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Can oral infection be a risk factor for Alzheimer's disease?

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ABSTRACT

Alzheimer's disease (AD) is a scourge of longevity that will drain enormous resources from public health budgets in the future. Currently, there is no diagnostic biomarker and/or treatment for this most common form of dementia in humans. AD can be of early familial-onset or sporadic with a late-onset. Apart from the two main hallmarks, amyloid-beta and neurofibrillary tangles, inflammation is a characteristic feature of AD neuropathology. Inflammation may be caused by a local central nervous system insult and/or by peripheral infections. Numerous microorganisms are suspected in AD brains ranging from bacteria (mainly oral and non-oral *Treponema* species), viruses (Herpes simplex type I) and yeasts (*Candida* species). A causal relationship between periodontal pathogens/non-oral *Treponema* species of bacteria has been proposed via the amyloid-beta and inflammatory links. Periodontitis constitutes a peripheral oral infection that can provide the brain with intact bacteria and virulence factors and inflammatory mediators due to daily, transient bacteraemias. If and when genetic risk factors meet environmental risk factors in the brain, disease is expressed, in which neurocognition may be impacted, leading to the development of dementia. To achieve the goal of finding a diagnostic biomarker and possible prophylactic treatment for AD, there is an initial need to solve the etiological puzzle contributing to its pathogenesis. This review therefore addresses oral infection as the plausible aetiology of late onset AD (LOAD).

Keywords: *Alzheimer's disease*; pathogenesis; microorganisms; oral bacteria; direct cause

Alzheimer's disease (AD) is a neurodegenerative disease and the most common example of a group of diseases that manifest as dementia. It is associated with atrophy and specific neuronal death particularly in the hippocampal region of the brain (1). Research into AD pathogenesis, has flagged two main categories of the disease: the familial onset presentation accounts for around 2% of all AD cases and the sporadic form of late-onset AD also referred to as LOAD constitutes approximately 98% of the cases. LOAD displays genetic susceptibility traits of which the well-known risk factor is inheritance of the apolipoprotein (APOE ϵ 4) gene allele (2) and, appears to require an environmental factor for disease expression. For example a pathogen-host interaction can exacerbate neurocognition in some elderly individuals who if in their 80+ years likely become diagnosed with LOAD (3, 4). The rationale for this review therefore is to try to explain the aetiology in the vast proportion of LOAD cases that relies upon common risk factors. Several scientists have proposed, one of these to be peripheral infections (5-11), and the accompanying systemic and local inflammatory mediators (11-13). Of these, the plausible risk from oral infection is the main focus of this review.

PREVALENCE OF AD

AD is a burden of longevity resulting from the superior quality of health care provision for all. This factor is likely to contribute to quadrupling of AD subjects living in our society during the next 40 years (14). It is estimated that by 2050 about 13-14 million people are likely to suffer from AD in the USA with a rise in the total costs estimated to be more than \$1 trillion. The odds of having a diagnosis of AD when over 85 years of age exceed 1:3 (15). One in six people over 80 years in the UK has dementia (16). Estimates for the prevalence of AD in the USA indicate that more than 5 million individuals who are 65 years or older currently suffer from AD (1, 15). About 200,000 subjects have been diagnosed with the early-onset familial

AD form and health care costs for this disease are about \$200 billion per year (1). It is clear that AD is fast becoming a major health challenge in the USA and around the globe that will financially drain public health budgets and care giver services.

NEUROPATHOLOGICAL CHARACTERISTICS OF THE AD BRAIN

The AD brain is characterized by several neuropathological features of which two seminal hallmarks (Fig. 1) arise from proteostasis of the ongoing neurodegenerative processes and are essential for a definitive diagnosis of the disease post mortem (17). One of the hallmark proteins is made up of fibrils in the form of extracellular, insoluble plaques and consists primarily of amyloid-beta ($A\beta$) (18). These peptide deposits in variable sizes depend upon the secretase enzymes (α -, β - and γ -secretases) that cleave it from the longer amyloid precursor protein (APP). Initial reports suggested fibrillar $A\beta$ to be neurotoxic (19) as it has been shown to kill all types of cells by apoptosis induction (20). However, there are two known insoluble fibrillar $A\beta$ amyloid peptides comprised of $A\beta_{40}$ and $A\beta_{42}$ amino-acid residues which exhibit distinct physiological states within the human brain. There is a general consensus among scientists that the larger ($A\beta_{42}$) peptide is the neurotoxic form as the ageing brain of cognitive intact individuals also displays $A\beta$ plaques. However, in the cognitively intact brain they are fewer in number and usually of the diffuse $A\beta_{40}$ type that appears not to bear any, as yet known, pathological significance. In addition there are the soluble monomeric, dimeric and the multimeric forms of $A\beta$ (21). The relative neurotoxicity of these isoforms remains unclear (22).

More recently, the fibrillary forms of the $A\beta_{(40/42)}$ peptides released in the AD brain were also recognized as “defensin” or innate immune defense molecules that act to protect the host against infection (23). For example, both of the aforementioned amyloidogenic peptides can

bind to bacterial membranes and in that way lyse bacterial cells. Although A β is acting as an antimicrobial peptide (AMP), it may be a part of the brain's ancient/modern innate immune defense mechanism. AMPs are potent, broad-spectrum, pore-forming agents targeting Gram-negative and Gram-positive bacteria, enveloped viruses and protozoans (23), thereby supporting the hypothesis that AD has an infectious origin.

Furthermore, the senile plaques (A β_{42}) are recognized as triggers that stimulate activation of microglial cells and initiate local immune responses (24). Activated microglia are the most important contributors of inflammation in the central nervous system (CNS) (25). They secrete a number of proinflammatory cytokines (24-26) and recognize pattern associated molecular patterns (PAMPs) on bacteria and their cellular debris (27-30) in response to CNS infection.

