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REVIEW

Time to test antibacterial therapy in Alzheimer's disease

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Alzheimer's disease is associated with cerebral accumulation of amyloid-β peptide and hyperphosphorylated tau. In the past 28 years, huge efforts have been made in attempting to treat the disease by reducing brain accumulation of amyloid-β in patients with Alzheimer's disease, with no success. While anti-amyloid-β therapies continue to be tested in prodromal patients with Alzheimer's disease and in subjects at risk of developing Alzheimer's disease, there is an urgent need to provide therapeutic support to patients with established Alzheimer's disease for whom current symptomatic treatment (acetylcholinesterase inhibitors and *N*-methyl D-aspartate antagonist) provide limited help. The possibility of an infectious aetiology for Alzheimer's disease has been repeatedly postulated over the past three decades. Infiltration of the brain by pathogens may act as a trigger or co-factor for Alzheimer's disease, with Herpes simplex virus type 1, *Chlamydia pneumoniae*, and *Porphyromonas gingivalis* being most frequently implicated. These pathogens may directly cross a weakened blood–brain barrier, reach the CNS and cause neurological damage by eliciting neuroinflammation. Alternatively, pathogens may cross a weakened intestinal barrier, reach vascular circulation and then cross blood–brain barrier or cause low grade chronic inflammation and subsequent neuroinflammation from the periphery. The gut microbiota comprises a complex community of microorganisms. Increased permeability of the gut and blood–brain barrier induced by microbiota dysbiosis may impact Alzheimer's disease pathogenesis. Inflammatory microorganisms in gut microbiota are associated with peripheral inflammation and brain amyloid-β deposition in subjects with cognitive impairment. Oral microbiota may also influence Alzheimer's disease risk through circulatory or neural access to the brain. At least two possibilities can be envisaged to explain the association of suspected pathogens and Alzheimer's disease. One is that patients with Alzheimer's disease are particularly prone to microbial infections. The other is that microbial infection is a contributing cause of Alzheimer's disease. Therapeutic trials with antivirals and/or antibacterials could resolve this dilemma. Indeed, antiviral agents are being tested in patients with Alzheimer's disease in double-blind placebo-controlled studies. Although combined antibiotic therapy was found to be effective in animal models of Alzheimer's disease, antibacterial drugs are not being widely investigated in patients with Alzheimer's disease. This is because it is not clear which bacterial populations in the gut of patients with Alzheimer's disease are overexpressed and if safe, selective antibacterials are available for them. On the other hand, a bacterial protease inhibitor targeting *P. gingivalis* toxins is now being tested in patients with Alzheimer's disease. Clinical studies are needed to test if countering bacterial infection may be beneficial in patients with established Alzheimer's disease.

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Abbreviations: HSV = herpes simplex virus; MCI = mild cognitive impairment

Introduction

Alzheimer's disease is the most common cause of dementia, with a prevalence that dramatically increases with age; from 1990 to 2016 the global number of affected individuals increased by 117% to 43.8 million, and that number is likely to rise to around 152 million by 2050. Dementia is the fifth leading cause of death globally, accounting for 2.4 million annually (GBD 2016 Dementia Collaborators, 2019). The current annual cost of Alzheimer's disease of about a trillion US dollars is forecast to double by 2030. This figure includes an element for 'informal' (usually family) care-givers, whose annual burden is estimated at about 82 billion hours (Alzheimer's Disease International, 2018). Alzheimer's disease is characterized by progressive impairment of memory, orientation, language, problem solving and personality. It is associated with cerebral accumulation of amyloid-β peptide and hyperphosphorylated tau, but years of extensive effort to reduce brain amyloid-β accumulation in patients with Alzheimer's disease have been unsuccessful (Panza *et al.*, 2019). However, while further anti-amyloid-β strategies and new anti-tau therapies are being pursued, there remains an urgent need for therapeutic support to patients with Alzheimer's disease for whom symptomatic cholinergic and *N*-methyl D-aspartate (NMDA) therapies provide limited help.

