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Research article

Is Alzheimer's Disease Infectious?

Relative to the CJD Bacterial Infection Model of Neurodegeneration

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Abstract: Alzheimer's disease (AD) has been recently considered as a possible brain infection related to the Creutzfeldt-Jakob disease (CJD) transmissible dementia model. As with CJD, there is controversy whether the infectious agent is an amyloid protein (prion theory) or a bacterium. In this review, we show that the prion theory lacks credibility because spiroplasma, a tiny wall-less bacterium, is clearly involved in the pathogenesis of CJD and the prion amyloid can be separated from infectivity. In addition to prion amyloid deposits, the transmissible agent of CJD is associated with amyloids (A-β, Tau, and α-synuclein) characteristic of other neurodegenerative diseases including AD and Parkinsonism. Reports of spiroplasma inducing formation of α-synuclein in tissue culture and *Borrelia* spirochetes inducing formation of A-β and Tau in tissue culture suggests that bacteria may have a role in the pathogenesis of the neurodegenerative diseases.

Keywords: spiroplasma; Alzheimer's disease; prion; A-β; α-synuclein; prion, bacteria; Parkinson's disease; neurodegenerative disease; amyloid

1.   Introduction

Alzheimer's disease (AD) is presently a hopeless condition with cognitive deterioration spanning a decade in the patient's life. On rare occasion a more rapid form of AD is seen [1]. The diagnosis of AD is complicated by clinical similarities to other neurodegenerative diseases such as Lewy body disease and Creutzfeldt-Jakob disease (CJD) [2]; these conditions are usually more rapidly progressive and of shorter duration. Of these neurodegenerative conditions, CJD has been shown to be infectious, and approximately 13 percent of patients diagnosed with AD have CJD [3,4]. Recently there has been a resurgence of the idea that occurrence of AD is related to brain infections [5]. The revelation of AD being infectious would have significant impact on the therapeutic approach to AD and on epidemiological studies of the disease. A controversy has already arisen whether conventional pathogens are involved, or whether AD is a prion disease similar to CJD. In this review, the data supporting each side of the argument will be presented, relying on the CJD model of infection for comparison and determination of the best approach to investigate the exciting possibility that AD may be infectious.

2.   Is AD an Infectious Disease?

2.1.   Historical

The idea AD may be infectious comes from research on CJD. CJD was discovered to be experimentally transmissible to animals in the 1970s [6,7]. At that time, there was interest in whether AD was also infectious, but supporting data was lacking [6]. Then in 1988, Elias and Laura Manuelidis reported the transmission of AD to hamsters via blood from volunteers with family history of AD [8]. The Manuelidis had been conducting experiments on CJD transmission to mice [9], so their data showing that the blood buffy coats from five of the volunteers induced a spongiform encephalopathy in the hamsters similar to CJD was suspect. They concluded that the same organism was responsible for both CJD and AD, but their results were discredited by other neuroscientists who could not reproduce the data. My personal bias, as a practicing neuropathologist, was the Manuelidis had presented cases either contaminated with the transmissible agent of CJD from their laboratory studies of transmission of CJD [9] or from occurrence of the rarer CJD in association with the more common AD. Subsequently numerous examples of mixed CJD/AD cases have been presented at scientific meetings; AD changes are seen in 10.9% of CJD patients and 19.1% of control patients [10]. The neuropathology is distinctive for each condition [6]. AD may show some vacuolization of the neuropil beneath the pia over the parietal lobes, but the classic widespread perineuronal vacuolization of the neuropil of CJD in the lower third of the cortex is not seen in AD [6]. The case for combined diseases can easily be determined by recognizing the mating of these neuropathological patterns.