The other pathological characteristic of AD is an accumulation of intracellular hyperphosphorylated tau and heat shock proteins constituting the neurofibrillary tangles (NFTs). Hyperphosphorylated tau protein alters the polymerization and stability of microtubules compromising their function (31). NFTs in AD reflect the severity of disease; however, the significance of pathogen-host interaction to the occurrence of NFTs in the AD brain is poorly understood. Current genetic evidence is pointing to aberrant innate immune responses (32, 33) and cholesterol lipid genes (34) having greater significance in AD pathogenesis. A dysfunctional immune system and predisposition to hyperlipidaemia also support the role of reduced blood flow due to the vascular lesions and inflammation, A β deposition and microorganisms in AD.

In advanced AD pathology, synaptic dysfunction is another structural defect associated with a decline in memory (35-37). Although a circular argument, malnutrition plays a role in the gradual loss of synapses and fewer teeth during life is a known risk factor for AD (38).

Neurons are capable of responding to injury by expressing multiple neurotransmitters. In AD, selective loss of cholinergic neurons in the basal forebrain (39) also correlates with the loss of cognitive function (18, 35).

THE AMYLOID CASCADE HYPOTHESIS

Several hypotheses have been advanced regarding the development of AD. The amyloid cascade hypothesis serves as a model particularly for the familial form of AD (40) which is a disease caused by mutations involving the amyloid- β protein precursor, located on chromosome 21 and presenilin 1 and 2 on chromosomes 14 and 1 respectively that enhance the APP gene processing towards A β deposition (41, 42). The model, which was first proposed by Glenner and Wong (43), maintains that the neurodegenerative disease is due to an imbalance between the generation and clearance of A β . Genome wide association studies (GWAS) highlighted the complement receptor 1 (CR1) gene playing a role in AD pathogenesis (44). One recognized role of CR1, a membrane bound regulatory protein, is its ability to bind C3b opsonins (Fig. 2). It is abundantly expressed especially on erythrocyte membranes and as such participates in immune complex clearance by transporting waste to the liver and the spleen. As the CR1 gene is a risk factor for LOAD, this suggests loss of function as a possibility for the defective clearance of A β in the brain. Other tentative explanations suggest variation in CR1 protein isoforms (longer and shorter forms) (45), whereby the longer form is less involved in the disease process via its ability to bind more C3b and facilitate more effective clearance of A β in the brain (46). This is a process that inevitably fails favoring disease expression with more A β proteostasis buildup and complement pathway activation. The amyloid hypothesis has been modified several times, particularly due to the finding that soluble oligomers of A β may contribute to early preclinical

stages of the disease that initiate the cascade leading to synaptic dysfunction, atrophy and neuronal loss (47).

THE INFLAMMATORY HYPOTHESIS

The intrinsic model

Currently there are two models of the inflammatory hypothesis of AD, an intrinsic and an extrinsic. The intrinsic inflammation model accounts for the intact “blood-brain barrier” (BBB) restricting entry of neurotoxic immune molecules and systemic lymphocytes to the brain. As a consequence, the brain glial cells are able to generate a local and complete innate immune system when challenged by foreign agents (26, 48-50). Historically, neuroinflammation has largely been viewed as being a downstream consequence of the amyloid hypothesis, whereby the presence of amyloidogenic peptides result in the activation of microglia initiating pro-inflammatory cascades and the release of potentially neurotoxic substances resulting in degenerative changes in neurons. GWAS now implicates innate immune genes (44, 51) as being a risk factor and supports a primary role for the inflammatory elements of AD pathology via inappropriate activation of the complement system (52-54) in association with A β plaques and NFTs (55).

The extrinsic model

The extrinsic model accounts for communication of the glial cells with the immune challenges presented via the blood vascular system using the circumventricular organs and the choroid plexus that are devoid of the BBB (56). The cells from this region of the brain are fully equipped with the CD14 receptor and the toll-like receptor 4 (TLR 4) to recognize LPS from the peripheral blood circulation (27, 28). Hence, elements of systemic infections such as those

originating from Gram-negative, highly virulent oral pathogens, bronchopneumonia and urinary tract infections (3, 4, 7, 57, 58) reach all organs including the CNS. Bacterial products entering the bloodstream trigger the innate immune responses of host cells via pattern recognition receptors (PPR) and TLRs that alert local and distant cells to the infectious threat by secreting immune mediators (cytokines) to confine and defeat the foreign agents. Increased risk of dementia in the elderly following multiple infectious episodes has been reported (4). In addition, systemic infections appear to contribute towards delirium in some clinically diagnosed AD patients and such episodes can exacerbate a premorbid cognitive status (3). Holmes et al. proposed that since cytokines are primary mediators released by the host to defend against infection, such secondary stimuli (IL-1 β and TNF- α) may mediate their effect on the brain and indirectly contribute to cognitive decline (3, 57).

NON-ORAL BACTERIA RELATED TO AD

Honjo et al. (59) using Bradford Hill's criteria for assessing the relationship between bacteria and disease found *Chlamydomphila pneumoniae* to be a likely infectious agent related to the pathogenesis of AD. Maheshwari and Eslick (60) reported a strong correlation between *C. pneumoniae* and AD, and according to Shima et al. (61) *C. pneumoniae* is currently the most plausible of all infectious agents proposed to be involved in AD. Lim et al. (62) suggested that the pro- and chronic inflammatory states in AD pathogenesis may in part be due to *C. pneumoniae* infection of monocytes. *C. pneumoniae* antibodies from typical intracellular and atypical *C. pneumoniae* antigens have been identified both in the frontal and temporal cortices of brains from AD patients (63). Amyloid deposit and NFTs were detected in the same regions in apposition to one another suggesting that *C. pneumoniae* infection is involved in the development of AD pathology.

