacterial virulence (Refs 329, 330, 331, 332). Iron has been shown to increase the formation of reactive oxygen intermediates, resulting in

lipid peroxidation and subsequent oxidative damage of proteins and nucleic acids (Refs 330, 331, 332). By affecting T-cell generation, iron also influences antigen-specific cellular responses, T-cell functions and the production of proinflammatory cytokines by macrophages (Ref. 333). Iron, which has a fundamental role in infection, also accumulates in senile plaques in AD (Refs 330, 334, 335, 336).

Activation of macrophages and other host cells by bacteria or LPS causes inducible nitric oxide synthase (iNOS) synthesis, which in turn generates substantial amounts of nitric oxide from the amino acid L-arginine (Ref. 332). Nitric oxide is a critical component in the clearance of bacterial, viral, fungal and parasitic infections (Ref. 337). In a mouse model, genetic disruption of iNOS was associated with a significantly higher risk of dissemination and mortality of infection (Ref. 338). Pathogens have also evolved a broad array of strategies to limit nitric oxide production (Refs 338, 339, 340), which might contribute to their evasion from host immune responses (Ref. 340). Nitric oxide also has a central role in chronic degenerative diseases, including AD (Ref. 341).

Amyloidogenesis

Amyloidogenesis is the aggregation of soluble proteins into detergent-insoluble filamentous structures about 10 nm wide and 0.1–10 µm long. These amyloid fibrils have distinct biochemical and biophysical properties, including resistance to proteinase K treatment, β-sheet structure and affinity for binding thioflavin S and Congo Red.

Chronic bacterial infections (e.g. rheumatoid arthritis, leprosy, tuberculosis, syphilis, osteomyelitis) are frequently associated with amyloid deposition. Cortical and vascular amyloid deposition in the atrophic form of general paresis, caused by a spirochete (*T. pallidum*), as in AD, corresponds to Aβ.

On the basis of previous observations, it has been suggested that amyloidogenic proteins might be an integral part of spirochetes and can contribute to Aβ deposition in AD (Ref. 146). It was shown that the BH(9–10) peptide on a β-hairpin segment of *B. burgdorferi* OspA forms amyloid fibrils in vitro that are similar to those in human amyloidosis (Ref. 342). Recent observations indicate that amyloid proteins constitute a previously overlooked integral part

of the cellular envelope of many bacteria (Refs 343, 344, 345, 346). Amyloid fibril formation not only results in toxic aggregates, but also provides biologically functional molecules (Refs 343, 344, 347). Bacterial amyloids are involved in bacterial cell–cell interactions, in their attachment to inert solid surfaces, and in spore and biofilm formation (Ref. 347). Microbial amyloids, through interaction with host proteases, also contribute to bacterial virulence, to colonisation of the host and to invasion of host cells.

Bacterial LPSs and PGNs are used worldwide to generate in vitro and in vivo experimental inflammation and amyloidosis (Ref. 348). LPSs induce Aβ accumulation, increased APP levels and hyperphosphorylation of tau in vitro and in vivo (Refs 261, 349, 350, 351). Increased APP mRNA in response to LPS was also detected in the basal forebrain and hippocampus of the rat (Ref. 349). Aβ deposition occurs in the brains of rats chronically infused with LPSs (Ref. 349). In a triple-transgenic mouse model of AD, repeated challenges with LPSs were shown to exacerbate CNS inflammation and to cause increased tau phosphorylation (Ref. 351).

Genetic factors

Host responses to bacterial infections are genetically controlled. Promoter polymorphisms in the genes of proinflammatory cytokines are associated with susceptibility to infection (Ref. 352). Tumour necrosis factor alpha (TNF-α) is a critical mediator of host defence against infection. Polymorphisms in the gene encoding TNF-α might determine a strong cell-mediated immune response or a weak or absent cellular response, which reflects the genetic variability in cytokine production (Refs 352, 353). In the absence of cell-mediated immune responses, the microorganism can spread freely and accumulate in infected host tissues (Ref. 354). Accordingly, in *Mycobacterium leprae* infection, two distinct phenotypes can be distinguished: tuberculoid leprosy and the lepromatous leprosy. In the tuberculoid or paucibacillary form, there is strong inflammatory infiltration and the number of microorganisms is low. Conversely, in the lepromatous or bacillary form, the inflammatory infiltrates are poor or absent and the number of *M. leprae* is high. A similar polarity in host reactions – the infiltrative form with strong cell-mediated

immune responses and few spirochetes versus the atrophic form, lacking lymphoplasmocytic infiltrates but with numerous spirochetes – occurs in response to *T. pallidum* and *B. burgdorferi* infection (Refs 114, 115, 118, 149). The influence of TNF- α polymorphism on spirochetal infections has also been demonstrated by others (Refs 355).