2.2. *AD is an inflammatory process*

Inflammation appears to be important in leading to neuronal cell death in neurodegenerative disease processes such as Parkinson's disease (PD), AD, prion disease (CJD), multiple sclerosis and HIV-dementia [11]. The inflammatory process in these conditions is mediated by activated microglia (immune cells of the central nervous system [CNS]), which respond to neuronal damage and phagocytize the damaged cells [12]. Microglia become less efficient in this scavenging as we age, with tendency for the microglia to become over-activated, therein causing too potent a reaction leading to further neuronal damage [11]. The pathological deposition of insoluble aggregates of amyloid-β (A-β) in AD activates microglia and initiates an inflammatory response [11,13]. These activated microglia release cytotoxic molecules such as pro-inflammatory cytokines, reactive oxygen intermediates, proteinases and complement proteins [12]. The IL-6 released by reactive microglia causes neuronal injury [14]. There are similarities between clinical and neuropathological features of AD and CJD such as progressive dementia, deposits of abnormally folded proteins in diseased tissue, and prominent neuronal loss. In both conditions there is generation of oxidative stress molecules and complement activation [14]. The phagocytic NADPH oxidase (NOX2) that plays a fundamental role in host defense and innate immunity is markedly up-regulated in microglia within affected brain regions of AD and CJD patients [15]. The endoplasmic reticulum (ER) responds to this by the activation of adaptive pathways, which is termed unfolded protein response [16]. The unfolded protein response and autophagy are protective cellular mechanisms that respond to ER stress [17–19]; these processes are activated in AD, and correlate with deposits of abnormally phosphorylated Tau in AD [14]. These homeostatic mechanisms are detected in prion diseases when concomitant neurofibrillary pathology is present [14]. Thus the pathogenic process in the AD brain involves deposition of insoluble aggregates of amyloid β-peptide, oxidative stress and calcium dysregulation in neurons, and activation of inflammatory cytokine cascades involving microglia [20], therein suggesting that AD may be an infectious process similar to CJD.

3. The CJD Model for an Infectious Dementia

3.1. *The transmissible spongiform encephalopathies (TSE) of man and animals*

Creutzfeldt-Jakob disease and the other TSEs share pathologic features and infectious mechanisms, but have distinct differences in transmission and epidemiology due to host factors and strain differences [21]. The TSEs include scrapie in sheep, bovine spongiform encephalopathy (BSE or 'mad cow disease') in cattle, chronic wasting disease (CWD) in deer and elk, transmissible mink encephalopathy (TME) in farmed mink in Wisconsin, and related disease in exotic animals. All are characterized by vacuolization of the neuropil [6]. There is minimal immune pathology in TSE-affected brains, with microglial proliferation indicative of an inflammatory component [22].

CJD typically presents as a rapidly progressive disorder that affects the central nervous system leading to memory loss, personality changes, ataxia and neurodegeneration [6]. TSEs are universally fatal in both in animals and humans showing a rapid clinical decline following onset of clinical disease. CJD patients typically die within a few weeks of diagnosis; 90% of CJD patients die within the first year. CJD usually occurs as a sporadic form or rarely (15% of CJD cases) as a hereditary form called Gerstmann-Straussler-Scheinker (GSS) syndrome. In GSS, there are missence mutations in the gene that encodes for prion protein, a misfolded protease-resistant isoform of a normal host protein (PrP). The misfolded prion is deposited in the tissues throughout the course of the disease [23]. The prion is the most important biomarker for these diseases, but is absent in 10% of cases [6]. Currently the prion can only be determined at autopsy or in brain biopsies as a diagnostic test. There are no other reliable *in vivo* tests for CJD, and often (50% of cases) the diagnosis turns out to be an unrelated condition such as lymphoma or cerebral vascular amyloid disease. CJD has to be differentiated from other rapidly progressive dementias including non-prion diseases such as Lewy body disease or a rapidly progressive form of AD [1,24]. Creutzfeldt-Jakob disease can be spread iatrogenically from CJD infected cadaver tissues used for corneal transplants or dural graphs, or from use of surgical instruments contaminated from neurosurgery or eye surgery on a prior case of CJD [6,25]. Creutzfeldt-Jakob disease is clearly infectious since the disease can be readily transmitted to laboratory animals [6].