Using various techniques Balin et al. (9) found *C. pneumoniae* in 80-90% of LOAD brain tissue specimens. *C. pneumoniae* infection was correlated with the APOE ϵ 4 allele expression. The same researchers subsequently demonstrated that astroglia, microglia, neurons, endothelial cells and monocytes in the LOAD brain are permissive to this bacterium. The mechanisms of pathogenesis differ between actively- and persistently-infecting chlamydiae and it is in the persistent state that these organisms cause chronic disease (64, 65). *C. pneumoniae* was cultured from two AD brain samples after one or two passages in HEp-2 cells (66). Interestingly, the study indicated that brain isolates were more related to respiratory than to vascular/atheroma strains of *C. pneumoniae*. This suggested that *C. pneumoniae* infection of the brain was secondary to bronchopneumonia and at the end stages of LOAD.

It has been suggested that the phages phiCPAR39 and phiCPG1, associated with *C. pneumoniae*, may enter mitochondria of the bacterial host and work as slow viruses initiating AD (67). These authors hypothesized that mitochondrial recruitment by *C. pneumoniae* phages may be the primary initiating event in the pathogenesis of neurodegenerative disorders.

In a meta-analysis based on 25 relevant, primarily case-control studies Maheshwari and Eslick (60) found a statistically significant association between AD and detectable evidence of infection caused by *C. pneumoniae* or spirochetes. They reported over a ten-fold increased occurrence of AD when there was evidence of spirochetal infection (OR: 10.61; 95% CI: 3.38-33.29) and over a four-fold increased occurrence of AD with a conservative risk estimate (OR: 4.45; 95% CI: 2.33-8.52). There was a five-fold increase in occurrence of AD with *C. pneumoniae* infection (OR: 5.66; 95% CI: 1.83-17.51). Accordingly, a strongly positive association between bacterial infection and AD was shown for both types of bacteria, but it was strongest for spirochetes.

It is generally accepted that the syphilis spirochete *Treponema pallidum* can cause chronic neuropsychiatric disorders including dementia as well as other neurodegenerative disorders (11). *T. pallidum* causes brain atrophy and A β deposition in the atrophic form of general paresis (68, 69) and is a strong indication for involvement of spirochetes in AD pathogenesis. Chronic diseases such as syphilis are frequently associated with deposition of amyloid (68, 69). Amyloid is an integral component of spirochetes which may contribute to amyloid deposition in AD (70). Spirochete accumulation in the cerebral cortex in the context of syphilis will also lead to formation of senile plaques, NFTs and granulovacuolar degeneration (71).

Miklossy (68, 69) analyzed data on the ability of spirochetes to induce pathological and biological hallmarks of AD in vitro following Koch's and Hill's postulates and demonstrated a plausible causal relationship between neurospirochetosis and AD. The data revealed a statistically significant association between spirochetes and AD ($P = 1.5 \times 10^{-17}$, OR = 20, 95% CI = 8-60, N = 247). When mammalian cells were exposed to spirochetes, the pathological and biological hallmarks of AD were reproduced in vitro (68, 69). Historical observations supported the conclusion that chronic spirochetal infections can cause dementia and reproduce the neuropathological hallmarks of AD (72). According to Miklossy (72), these observations represent further evidence in support of a causal relationship between various spirochetal infections and AD.

Another spirochete also implicated in AD is *Borrelia burgdorferi*, the causative agent of Lyme disease which is transmitted to humans via tick vectors. There are great similarities in the clinical and pathological manifestations of syphilis and Lyme disease (72, 73). The occurrence of *B. burgdorferi* in the brains of AD patients was first reported by MacDonald and Miranda (74) and was confirmed later by MacDonald (75, 76), Riviere et al. (5) and Miklossy et al. (77). Interestingly, Bu et al. (78) found that the infectious burden consisting of

B. burgdorferi, *C. pneumoniae*, *Helicobacter pylori*, cytomegalovirus and Herpes simplex-1 (HSV-1) is associated with AD. In contrast, Gutacker et al. (79) and Pappolla et al. (80) found no evidence for an association between *B. burgdorferi* and AD.

Among other bacterial species, *H. pylori* (mono-infection) has been found to be related to AD (59). These authors suggested that AD pathology can be initiated and exacerbated by some microorganisms with inflammatory and oxidative responses which may affect the brain continuously and gradually over time. However, the *H. pylori* status was not associated with AD in a study from Japan, probably due to the high prevalence of the organism in controls (81). This was refuted by Kountouras et al. (82) who had previously found that successful eradication of *H. pylori* infection was associated with significantly lower mortality risk in AD patients [HR (95% CI)=0.287 (0.114-0.725), p=0.008] (83).

ORAL BACTERIA RELATED TO AD

The oral cavity harbors an impressive range of bacterial phylotypes (84). Molecular identification methods have detected close to 900 different predominant bacterial species of which 35% cannot yet be cultured (85). The oral microbiome profiles appear to be individualized (86), meaning that bacterial microbiomes can vary both qualitatively and quantitatively between individuals, although there are also significant overlaps. Each individual can harbor up to 200 different bacterial taxa in their mouth and there is a large variation in the microbiota in different oral sites (84, 87). Furthermore, the composition of the oral microbiota irrespective of being indigenous or pathogenic in the oral cavity keeps changing in view of major oral diseases (caries, gingivitis, aggressive and chronic periodontitis, periodontal-endodontic lesions, peri-implantitis and mucositis) (88-94). Particularly plaque-induced oral diseases such as periodontitis are associated with a change in

the oral microbiota. There is a predominance of anaerobic bacteria in the oral cavity. Many of the major periodontal microorganisms are anaerobic, e.g., *Porphyromonas gingivalis*, *Treponema denticola* and *Tannerella forsythia*. The abundance of anaerobes tend to increase with the development of plaque-induced oral diseases.