Class II major histocompatibility genes influence host immune responses to bacterial and viral infections. The major histocompatibility complex phenotype of the antigen-presenting cell can modulate Th1-like versus Th2-like cell activity against *M. leprae* and other pathogens. In general, it has been acknowledged that human leukocyte antigen (HLA) DR isotypes are associated with a protective response, whereas DQ isotypes are associated with the multibacillary lepromatous form with a limited cellular response. HLA gene polymorphism is a dominant marker of susceptibility to various infections, including *B. burgdorferi* infection (Ref. 356). The important role of the HLA system in controlling cell-mediated responses suggests that differences in HLA haplotypes could contribute to the wide spectrum of immune responses observed in leprosy and in other infections (Ref. 357). It is noteworthy that TNF- α and HLA polymorphisms, which are risk factors for infection, substantially influence the risk of AD (Refs 61, 358, 359, 360, 361, 362).

Association between AD pathology and IL-1 β expression patterns (Ref. 363), and between disease risk and IL-1 β gene polymorphisms, has been reported (Refs 62, 364). Genetic variation in the expression of microbial PRRs, including TLRs, and *CD14* gene polymorphisms predispose to various infections and are also associated with AD (Refs 365, 366).

Genetic mutations in *APP*, *PS1* and *PS2* are related to the processing of APP (Ref. 39). Because APP has an important role in the regulation of immune system reactions and in T-cell differentiation (Refs 16, 17, 18), genetic defects in such genes should also result in increased susceptibility to infection.

APOE $\epsilon 4$ enhances the expression of inflammatory mediators (Refs 367, 368) and has a modulatory function in susceptibility to infection by various bacteria, viruses and protozoa (Refs 367, 368, 369, 370, 371, 372). *APOE* genotyping of three AD cases, where *B.*

burgdorferi was detected and cultivated from the brain, showed that two of them were *APOE* $\epsilon 4$ carriers. The low number of cases does not allow any conclusion for an eventual link between spirochetal infection and the *APOE* $\epsilon 4$ allele (Ref. 149). Sixty-four per cent of AD cases with positive *C. pneumoniae* PCR had at least one *APOE* $\epsilon 4$ allele (Ref. 207). ISH and quantitative real-time PCR analyses indicated that the number of *C. pneumoniae*-infected cells and the bacterial load in affected brain regions of *APOE* $\epsilon 4$ carriers in AD were significantly higher compared with those lacking that allele (Ref. 200). Furthermore, HIV-1-infected subjects carrying the *APOE* $\epsilon 4$ allele have higher recorded levels of dementia (Ref. 369), and the $\epsilon 4$ allele has also been shown to modulate HSV-1 infection (Refs 240, 259, 260, 371).

Outstanding research questions and conclusions

The analysis of all positive and negative data available in the literature on the association of pathogens with AD indicates a statistically significant association between various types of spirochetes, *C. pneumoniae* and AD. There is no significant difference between the frequency of HSV-1 in AD cases compared with age-matched controls. However, several authors found a significant difference between the frequencies of HSV-1 in *APOE* $\epsilon 4$ carriers and noncarriers. The occurrence of positive anti-HSV-1 IgM was reported to be a risk factor for AD (for a recent review, see Ref. 373).

Lesions that are similar to senile plaques, neurofibrillary tangles and neuropil threads, and granulovacuolar degeneration, accumulation of A β , increased APP levels and phosphorylation of tau have all been induced by exposure of mammalian neuronal and glial cells and CNS organotypic cultures to spirochetes. A β -positive plaques were induced by inhalation of *C. pneumoniae* in mice in vivo, and exposure to HSV-1 increased the A β level and produced tau phosphorylation in neurons in vitro and in vivo.