3.2. *Nature of the transmissible agent of CJD and biologic markers*

The transmissible agents associated with TSE are defined by their virulence for particular animal species (species barrier), their pattern of spread in the brains of affected animals, their incubation times needed to cause disease, and their neuropathological sequelae including pattern of prion glycosylation on Western blots [6]. The nature of the transmissible agent has been studied extensively by experimentation on scrapie, known for over 300 years as a transmissible fatal neurodegenerative disease in sheep [6]. There are over 20 known scrapie agent strains as defined by variation of neuropathological patterns in transmission studies in mice [6]. There are parallels between types of sporadic CJD and types of sheep scrapie showing that distinct groups of prion disease exist in different species [26]. Serial transmission of scrapie isolates in mice have produced prion proteins phenotypically identical to those causing sporadic CJD therein suggesting a possible link between animal and human TSEs [27]. Similar transmission studies of CJD in hamsters by Manuelidis has similiarly defined the properties of the transmissible agent responsible for CJD. The transmissible agent/s of TSEs possess unique biologic properties [6]. The agent is viral-like in size and will pass through a 100 nm filter but not through a 35 nm filter. The agent shows significant resistance to UV and ionizing radiation, and to extremes in other physical and chemical treatments ie. fixatives including glutaraldehyde, and survival following extreme heat treatments. For these reasons, the transmissible agent/s of CJD and the other TSEs have not been considered to be a typical conventional microbe. In addition to the prion, another biomarker of TSE infection are the

scrapie-associated fibrils (SAF)[28] seen on negative stain electron microscopy of ultra-centrifuged synaptosomal fractions as protease-resistant twisted 4 nm fibrils with 200 nm straight segments [6,28]. SAF are consistent and unique markers of TSE infection, whereas the prion is occasionally absent. In the author's opinion, SAF likely represents part of the agent, whereas the prion is a reaction product of the infection since the prion can be readily separated from infectivity [6].

4.   Is AD a Prion Disease?

4.1.   Experimental evidence supporting AD as a prion disease

Misfolded protein (amyloid) deposits are characteristically seen in brains of patients affected with neurodegenerative diseases that are associated with cognitive impairment. Prion protein is deposited in CJD-affected tissues, α-synuclein is present in Lewy body dementia and PD, Tau is found in fronto-temporal dementia, and A-β is associated with senile plaques in AD. Tau is seen as neurofibrillary tangles in AD along with amyloid angiopathy [29]. The amyloid plaques occur before onset of cognitive loss, whereas neurofibrillary tangles, neuron loss and synaptic loss parallel the progression of cognitive decline [30]. Increased deposits of A-β causes damage to neuronal synapses with reduction and loss of dendritic spines [31]. The spread of a toxic agent is likely responsible for the widespread amyloid deposition in brains affected with neurodegeneration [31]. The other possibility is that A-β aggregates may be transmissible themselves [32]. Deposits of phosphorylated Tau have been shown to spread to neurons at other sites in the brain suggesting a trans-synaptic mechanism [32,33]. There appears to be a spread of amyloid plaques and Tau tangles into specific regions of the brain at different stages of AD progression [30,35]. Injection of AD-affected brain tissue homogenates into brains of transgenic mice capable of making human A-β has led to spread and accumulation of A-β in the mouse brain suggesting a prion-like process [36]. Similarly injection of synthetic α-synuclein into brains of mice led to spread and formation of aggregates suggestive of Lewy bodies [37,38]. Injection of synthetic A-β into mouse brains accelerated formation of amyloid plaques [39]. Insertion of stainless steel wires coated with A-β particles into brains of mice simulating surgical instruments resulted in amyloid plaque formation around the wires [35]. Peripheral inoculation of A-β did not induce amyloid plaque formation in mice [35]. Although this process in AD is compared to prion diseases, the normal prion protein isoform (PrPc) appears not to be important in A-β-induced neurological degeneration [31]. The co-distribution of A-β plaques and CJD-associated changes suggests that PrPc may be involved in A-β formation and A-β pathology [40]. Further argument for the prion theory of generation of A-β in Alzheimer's disease is that there is similar generation of A-β using the protein misfolding cyclic amplification (PMCA) method of repeated sonications [41]. These data have led to the concept of cross-seeding of A-β as the punative mechanism for development of AD [42]. On the other hand, A-β amyloid can be seeded and