Periodontal bacterial pathogens are related to AD

Major pathogens of chronic periodontitis such as *P. gingivalis*, *T. forsythia* and *T. denticola* are implicated in the development of several inflammatory diseases at remote organ sites. Except for *T. forsythia*, all three of the above-named organisms of which *T. denticola* represents a spirochete, have been found in the AD brain (5, 8). Spirochetes are strongly neurotropic. They can spread along nerve fibers and via lymphatics (67, 68) and have been detected in the trigeminal nerve and trigeminal ganglia (95). Spirochetes and their antigens as well as DNA have been found associated with AD and are strongly implicated as the causative agents leading to dementia (68, 69). In 14 studies spirochetes were detected in AD by different authors in different laboratories and countries by means of different techniques (for reviews see Miklossy (68, 69). Riviere et al. (5) demonstrated the presence of seven different oral *Treponema* species in 14 out of 16 AD brain specimens (Fig. 3). Spirochetes were even cultivated from the brains of AD patients indicating that they were viable in the brain (67, 68, 77). Miklossy suggested a co-infection by several spirochetes in AD including the oral varieties (*T. socranskii*, *T. pectinovorum*, *T. denticola*, *T. medium*, *T. amylovorum* and *T. maltophilum*) as demonstrated by Riviere et al. (5). Spirochetes reproduced the biological and pathological hallmarks of AD after exposure of mammalian neuronal and glial cells in organotypic cultures (68, 69).

It was demonstrated that LPS from periodontal bacteria can access the AD brain during life while detection in corresponding controls, with equivalent or longer postmortem interval was absent (8). This study supports the literature on elevated antibodies to periodontal disease-associated bacteria such as *P. gingivalis*, being found in AD patients (7). Furthermore, in 2,355 people 60 years and over, the third NHANES study found associations between periodontitis and cognitive impairment and between measures of immunoglobulin to *P. gingivalis* and cognitive test performance (96, 97). In this study all participants were cognitively intact at baseline. Those who went on to develop AD had higher levels of serum antibodies to periodontal pathogens at baseline. The study suggested a temporal relationship in that the periodontal disease came before AD.

Other important periodontal pathogens related to AD are *Fusobacterium nucleatum* and *Prevotella intermedia*. In the NHANES study antibody levels to these organisms were significantly increased ($\alpha = 0.05$) at baseline serum in patients with AD compared to controls (97). The results were significant after controlling for baseline age, Mini-Mental State Examination score, and allele APOE ϵ 4 status. Noble et al. (98) found that a high anti-*Actinomyces naeslundii* titer (> 640 ng/ml, present in 10% of the subjects) was associated with increased risk of AD (HR=2.0, 95% CI: 1.1-3.8). This association was stronger after adjusting for other significant titers (HR=3.1, 95%CI: 1.5-6.4) and confirmed that periodontal pathogens may be associated with AD.

Possible consequences to the brain carrying oral bacterial pathogens

The fact that inflammation is sustained in the AD brain suggests that local immunogenic hallmark proteins and/or peripheral infections are key perpetrators. This is supported by reports highlighting microorganisms and their toxic products as well as DNA in brain tissue

of AD patients and experimental animals (see below). Bacteria activate pathways that include the integrin receptor CR3 (CD11b/CD18) and TLR signalling (99) and the complement cascade (100). The NF- κ B signalling pathway for cyto/chemokine release (TNF- α , IL-8) (101) produces free radicals, nitric oxide triggers and apoptosis (102). The oral cavity, lungs and gastrointestinal and urinary tracts are plausible sources of brain microorganisms. The likely passage of the microorganisms of interest from their original sites to the brain is described below.

Infections with spirochetes can cause cerebral hypoperfusion (103), cerebrovascular lesions and a severely disturbed capillary network (68, 69). Chronic spirochetal infections can also induce slowly progressive dementia, cortical atrophy, chronic inflammation and A β deposition, indistinguishable from that occurring in AD brains (for reviews see 68, 69, 72). Furthermore, cultured neuronal cells exposed to spirochetes produce A β (104). Spirochetes are also able to form plaque-, tangle- and curly fiber-like lesions (72, 105). They induce a latent and slowly progressive infection by evading host defenses. This promotes their survival and proliferation in the brain by blocking the complement cascade. Spirochetes may even survive and proliferate in hosts that are immune-competent—Interestingly, the remarkable ability of *T. pallidum* to evade clearance from the immune system has earned it the designation “stealth pathogen” (106). The activated complement cascade following spirochete infections (11) may be used as a non-specific marker of CNS inflammation. Spirochete-host interactions initiate and sustain chronic inflammation triggering various immune responses that activate the innate and adaptive immune system, free radical production, apoptosis and amyloid deposition typically seen in AD brains (107).

P. gingivalis has been designated as one of the “keystone” periodontal pathogens because it is able to establish and maintain the periodontal disease-associated “inflammophilic” microbiota (108). It is able to perform this task as it possesses an awesome variety of

virulence factors, recently reviewed by Singhrao et al. (109), to evade the host immune defenses, thus serving two major functions: initial survival of *P. gingivalis* itself via a sustainable inflammatory milieu and sustainment of nutritional sources by eliminating microbial competitors (108).

The *P. gingivalis* endotoxin LPS demonstrates differences in the number of phosphate groups together with both the amount of lipid A fatty acids and their specific position. The presence of multiple lipid A structures makes it more difficult for the innate host responses to recognize the molecule thereby aiding the virulence of *P. gingivalis* (110). The consequences of finding *P. gingivalis* LPS in the host's body, e.g. the brain (8), include priming of immune cells for differential activation of the TLR-mediated NF- κ B signalling pathway (111) leading to cytokine liberation, complement activation and maintenance of intracerebral inflammation.

P. gingivalis evades circulating phagocytes by adhering to erythrocytes (112). An active invasion of *P. gingivalis* and infection-induced complement activation with bystander neural injury was detected in the brains of ApoE^{-/-} mice (113). This supported previous notions that bacterial infections can contribute to the development of AD pathology via mechanisms involving acute phase proteins such as cytokines and the complement cascade where neurons would be attacked.