In the early 1980s, molecular virologist Ruth Itzhaki began seeking a causal connection between infection and neurodegenerative disorders. In 2016, she and 33 other scientists published a review presenting evidence for a causal role of pathogens in Alzheimer's disease (Itzhaki *et al.*, 2016), although possible mechanisms have yet to be fully elucidated (Vojdani *et al.*, 2018). This review aims to summarize the current evidence for this association, to describe recent clinical studies with antimicrobial agents in patients with Alzheimer's disease, and to anticipate future therapeutic developments.

Chronic infections and Alzheimer's disease

Cognition in ageing individuals is frequently unimpaired until they suffer a major health challenge such as severe infection. Patients with Alzheimer's disease often worsen during infections when the associated inflammatory response (Perry *et al.*, 2007) accelerates cognitive decline (Schott *et al.*, 2015). Neuroinflammation caused by local deposition of amyloid-β in the brain contributes to the pathogenesis and progression of Alzheimer's disease. However, a potential association between certain infectious diseases and Alzheimer's disease, either through direct invasion or indirectly by modulating immune response, has been suggested recently. A cross-sectional study investigating associations between Alzheimer's disease and prior infection with herpes simplex virus 1 (HSV-1), *Cytomegalovirus*, *Borrelia burgdorferi*, *Chlamydia pneumoniae* and *Helicobacter pylori* showed that patients with Alzheimer's disease were significantly more likely than age-matched controls to have evidence of prior infection with *Cytomegalovirus* (odds ratio: 2.3) or *C. pneumonia* (odds ratio: 2.4) (Bu *et al.*, 2015). Alzheimer's disease was associated with the highest odds ratio (4.1) for subjects who were serum-positive for four or five microorganisms. Serum amyloid-β levels and inflammatory cytokines in individuals exposed to four to five organisms were significantly higher than in those exposed to fewer pathogens. Other large studies have associated systemic inflammation during midlife with cognitive decline over a 20-year period (Walker *et al.*, 2019). These findings support the hypothesis that age is a vulnerability factor that increases the likelihood that an immune challenge will lead to cognitive impairment; this is possibly mediated by age-related changes in the glial environment that exaggerate brain inflammatory response to infection.

These studies highlight a possible pathogenic role for chronic microbial infections and systemic inflammation as drivers of cognitive decline and possibly dementia in the elderly (Fulop *et al.*, 2018*a*). The 'microbial hypothesis' suggests that chronic infection with viral, bacterial, and/or fungal pathogens may be a trigger for sporadic Alzheimer's disease onset during ageing—probably through inflammatory processes—risk of which appears particularly high in apolipoprotein E (*APOE*) ε4 allele carriers (Itzhaki *et al.*, 1997). Candidate pathogens proposed over the years include latent viruses [HSV-1, herpes simplex virus type 2 (HSV-2), human herpesvirus 4 (HHV-4), human herpesvirus 5 (HHV-5), human herpesvirus 6 (HHV-6) and 7 (HHV-7)], gut bacteria (*H. pylori*), periodontal bacteria (*P. gingivalis*), pulmonary bacteria (*C. pneumoniae*), spirochetes (*B. burgdorferi*) and others (Fulop *et al.*, 2018*b*). These pathogens may invade the CNS directly via the trigeminal nerve or the oral-olfactory pathway, or by systemic circulation from the gastrointestinal tract (Dando *et al.*, 2014). Increased Alzheimer's disease risk has been found

to be associated with chronic periodontitis (Chen *et al.*, 2017), while herpes simplex virus (HSV) infection significantly correlates with a higher risk of dementia later in life (Tzeng *et al.*, 2018). These findings have renewed interest in the viral hypothesis of Alzheimer's disease (Li *et al.*, 2018), but do not preclude a role for bacteria, in particular *C. pneumoniae*, *P. gingivalis*, and *H. pylori*. One or more such pathogens might be involved, likely simultaneously, in contributing to a significant proportion of Alzheimer's disease cases. We will now briefly review individual major pathogens associated with Alzheimer's disease.