increased by self-assembly [43] suggesting to the author that those espousing the prion concept have not taken this phenomenon into consideration.

4.2. *The significance of other amyloid proteins in CJD and AD*

If various amyloid proteins function individually as replicating prion-like infectious agents, then how do you explain the mixture of amyloid proteins presumably specific for different neurodegenerative diseases to be present along with prion deposits in familial CJD? A-β plaques have been seen in familial CJD in the same distribution of the spongiform encephalopathy [39]. Genetic CJD, which has been shown to be transmissible [44], show deposits of A-β, Tau and α-synuclein along with amyloid angiopathy and neurofibrillary tangles [45]. A sporadic case of CJD was reported with A-β and α-synuclein deposits [46]. Alpha-synuclein and Tau levels were elevated in a CJD patient's cerebrospinal fluid (CSF) while not in control CSF [47]. These studies suggest that the transmissible agent of CJD is capable of misfolding other host proteins in addition to prion, which is more likely than involvement of a complex mixture of infective prion-like proteins in the disease. Cross-seeding interactions between diverse forms of amyloid are suggested as important mechanisms in the pathogenesis of AD [48]. Amyloid variations lead to different cross-seeding efficiency [49]. Amyloid seeding and cross-seeding along with self-assembly may account for the spread of amyloid in the tissues [50] rather than the amyloid itself being infectious. Bacterial amyloids are capable of cross-seeding often with diverse amyloids [51] thus possibly representing a trigger mechanism.

5. Prion Diseases and their Causality

5.1. *Experimental evidence does not support TSE as an exclusively prion disease*

The prion theory is complex and does not explain all aspects of the disease. It is presumed that the prion amyloid proteins deposited in tissues of TSE cases are the transmissible agents. [52]. The abnormally folded prion amyloid is thought to evolve by spontaneous generation, and once formed can serve as a template for formation of more prion copies [53]. There is no data directly supporting this type of replication [54]. Accumulation of the abnormally folded isoform of the prion protein (PrPsc) in the prion encephalopathies, and the formation of A-β protein from its precursor in AD takes place in lysosome-related organelles; the release of hydrolytic enzymes from the lysosomes may be the primary cause of neuronal damage in these conditions [55]. Another assumption is that the infectious form of prion shows different conformations that represent distinct TSE agent strains without involvement of nucleic acid [52]. Correlations between prion protein polymorphisms and disease have been found, however in what way these polymorphisms influence the conversion processes remains an enigma [56]. The heritable nature of this process has been determined from studies of yeast and fungal prions [57,58], although the prions generated by yeast are non-toxic in

contrast to those associated with prion diseases [58]. The exact mechanism by which mutant prion proteins affect the central nervous system and cause neurological disease is also not well understood [23]. It is interesting that a misfolded form of murine prion in a GSS model in *Drosophila melanogaster* causes motor abnormalities, but the process can be reversed [23]. Conformational transitions of prion induced at acid pH (pH 2) are reversible during the early moments of the misfolding transition [53]. The basis of the prion concept is open to question since experiments showing lack of susceptibility of scrapie infectivity to psoralen are flawed [59]. Psoralen cross-links nucleic acids making them susceptible to UV, and the lack of response of scrapie brain homogenates to these treatments indicated that there were no nucleic acids associated with scrapie infectivity. Prusiner [59] also added RNase and DNase with no effect. This author consulted Dr. John Hearst, a psoralen expert [60] who worked with Prusiner on that experiment. Dr. Hearst [personal communication] indicated that the results of Prusiner's experiments [59] were over-interpreted; Dr. Hearst stated 'if the chemical can't get in, it can't cause a problem'. He gave poliovirus and *Bacillus subtilis* as examples of conventional organisms that can survive these psoralen treatments. Therefore something else in involved in the pathogenesis of prion diseases, and may act as a trigger for the prion transformation.