ORAL VIRUS RELATED TO AD

Herpes simplex virus (HSV) is present in more than 70% of the population after 50 years age (114-116). It persists latently in the peripheral nervous system and is periodically reactivated. Characteristically, HSV-1 has been designated as the enemy within (10). Herpes viruses, including Epstein-Barr virus and cytomegalovirus, are found in high copy counts in aggressive periodontitis, and may interact synergistically with periodontopathic bacteria in the

pathogenesis of this disease (117). Periodontal infections activated by Herpes virus may impair local host defenses and thus increase the aggressiveness of resident periodontopathic bacteria. The bacteria, in turn, may augment the virulence of the herpes viruses.

High proportions of viral-associated proteins in amyloid-containing plaques and/or NFTs corroborate with the involvement of HSV-1 in AD pathology (118). Notably, De Chiara et al. (119) reported an association between A β accumulation in the brain and HSV infection. Itzhaki et al. (120) suggested that not only does HSV-1 produce the main components of amyloid plaques and NFTs (i.e. A β and hyperphosphorylated tau), but it also interferes with the autophagic events that prevent degradation of these proteins and eventually leading to their accumulation in the AD brain. Further, in vitro and in vivo investigations in murine models following HSV-1 infections demonstrated A β accumulation (121).

A number of scientists have suggested that there is imbalance between production and clearance of β -amyloid in the brain, a premise first proposed by Wisniewski et al. (122) based on the discovery of soluble species of this protein and later confirmed by Zlokovic et al. (123). It is now widely accepted that defective clearance of this protein is a hallmark of AD brains leading to its accumulation in the form of insoluble A $\beta_{40/42}$ plaques. Although HSV and cytomegalovirus have been detected in the brains of older adults with and without AD (124-126), HSV-1 viral DNA is present in a higher proportion of AD patients (127). It is particularly seen in the temporal and frontal cortices which are the brain regions that are most damaged in AD (128, 129). The relevance of this association is still under investigation; however a plausible role for the HSV-1 viral DNA could be associated with the plaque maturation process. Jamieson et al. (127) found that the virus was absent from the brains of most young people, probably because it enters the brain during old age either with immune senescence (130) or the virus itself is initially responsible for weakening the host's immune defenses. This latter explanation is likely and is supported by us and others (131).

HSV-1 is a strong risk factor for AD in the brains of those with the APOE ϵ 4 allele (125, 132). This virus is not only a dormant passenger but can persist in the latent form in neurons or replicate at a very low level in neuroglia (133). During persistence it may release toxic products continuously and induce pro-inflammatory cytokines at low levels which become an additional burden to a host already challenged by age, poor diet, restricted exercise as well as any genetic susceptibilities. Itzaki and Wozniak (10) suggested that stress or peripheral infection can reactivate the virus periodically from latency in the brain. This may cause an acute but presumably localized infection, and subsequent damage modulated by the APOE gene can lead to formation of A β plaques and NFTs.

The presence of anti-HSV IgM, a sign of reactivated infection, almost doubled the risk for AD while anti-HSV IgG did not influence the risk (134). Kobayashi et al. (135) suggested that the anti-HSV-1 Ig antibody avidity index could be a useful biomarker for early diagnosis of amnesic mild cognitive impairment, which is prodromal to AD, as well as for AD sufferers.

Reactivation of HSV seropositivity is highly correlated with incident-AD (136). Letenneur et al. (136) speculated that AD pathology starts many years before frank dementia and recurrent reactivation of HSV can act as a potent stimulus to brain microglia, increasing cytokine levels, and triggering a positive feedback cycle leading to increasing accumulation of neurohistopathological changes. In other words, infection, followed by local CNS inflammatory reaction is the likely primary stimulus whereas proteostasis is a consequence of the primary event leading to the development of AD.

Hill et al. (137) suggested a role for HSV-1-induced miRNA-146a in the evasion of HSV-1 from the complement system which is a major first-line host defense mechanism, and the

activation of key elements in the arachidonic acid cascade known to contribute to AD-type neuropathological changes.

ORAL YEASTS RELATED TO AD

Oral yeast infection represents a secondary opportunistic infection particularly involving *Candida albicans*, but increasingly non-*albicans* species, e.g. *Candida glabrata*. With a growing population of elderly, severe systemic fungal infections have increased dramatically in this age group during the last 30 years (138, 139). Oral yeasts can be found in periodontal pockets, in root canals, on the mucosae and underneath dentures (denture stomatitis) (140-142). Denture stomatitis is prevalent in elderly wearing dentures that are heavily contaminated with yeasts which can be a source of systemic mycosis. Disseminated mycoses have recently been reported in AD patients (143, 144). Fungal molecules including proteins and polysaccharides [(1,3)- β -glucan] were detected in peripheral blood serum, and fungal proteins and DNA were demonstrated by PCR in brain tissue of AD patients. Chitin-like fungal structures have also been found in the AD brain (145) and chitinase activity has been proposed as a powerful biomarker of AD (146). In AD brains, cytoplasmic material in a small number of cells was targeted by antibodies with immunoreactivity to yeast cells (147). These findings were consistent with the idea that neurons can be infected by fungi. Interestingly, antifungal treatment reversed the clinical symptoms of some AD patients (148, 149).

HOW DO ORAL MICROORGANISMS REACH THE BRAIN?

Blood stream dissemination

The most likely pathway for dissemination of oral microorganisms to the brain is through the blood stream (150). Dental treatment as well as brushing, flossing, chewing and use of tooth picks in a patient with periodontitis will release a bacteraemia (151). This can occur several times during the day and has been estimated to last for up to 3 hours for oral bacteria (152). The bacteraemia is usually contained by immune cells of the body. However, in people with reduced immune defense, e.g. older individuals, bacteria may localize to crevices of the oral cavity and vascular channels (150).