Through TLRs and other PRRs, pathogens or their toxic components induce gene expression and activation of proinflammatory molecules by host cells. Both the classical and alternative complement pathways are activated in AD. MAC(C5b-9), which is intended to lyse bacteria or encapsulated viruses, and activated microglia that are designed to clean up debris and foreign

bacteria are both associated with cortical AD lesions.

Evasion of pathogens from host defence reactions results in sustained infection and inflammation. The microorganisms and their toxic components can be observed in affected brains, along with host immunological responses.

Spirochetes, *C. pneumoniae* and HSV-1 disseminate from the primary site of infection to the brain usually through systemic infection. As in syphilis, systemic infection and inflammation precede the development of dementia by years or decades. Detection of infection in its early, peripheral stage can hamper its dissemination to the CNS and prevent dementia. Consequently, peripheral infections can have a role in the initiation and progression of neurodegeneration in AD (Refs 146, 374, 375, 376). Worsening of peripheral and systemic infection will deteriorate CNS involvement. One example is periodontitis, which is caused mostly by Gram-negative bacteria, including periodontal spirochetes, which represents a risk factor for AD (Refs 156, 377).

It is important to consider that several types of spirochetes and several types of pathogens can occur in AD. Coinfection of *B. burgdorferi* with various periodontal spirochetes (Ref. 147) or with *T. pallidum* (Ref. 378) is well documented. *T. pallidum* frequently coinfects with other bacteria and herpes viruses in syphilis (Ref. 167). In Lyme disease, coinfection of *B. burgdorferi* with *C. pneumoniae* and HSV-1, which are also associated with AD, can be observed. Coinfection by several microorganisms can accelerate the degenerative process, exacerbate CNS damage and worsen dementia.

An infectious origin of AD is in harmony with recent observations showing that A β belongs to the group of AMPs, which are potent, broad-spectrum bactericides targeting Gram-negative and Gram-positive bacteria, enveloped viruses, fungi and protozoans (Ref. 31). A β has the capacity to associate with lipid bilayers of bacterial cell membranes and to exert antimicrobial activity by membrane permeabilisation and by alteration of calcium homeostasis (Refs 31, 379). The microtubule-binding site of tau protein also exhibits antimicrobial properties (Ref. 380).

Inflammation has a primordial role in the elimination of invading pathogens and infected host cells. If infection is eradicated, it helps the

healing process. Some proinflammatory molecules, such as cytokine IL-1 β , in addition to their harmful effects, have a beneficial and protective effect (Ref. 381). Therefore, long-term use of anti-inflammatory drugs alone might weaken the elimination of pathogens and facilitate their evasion, survival and slowly progressive proliferation. Combined antibiotic, antiviral and anti-inflammatory therapy is suggested as the treatment of choice.

In conclusion, the data available indicate that infectious agents can initiate the degenerative process in AD, sustain chronic inflammation, and lead to progressive neuronal damage and amyloid deposition. The accumulated knowledge, views and hypotheses proposed to explain the pathogenesis of AD fit well with an infectious origin of the disease. The outcome of infection is determined by the genetic predisposition of the patient, by the virulence and biology of the infecting agent, and by various environmental factors, such as exercise, stress and nutrition.

More attention and support is needed for this emerging field of research. Infection starts long before the manifestation of dementia; therefore, an adequate treatment should start early. Because antibacterial, antiviral and anti-inflammatory therapy is available, as in syphilis, one could prevent and eradicate dementia. The effect on the suffering of patients and on the reduction of healthcare costs would be considerable.

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Features associated with this article

Figures

Figure 1. Distribution of spirochetes in the atrophic form of general paresis and in the frontal cortex of an Alzheimer disease (AD) patient with Lyme neuroborreliosis.

Figure 2. Spirochetes detected in the frontal cortex of neuropathologically confirmed Alzheimer disease cases.

Figure 3. Sources and dissemination of pathogens associated with Alzheimer disease

Figure 4. Schematic representation of signalling pathways implicated in host–pathogen interactions in Alzheimer disease.

Tables

Table 1. Detection of spirochetes in AD.

Table 2. Detection of *Chlamydothya pneumoniae* and other bacteria in AD.

Table 3. Detection of HSV-1 in AD.

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