5.2. *Additional evidence that the prion is not the causative agent of TSEs*

Manuelidis recently showed that there is a disconnect between prion protein and infectivity in a tissue culture model, with continuing increase in prion amyloid in spite of a fall in infectivity [61]. In the author's opinion, the fall in infectivity shown by Manuelidis is more consistent with the lag phase of the growth curve of a conventional bacterial agent, while the prion amyloid continued to increase unabated more analogous to self-assembly of an amyloid protein [43]. This important study suggests that the initiating infectious agent may be absent by the time the tissues are evaluated, when only the resulting prion amyloid remains. Manuelidis has most convincingly shown in a sucrose gradient study that prion protein sediments at different gradient densities compared to infectivity [62]. Another example of this disparity between prion and infectivity is evaluation of the protein misfolding cyclic amplification (PMCA) test [63] used extensively for detection of miniscule amounts of prion in test samples. In this procedure, test samples are mixed with normal mouse brain to provide a source of PrPc then submitted to repeated sonication cycles. The result is recovery of increasing levels of prion amyloid but with no comparable increase in infectivity [64], suggesting that the prion is not the surrogate marker for the transmissible agent of CJD. It is interesting that studies using a 29 polymerase and chromatography strategy, highly infectious culture and brain homogenates of three different geographic TSE preparations all contained novel circular DNAs [65], therein challenging the prion concept. The neurotoxicity in prion diseases can be prevented by deletions of the octapeptide repeats within the flexible tail of the prion protein [66] suggesting prions are likely toxic factors generated during the course of the disease. Scrapie agents display doubling times that are 8× to 33× faster in cell cultures than *in vivo*, indicating release from complex innate immune responses, suggesting a foreign pathogen rather than an infectious form of host prion protein

(PrPsc) [67]. Manuelidis also presented evidence that the normal host prion (PrPc) serves as a receptor for the infectious agent of CJD, and that infection spreads from cell to cell by the agent reacting with the prion receptor. This idea explains the likely mechanism of spread of amyloids observed in the AD brain [34]. Interestingly PrPc serves as a receptor for *Brucella* sp. [68], which fits with our data that shows a bacterium is involved in the pathogenesis of CJD [69,70]. This controversy shows that the prion alone is not the cause, but rather another factor is involved that likely triggers the onset of the disease.