The blood- brain barrier

An intact blood-brain barrier (BBB) prevents microorganisms in the blood from accessing the brain. However, aging favors overgrowth of oral microorganisms, particularly anaerobic bacteria and facultative yeasts that established earlier in life and provoked pro-inflammatory responses that weakened the BBB (16). Notably, magnetic resonance imaging (MRI) confirmed loss of BBB integrity in a mouse model of disseminated candidosis (153). Loss of integrity allows microorganisms to spread through the blood stream and quietly contribute in the pathogenesis of AD. During immunosenescence, the innate immune system gradually takes over for the acquired immune system. This contributes to a rise in circulating proinflammatory cytokines such as TNF- α (16). Indeed, proinflammatory mediators can cross the BBB (3, 7, 154). APOE ϵ 4, TNF- α and perhaps Ephrin Type-A Receptor 1 (EphA1) may influence BBB integrity and thus be important for penetration of bacteria, LPS and other toxic bacterial products as well as yeasts into the brains of AD patients (16). APOE ϵ 4 affects the integrity of the BBB by activating the cyclophilin A matrix metalloproteinase MM-9 pathway (155).

It is also plausible to suggest that the permeability of the BBB increases with age and thus promotes AD pathogenesis making the brain accessible to microorganisms. Mice with a mutation in the amyloid precursor protein gene which is related to early-onset AD in man,

showed increased permeability of the BBB and increased formation of senile plaque as compared to control mice (156). The changes increased with age.

Circumventricular organs and perivascular spaces

Circumventricular organs (permit polypeptide hypothalamic hormones to leave the brain without disrupting the BBB) are not dependent on the BBB (56) and may act as another entry portal to the brain for bacteria (157). Poole et al. (8) postulated that bacteria and their products may also directly access the brain via the systemic circulation through the perivascular spaces.

The olfactory hypothesis

The “olfactory hypothesis” suggests the olfactory tract as a potential route for pathogenic bacteria to enter the brain and thereby trigger the production of A β and NFTs (158). The olfactory and trigeminal nerves are known to be used by periodontal pathogens to bypass the BBB for direct passage to the CNS (5, 150, 159, 160). Identification of oral treponemes in the trigeminal ganglia supports such a route of dissemination (5). Further, spirochetes may spread along the fila olfactoria and tractus olfactorius (68, 69).

Olfactory unsheathing cells (OECs) engulf bacteria and migrate towards TNF- α released by activated astrocytes (161). Therefore, OECs could be a vehicle for transporting live bacteria to the brain (i.e., Trojan horse). The olfactory bulb was the first area where NFTs and A β deposition were detected in the neuropathological trajectory of AD in humans (162) and in mouse models of AD (163).

GENETIC, NUTRITIONAL AND ENVIRONMENTAL FACTORS PROMOTING AD

While early-onset AD is genetically determined, LOAD is thought to result from interaction between genetic and environmental factors (12). Several mutated genes are associated with the familial AD, such as the amyloid beta ($A\beta$) precursor protein ($A\beta$ PP) gene and the presenelin-1 (PSEN-1) and PSEN-2 gene (164-166). A major risk factor for LOAD is polymorphism in the $APO\epsilon 4$ allele (2). Also cytokine-related genes seem to be involved in the susceptibility to inflammation in both LOAD (167, 168) and periodontitis (169-171). Thus, polymorphisms that increase $TNF-\alpha$ also increase the risk of both AD and periodontitis (172, 173). Lambert et al. (174) found that 20 different loci can increase host susceptibility to AD including polymorphisms in genes associated with interleukin-1 (IL-1) (71, 175-178) and $TNF\alpha$ (71, 172, 179-181). The $APO\epsilon 4$ gene which is one of these 20 loci is highly correlated with AD (182) but it is also a risk factor for infection and increases the expression of inflammatory mediators (11). Recently, genetic overlap between AD, C-reactive protein (CRP) and plasma lipids was demonstrated by using summary statistics from GWAS of over 200,000 individuals (183). There may also be interplay between genetic risk and environmental risk factors such as toxins and or bacterial, viral and fungal pathogens in LOAD reflecting its complex and multifactorial etiology (1).

Diet with its content of essential B-vitamins, phospholipids and other micronutrients are important for forming new nerve synapses (184). Nutritional deficiencies are common both in elderly and in dementia subjects as briefly discussed by Singhrao et al. (150).

ASSOCIATION BETWEEN CHRONIC PERIODONTAL DISEASE AND AD

There is increasing evidence for an association between chronic periodontitis and LOAD (185). Cross-sectional and longitudinal studies have demonstrated that gingival bleeding, loss

of periodontal attachment, periodontal probing depth, alveolar bone loss and antibodies to periodontal pathogens are significantly associated with lower cognitive function and decline after adjustment for co-variables (for a review see (12)). Acute phase proteins, including cytokines are possible indirect links between periodontal pathogens and/or their virulence factors (12, 13). Elderly often show neglect of oral hygiene which can stimulate recurrent chronic oral infection (150). This again promotes inflammation which can lead to confusion and dementia (3, 4, 154). In 152 subjects 50-70 years of age who were followed for 20 years, greater levels of periodontal inflammation correlated with lower cognitive levels (186). Furthermore, gingival bleeding and loss of periodontal attachment were significantly associated with cognitive impairment in a cohort of 5,138 people aged 20-59 years (187). In 144 nuns, those encoding APOE ϵ 4 and who had fewer teeth experienced more rapid cognitive decline than those with neither or either of these risk factors (188). Clinical and epidemiological studies showed that loss of teeth is associated with poor memory (6, 96, 187, 189). In another study of 597 community dwelling men followed for 32 years, tooth loss, increasing periodontal pocket depths and progression of alveolar bone loss were associated with impaired cognition particularly in those over 45 years of age (190). Recently, de Souza Rolim et al. (191) found that periodontal infections were more frequent in patients with mild AD than in healthy subjects. Another interesting feature related to the pathogenesis of AD is the low level of infection by “commensals on the loose” (16). These “immuno-tolerated” bacteria may silently multiply in sites outside of their primary niche and an ongoing infection at their secondary location may have significant deleterious effects upon the health of the elderly or demented host with an existing immunocompromised status.