Viruses associated with Alzheimer's disease

Figure 1 summarizes the major viruses that have been associated with Alzheimer's disease. The *herpesviruses* are a large family of DNA viruses that can cause latent, recurring infections. More than 90% of adults have been infected with at least one herpesvirus, and a latent form of such viruses remains in almost all humans. Accumulating evidence associates infection with several herpesviruses with increased risk of Alzheimer's disease.

Human herpesvirus 1

Human herpesvirus 1 (HHV-1) or HSV-1 is a common neurotropic virus with seroprevalence of 54% between 2005 and 2010 (Bradley *et al.*, 2014). Most humans acquire herpesvirus early in life, which persists latently in the peripheral nervous system, usually in the trigeminal ganglia. Periodical reactivation may present as herpes labialis (cold sores) or be symptom-free, and has been suggested to trigger Alzheimer's disease, where distribution studies reveal that viral DNA is located primarily within senile plaques. In 1979, HSV-1 DNA was detected in brain samples of psychiatric patients using *in situ* hybridization (Sequiera *et al.*, 1979). The association with Alzheimer's disease was initially proposed by Ball (1982), who noted that early Alzheimer's disease affects the same areas as the brain inflammation caused by HSV-1. HSV-1 DNA has been detected in the brains of older people with and without Alzheimer's disease (Jamieson *et al*, 1991), a strong association found between HSV-1 infection and carriage of the *APOE* ε4 allele (Itzhaki *et al.*, 1997), and a striking localization of HSV-1 DNA within amyloid-β plaques identified in six patients with Alzheimer's disease (Wozniak *et al.*, 2009). In a study involving 3432 individuals (mean age at inclusion: 63 years, mean follow-up: 11 years), the presence of anti-HSV IgG antibodies was not associated with increased risk of Alzheimer's disease, but the presence of anti-HSV IgM at baseline (a sign of reactivated infection) was associated with a 2-fold increased risk (Lövheim *et al.*, 2015). A retrospective cohort study of 33 448 subjects revealed that those with HSV infection have a 2.6-fold increased risk of dementia; treatment with anti-herpetic medications dramatically reduced that risk by 90.8%, with prolonged therapy producing a better outcome (Tzeng *et al.*, 2018). This study was contested by other authors on methodological grounds (Nath, 2018), but the results are impressive. In contrast, a study involving 36 subjects with amnestic mild cognitive impairment (MCI) found elevated baseline HSV-1-specific antibody titres in

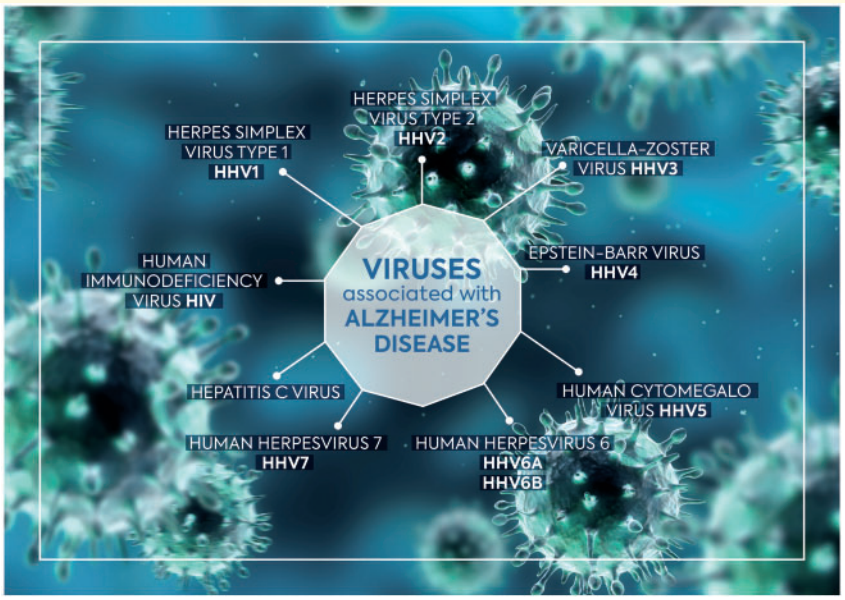

Figure 1 Main viruses associated with Alzheimer's disease. Several viruses have been associated with Alzheimer's disease. The most robust evidence relates to herpes simplex virus type 1, human herpesvirus 6A and human herpesvirus 7.