6. Experimental Evidence Supporting AD as Caused by a Transmissible Bacterial or Viral Microorganism

6.1. The candidate agent/s of AD

Both CJD and AD have an inflammatory component with the neuroinflammation evidenced by presence of microglial proliferation in each [1,71–73], suggesting involvement of infectious agent/s capable of inducing amyloid formations that have become primary markers of these diseases. A search for a possible conventional etiologic agent in AD has led to investigation of several candidates. Gastrointestinal and mouth bacteria are often seen in association with AD [5,74–76]. Periodontal pathogens such as *Porphyromonas gingivalis, Tannerella forsythia, and Treponema denticola* have been implicated [77]. The importance of periodontal disease in pathogenesis of AD makes sense because of chronic inflammation related to daily episodes of bacteremia due to teeth brushing or flossing, and large injections during dental procedures possibly allowing bacteria to disseminate to the CNS [77]. Chronic bacterial inflammation is associated with amyloid formation, and spirochaetes contain amyloidgenic proteins [73,78]. *Borrelia burgdorferi* spirochetes have induced A-β and Tau formation in mammalian tissue culture cells [73]. Amyloid proteins in bacterial microbiota in the gut are able to cross-seed amyloid misfolding in the host tissues and induce oxidative stress [79]. AD pathology has been found after intranasal infection of mice with *Chlamydia pneumonia* that had been isolated from an AD-affected brain [80]. Another consideration is whether the culprit is a mixed infection with *Mollicutes* associated with other bacteria. The tiny wall-less *Mollicutes* could be missed on standard culture testing [6]. I base this suggestion on prior studies of *S. citri* and *Wolbachia* as mixed infections in insect vectors [81]. The association between *S. citri* or *Wolbachia* is complex in that each bacterium alone grow to high titer but as a mixture, the *Wolbachia* growth is subdued, while *S. citri* flourishes. Presumably *S. citri*, the smaller bacterium, is utilizing some growth factor or excretion product from the larger bacterium.

6.2. Mechanics of Amyloid formation in bacterial infection

Currently the initiating factors involved in the misfolding leading to amyloid deposits are unknown [79], but the amyloids when formed can increase by self-assembly [82]. It has been

proposed that cross-seeding of amyloid misfolding, altered proteostasis and oxidative stress may occur in response to amyloid proteins within bacteria in the microbiota of the gut [79]. 'Similarities of tertiary protein structure may be involved in the creation of these prion-like agents through molecular mimicry'[79]. Viruses of animal or plant origin may mimic nucleotide sequences of microRNAs and influence protein expression, therein leading to formation of the amyloid proteins [79]. The most compelling study is *in vitro* formation of amyloid deposits similar to those seen in AD-affected brain in glial and neuronal cell cultures exposed to *Borrelia burgdorferi* and bacterial lipopolysaccharides [73]. A-β and hyperphosphorylated Tau were detected in cell extracts in this study by Western blots suggesting that A-β plaques can occur either by contact with the bacterium or bacterial toxic products. A mechanism of involvement of bacteria in amyloid formation may be related to discovery of curli fibers, otherwise known as bacterial amyloid [83,84]. Curli fibers are functional amyloids produced in bacterial biofilm and are involved in attachment of the biofilm to surfaces. Interestingly curli fibers can bind with host proteins and cause misfolding of those proteins (amyloid formation) [83,84]. Injection of bacterial curli fibers from bacterial biofilms into susceptible mice has resulted in formation of disease-associated amyloid A [85]. There are no studies that directly show curli fibers are involved in A-β formation, although cross-seeding is a possibility [51]. The ability of bacteria to trigger formation of amyloid suggest that a bacterial infection could possibly be involved in AD.

7. CJD is a Bacterial Disease

7.1. *Experimental evidence supporting TSE is caused by a bacterial infection*

The biggest thorn in the prion theory is our evidence that CJD is a bacterial disease. Spiroplasma, a wall-less motile *Mollicute,* was found by transmission electron microscopy (TEM) of brain biopsy and autopsy brains from several CJD patients [86–89]. Ribosomal DNA specific to spiroplasma has been found in brain tissues from all forms of TSE including CJD, scrapie in sheep, and CWD in deer [90]. One negative poorly controlled study [70,91] is discounted since different primers were used even though our prior study presenting the workable primers was referenced [90]. *Spiroplasma* sp. have been grown in cell-free media from brain tissues from all forms of TSE [92], and recently from eyes of sheep with terminal scrapie [93]. The laboratory strain of spiroplasma (*S. mirum* isolated from rabbit ticks) intracranially (IC) inoculated into deer produced classic clinical signs of CWD at 4 months post injection [94]. The deer show neuropathological evidence of spongiform encephalopathy with demonstration of the organism in the obex (brain stem) of the deer with CWD clinical signs by immunohistochemistry (IHC) and TEM. Spiroplasma isolates from scrapie-affected sheep brain and CWD-affected deer brain inoculated IC into sheep and goats induced neuropathology in the obices of these animals identical to that seen in naturally occurring TSE in ruminants [94]. Experimental spiroplasmosis in the rodent has been shown to be dose