PUTATIVE TREATMENT AND PROPHYLAXIS OF AD

There is no effective treatment or prophylaxis yet for AD, but several approaches have been proposed. Efforts in this respect are important. If we could delay onset of dementia by only 2 years we might lower the prevalence of AD by more than 22 million cases over the next 40 years (14). Notably, the inheritance of the APOE ϵ 4 allele in the very old (90+) age group, appears to confer protection (192), having bypassed a period of being at risk around 85+ years of age.

If periodontal disease is implicated in AD, periodontitis prophylaxis could be of help. It would be interesting to see if this has any effect on the initiation and aggravation of AD but an observation period of decennia is probably needed.

In a study of subjects with mild to moderate AD, a 3-month course of doxycycline and rifampicin reduced cognitive deterioration during a 6 months' follow-up interval (193). It was concluded that use of antibacterial compounds may not have had any effect on the treatment of *C. pneumoniae* but had a beneficial effect on cognitive decline in AD (193). This might be related to prevention or attenuation of a number of peripheral infections or dampening down the proinflammatory cytokine response. Minocycline was found to correct early, pre-plaque neuroinflammation and inhibit the APP cleaving enzyme 1 (BACE-1) in a transgenic model of Alzheimer's disease-like amyloid pathology (194). It was suggested that interfering with inflammation could be a useful therapeutic approach in early, pre-plaque stages of AD-like amyloid pathology.

Anti-inflammatory drugs given for at least 2 years before the onset of dementia delayed the disease process (195-197). It may also be beneficial to combine anti-inflammatory agents with antibacterials (193). Examination of several available Non-steroidal Anti-Inflammatory Drugs (NSAIDs) showed that only a few of them had any useful A β -modifying or other activity of therapeutic use in LOAD (for a review see (1)).

Itzhaki and Wozniak (10, 198) suggested that antiviral therapy and perhaps vaccination against HSV-1 in early life could be useful. If HSV-1 is implicated in AD, vaccination could prevent the excessive accumulation of A β in the brain. Vaccination with mixed HSV glycoproteins prior to HSV infection protected against viral latency in mouse brains (199). Also Mori (200) maintained that antiviral approaches including chemotherapy and vaccination are promising for prevention and treatment of AD and remain to be validated. Furthermore, Carter (118) suggested that vaccination or antiviral agents and immune suppressants may be considered as therapeutic options before or during the early stages of AD. Interestingly, exposure of HSV-1-infected cell cultures to intravenous immunoglobulin acting via anti- β -amyloid antibodies reduced the accumulation of A β and phosphorylated tau (201).

Angiotensin-converting enzyme (ACE) from *Stigmatella aurantiaca* may cleave the A β peptide similar to human ACE and may be used as a novel form of treatment against AD (202). Furthermore, Chiarini et al. (203) maintained that calcilytics could halt AD progression and preserve the patients' cortical neurons, cognitive abilities, and eventually life if given at minimal cognitive impairment or at earlier stages. Studies using mice suggested the use of tau aggregation inhibitors as potential drugs for the treatment of AD and other tauopathies (204).

Resveratrol is a polyphenol present in red wine. Its capability of directly interfering with the toxic β -amyloid protein aggregation in AD has recently been shown (205). Resveratrol was found to reduce A β -induced toxicity in a *Caenorhabditis elegans* model of AD by targeting specific proteins involved in proteostasis and thereby reducing the amount of aggregated A β (206). This is in concert with our previous finding that the effect of a drinking pattern of 2-7 times per week reduced the risk of myocardial infarction among men who had a history of tooth extractions due to periodontal/dental infection (207).

Potent inhibitors of A β oligomer formation or A β -induced cell toxicity have proven to be attractive means for therapeutic intervention of AD. Song et al. (208) found that the anti-Alzheimer effects of centipede grass, which contains several C-glycosyl flavone constituents, occurred through inhibition of neuronal cell death by intervening with oligomeric A β formation and reducing beta-site amyloid precursor protein cleaving enzyme 1 activity. The authors suggested that Maysin, a major flavonoid of corn silk, in centipede grass could be an excellent therapeutic candidate for the prevention of AD.

Active immunization against important domains of Alzheimer tau eliminated tau aggregation and neurofibrillary pathology (209). The AD type of tau hyperphosphorylation was abolished in transgenic mice by vaccination across a wide range of AD phospho-epitopes. Kontsekova et al. (209) demonstrated that active immunization of rats with a tau peptide encompassing the epitope revealed by monoclonal antibody DC8E8 led to elimination of all major hallmarks of neurofibrillary pathology involving a 95% reduction in the AD-type hyperphosphorylation of tau.

CONCLUSIONS

LOAD which is the predominant form of AD, does not seem to have a single cause. On the contrary, a multitude of factors may be involved and they may act in concert. Among others both genetic and environmental factors may be involved. Even among microorganisms, cooperation may occur since the brain can hardly differentiate between different microbial insults which collectively contribute capacity for enhancing inflammation. Irrespective of the cause, systemic inflammation may predict the onset of dementia. Organisms such as spirochetes, *P. gingivalis*, *C. pneumoniae*, *H. pylori*, Herpes simplex type I virus and *Candida* are among the prime candidate pathogens in AD brains. In the cascade of events causing AD,

oral microorganisms may play a role, particularly anaerobic bacteria such as treponemes, *P. gingivalis*, *Prevotella* spp., *Fusobacterium* and *Actinomyces*, but also facultative anaerobic *Candida* species. It is important to recognize that infection can occur decades before the manifestation of dementia. The most convincing evidence for a causal relationship between oral bacteria and AD is noted for spirochetes which are both neurotropic and motile. It is likely that oral infection can be a risk factor for Alzheimer's disease but it is not the only one. Experiments in humans may require long exposure time to disclose key events and mechanisms of AD. There is, as yet, no cure for AD despite concerted efforts and investment by industry. Prevention of AD through long-term use of antibiotics may be impractical and could select for resistant bacteria. This is worrisome as the prevalence of AD and the public expenses related to its management are expected to increase greatly in the next decade. If anaerobes of periodontitis have a major role in AD, dental hygiene and treatment will provide the AD prophylaxis from an early age as periodontitis is modifiable. However, improving oral hygiene and treating periodontal disease in the AD patient can be challenging since patients are often uncooperative. There is also need for training care-givers to assist with oral care in such patients.