those who did not convert to Alzheimer's disease in a 2-year follow-up period, compared to those who did progress. HVS-1 specific IgG avidity was also significantly higher in MCI-non-converters than MCI-converters indicating higher functional affinity of antibodies for HSV-1. HSV-1 antibody titres also correlated positively with left hippocampus and amygdala volume (Agostini *et al.*, 2016*a*). A recent study found that repeated virus reactivations of HSV-1 infection in mice—as occurs in humans suffering recurrent infection—triggered progressive accumulation of Alzheimer's disease (amyloid-β, hyperphosphorylated tau) and neuroinflammatory [astrogliosis, interleukin (IL)-1β, and IL-6] biomarkers in neocortex and hippocampus that correlated with cognitive deficits (De Chiara *et al.*, 2019). Another study in HSV-1 infected mice indicated that the virus is transported from the trigeminal ganglia to the CNS following reactivation, but did not exclude the possibility of direct reactivation in the CNS (Doll *et al.*, 2019).

Human herpesvirus 2

Human herpesvirus 2 (HHV-2) or HSV-2 is a neurotropic virus that establishes lifelong latent infection in neurons, is responsible for most genital herpes, and can cause encephalitis and meningitis (Berger and Houff, 2008). An estimated 17% of US adults are infected (Koelle and Corey, 2008). Studies have indicated an association between exposure to HSV-2 and decreased cognitive function (Strandberg *et al.*, 2003), accumulation of hyperphosphorylated tau and amyloid-β in human SK-N-MC neuroblastoma cells (Kristen *et al.*, 2015), and greater temporal cognitive decline (Nimgaonkar *et al.*, 2016).

Human herpesvirus 3

Human herpesvirus 3 (HHV-3) or varicella zoster virus can reside latently in the peripheral nervous system; it usually reactivates only once, as shingles, and lacks asymptomatic shedding. A small study looking for HHV-3 DNA in 17 patients with Alzheimer's disease and 12 aged healthy subjects did not identify in any (Lin *et al.*, 1997), but a recent population-based study showed increased risk of dementia in individuals with herpes zoster (Chen *et al.*, 2018). A retrospective cohort study of 846 patients and 2538 controls found the incidence of dementia during 5-year follow-up was 2.97-fold greater in those experiencing herpes zoster ophthalmicus than in controls (Tsai *et al.*, 2017). Finally, prescription of antiviral therapy for herpes zoster was associated with a substantially reduced risk of dementia (Chen *et al.*, 2018).

Human herpesvirus 4

HHV-4 or Epstein-Barr virus (EBV) causes infectious mononucleosis and is associated with various lymphoproliferative diseases. A 2-year prospective study of 1391 elderly subjects found high titres of anti-EBV antibodies predicted development of amnestic MCI (Shim *et al.*, 2017), while EBV stimulates the production of anti-amyloid-β antibodies in patients with Alzheimer's disease (Xu and Gaskin, 1997).

Human herpesvirus 5

HHV-5 or human cytomegalovirus (HCMV) is prevalent in older adults and is implicated in many chronic diseases. A relationship with Alzheimer's disease risk was initially proposed in 1979 (Renvoize *et al.*, 1979), while HCMV seropositivity was associated with a 2-fold increased risk of Alzheimer's disease and faster cognitive decline in 849 elderly subjects (Barnes *et al.*, 2015) and with increased inflammatory markers (Westman *et al.*, 2014). Anti-HCMV IgG levels are also significantly associated with faster cognitive decline (Nimgaonkar *et al.*, 2016), with neurofibrillary tangle (NFT) density, and (marginally) with amyloid-β load in elderly subjects (Lurain *et al.*, 2013).