dependent and the organism can be re-isolated from the tissues [95]. Spiroplasma have been shown to be neurotropic in the rodent model [96]. Identification of spiroplasma in brain tissues by standard TEM is difficult since spiroplasma show morphological variation, and therefore are mostly seen as membranous forms within the tissues [94]. Spiral forms are rarely seen by TEM in experimental infection [94], but are identical to those originally seen in the brain biopsy of a CJD patient [86]. Immunehistochemistry studies of the brains of rats infected with the laboratory spiroplasma (*S. mirum*) strain show the bleb-like organisms lining the walls of the vacuoles [94]. Sheep inoculated with this organism show localization of the infection to the eye, involving primarily the retina, vitreous and corneal endothelia [92]. The spiroplasma scrapie isolates infected mouse neuroblastoma cells, inducing a vacuolar cytopathology and numerous apoptotic cells [66]. Bovine corneal endothelial (BCE) cells infected with scrapie spiroplasma isolates induced similar cytopathology [92]. Immune florescent antibody (IFA) studies of BCE cell cultures either infected with the scrapie isolates or the laboratory strain (*S. mirum*) using hyperimmune sera against *S. mirum* showed presence of spiroplasma in the positive control, both in the cells and in the surrounding media [92]. By contrast, there was no immunoflorescent staining of spiroplasma in BCE cells infected with the scrapie isolate in the IFA study, although the scrapie-associated bacterium was identified in the infected BCE cells by TEM [92]. Therefore, we can conclude that the scrapie spiroplasma isolate is immunologically distinct from the *S. mirum* laboratory strain. This immunological difference between the laboratory spiroplasma strain and the scrapie isolate suggests epitope differences of surface proteins on the scrapie spiroplasma isolate versus the laboratory spiroplasma strain, which can be further investigated for the purpose of developing a serological test for the disease.

7.2. *Spiroplasma as a pathogen for CJD*

Spiroplasma share morphological characteristics and biologic stringent properties with the transmissible agent of CJD. Spiroplasma phenotypic forms measuring 40 nm in diameter have been seen in broth culture during all stages of the growth cycle [65,66]. These tiny filaments have been seen by TEM of rat brains experimentally inoculated with *S. mirum* [93] and are remarkably similar to inclusions seen in scrapie-affected brain tissues by TEM [97]. Spiroplasma can easily pass through a 100 nm filter [6]. Prion amyloid deposits have not been clearly documented in experimental spiroplasmosis using the laboratory spiroplasma strain (*S. mirum*) isolated from ticks [Dr. M Hall, APHIS, personal communication, 2006]. On the other hand, this spiroplasma (*S. mirum*) produces α-synuclein in BCE tissue culture [Feng, personal communication, 2012]. It is noteworthy that SAF bears a striking morphological resemblance to the internal fibrillar network of spiroplasma responsible for the locomotor machinery of the cell [65,98]. Scrapie-specific hyperimmune sera (courtesy Patricia Merz, New York State Institute of Basic Research) react by Western blot with the spiroplasma fibrils obtained from protease-digested spiroplasma cell-free broth cultures [99]. Spiroplasma form biofilm on glass, mica and stainless steel surfaces [100]. The reaction is particularly prominent on nickel. *Spiroplasma mirum* is resistant to all antibiotics, except for