Vaccination against key organisms and important domains of AD has had some beneficial effect. Also several agents interfering directly with the pathogenesis of AD have been tested. In order to find a cure, there is a need for clinical diagnostic information and knowledge of the causal agents for AD so that specific treatment options targeting these organisms can be developed. As for diagnostic biomarkers, increased antibody levels to specific oral pathogens in particular to *P. gingivalis* may be used as a monitoring tool years before clinical manifestation of AD. This is important because treatment will probably have to start early.

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CONFLICT OF INTEREST AND FUNDING

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Figure legends

Fig. 1. The pathological hallmarks of AD, numerous extracellular amyloid-A β plaques and intra-neuronal neurofibrillary tangles (NFTs). Although there are several NFTs, only one is picked out in boxes at x 10 and x 40 objective lens magnification.

Fig. 2. Immunofluorescence labelling (green dots) of hippocampal CA neurons opsonised by iC3b following monoinfection with *P. gingivalis* at 24 weeks of APO ϵ gene knockout (ApoE $^{-/-}$) mice. This is indirect evidence of an oral infection having affected the host's brain.

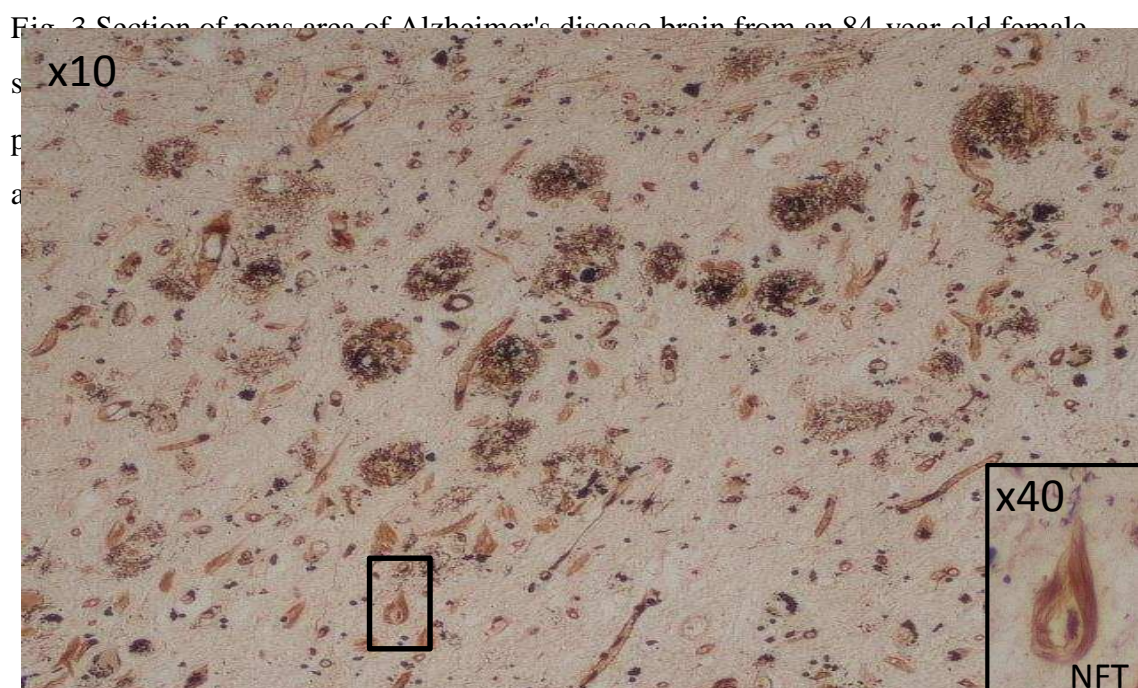


Fig. 1.

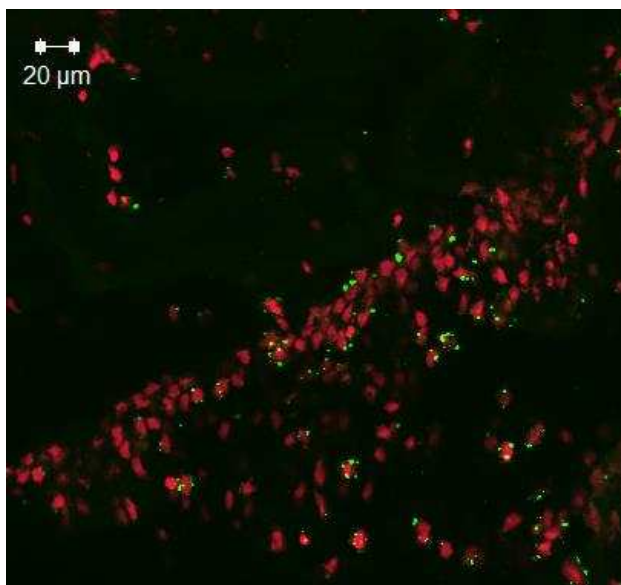


Fig. 2.

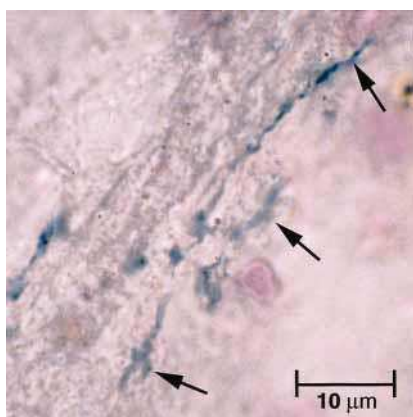


Fig. 3.