tetracyclines or erythromycin, which are bacteriostatic drugs [6]. There is currently no bacteriocidal antibiotic for spiroplasma [6]. It is interesting that experimental scrapie infection in hamsters can be prevented by adding tetracycline to the inoculum [101,102]. Spiroplasma within biofilm are resistant to 50% glutaraldehyde [100] simulating the biologic properties of the scrapie agent. *Spiroplasma mirum* strains have shown survival after treatment with extremes in heat, with no loss of viability until 87.5°C and surviving near 100°C (unpublished data). Spiroplasma survival is identical to data regarding resistance of scrapie agent to heat treatments as reported by Pattison [103,104]. In our study, the control *Escherichia coli* were dead at 80°C. *Spiroplasma mirum* showed significant resistance to irradiation sources (unpublished observation). This remarkable resistance to physical and chemical treatments has long been proposed as unique biological characteristics of the transmissible agent of CJD [6] and strongly suggests that spiroplasma are candidate causal agents of CJD and the other TSEs [65,66].

8.   Epigenetic Mechanism in AD-role of a Bacterium

Both AD and CJD fit the epigenetic model wherein there is alteration of the nuclear chromatin of the host without any changes in DNA sequence. DNA methylation and histone acetylation are two critical epigenetic mechanisms controlling chromatin structure and function in post-mitotic mammalian neurons [105,106]. Hypermethylated DNA recruits silencing transcription chromatin remodeling complexes with histone deacetylases, and promotes chromatin condensation. Hypomethylated DNA unfolds into a 'beads-on-a-string' structure in which histones are accessible for chromatin remodeling factors such as histone acetyltransferase, and the transcriptional co-activator implicated in epigenetic mechanisms controlling memory consolidation. There is a 'correlation between ubiquitin load and histone modifications' [105]. Epigenetic phenomena are multifactorial [107]. Obligate intracellular pathogenic bacteria possessing constrained compact genomes manipulate their host by recruiting chromatin modifying enzymes (histone decetylases and methyltransferases) or by acting directly on transcription [108]. Non-nuclear isoform of histone H1 expressed in neurons is upregulated in AD and also in neurons and astrocytes of brains affected with scrapie [108]. Epigenic changes occur in the mitochondrial genome [109]. This phenomenon likely is related to the mitochondrial dysfunction seen in AD, both in brain neurons and leukocytes leading to oxidative stress and neurodegeneration. Mitochondria are also swollen and dysfunctional in brains of scrapie-infected mice [110]. Spiroplasma, the candidate causal agent of CJD, is an intracellular wall-less bacterium that alters the cellular chromatin by means of hyper-methylation of chromatin and fuses with mitochondria leading to mitochondrial swelling and dysfunction [6]. Nearly 100% of *S. mirum* DNA CpG sequences are methylated suggesting the organism is a gene manipulator [111,112]. The term epigenetic mechanism has also been raised in the context of the prion protein, but with no supporting data; the term is used in this context only to try to interpret phenomena seen in the TSE literature that are difficult to explain based upon strict interpretation of the prion theory [113].

9.   Summary

Although there is no experimental evidence of the transmissibility of AD, the occurrence of inflammatory change in AD brain tissues and the association of AD with a wide variety of bacteria, supports the idea that AD may be infectious. If AD is infectious, it is likely that pre-amyloid proteins interact as receptor proteins with an infectious agent such as a bacterium [114]. The interaction of bacterial curli fibers with the precursor proteins could lead to formation of amyloids that damage the neuron. The epigenetic phenomena associated with AD supports a bacterial etiology [115]. The involvement of spiroplasma as a candidate causal agent of CJD serves as a model for investigating the possible role of a bacterium in the pathogenesis of AD. The consideration of amyloids as being infectious agents in AD is not plausible in light of the controversy regarding the prion theory in the causality of CJD. The finding of A-β, Tau, and α synuclein in hereditable forms of CJD suggest that the agent is able to transform other host proteins besides the normal host prion (PrPc). Future studies should look for wall-less bacteria as well in AD that would not be detectable unless one uses special growth media for testing. The determination that AD is a bacterial infection through further experimentation would revolutionize how we deal with this devastating illness.

Acknowledgments

Conflict of Interest

The author has no conflict of interest.

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