Human herpesvirus 6 and 7

Human betaherpesvirus 6A (HHV-6A) and human betaherpesvirus 6B (HHV-6B) are double stranded DNA viruses that infect most human populations. Human betaherpesvirus 7 (HHV-7) often acts together with HHV-6A and HHV-6B; all can cause a skin condition in infants known as exanthema subitem. Studies suggest that HHV-6 may be an environmental risk factor for cognitive deterioration and progression to Alzheimer's disease in older subjects. A 5-year study showed 23% positivity for HHV-6 in peripheral blood leucocytes samples from patients with Alzheimer's disease versus 4% from controls ($P = 0.002$); 17% of Alzheimer's disease brains were HHV-6-positive (Carbone *et al.*, 2014). However, other studies have failed to reproduce these findings (Agostini *et al.*, 2016*b*; Westman *et al.*, 2017). More recently, HHV-6A and HHV-7 RNA levels were found to be increased in multiple brain regions of patients with Alzheimer's disease compared with healthy controls, and that this correlated with amyloid plaque load, NFT densities, and dementia ratings (Readhead *et al.*, 2018).

Hepatitis C virus

Hepatitis C virus (HCV) is a positive-strand RNA virus that primarily infects hepatocytes but is also associated with extrahepatic changes including CNS abnormalities and cognitive dysfunction. A population-based cohort study of 58 570 subjects found HCV-infection increased risk of dementia (Chiu *et al.*, 2014), and HCV RNA has been detected in the CSF and brain of chronically infected patients with neuropathological abnormalities (Morgello, 2005). Brain imaging studies have demonstrated evidence of microglial activation positively correlated with HCV viraemia and altered cerebral metabolism in patients with mild hepatitis C (Grover *et al.*, 2012).

Human immunodeficiency virus

Human immunodeficiency virus (HIV) can cause HIV-associated neurocognitive disorders (HAND) (Clifford *et al.*, 2013). These conditions are classified into three groups: asymptomatic neurocognitive impairment, mild neurocognitive disorder, and HIV-associated dementia (Antinori *et al.*, 2007). Asymptomatic neurocognitive impairment HIV-positive patients present higher risk for developing cognitive impairments compared to controls and HAND patients have significantly decreased CSF amyloid-$\beta_{1–42}$ and increased total-tau and phosphorylated-tau concentrations, suggesting that HAND may be associated with an Alzheimer's disease-like process (Brew *et al.*, 2005; McArthur *et al.*, 2010). It is proposed that HIV infection may represent a risk factor for Alzheimer's disease (Canet *et al.*, 2018).

Bacteria associated with Alzheimer's disease

Figure 2 summarizes the major bacteria that have been associated with Alzheimer's disease.

Chlamydia pneumoniae

C. pneumoniae is a major cause of pneumonia that has been associated in 25 case-control studies with a >5-fold increased occurrence of Alzheimer's disease in infected subjects compared to controls (Maheshwari and Eslik, 2015). *C. pneumoniae*-specific DNA was first identified in the brains of 17 of 19 patients with Alzheimer's disease versus 0 of 19 controls by Balin *et al.* (1998). It has been proposed that *C. pneumoniae* infection may promote CNS vascular inflammation and be a key factor in the initiation of Alzheimer's disease (MacIntyre *et al.*, 2003). Indeed, mice infected with *C. pneumoniae* develop amyloid plaques and deposits consistent with those in the Alzheimer's disease brain (Itzhaki *et al.*, 2004), which appears to resolve with reduction of the *C. pneumonia* antigen burden over time (Little *et al.*, 2014). While some studies demonstrate a clear association between *C. pneumoniae* infection and Alzheimer's disease (Gérard *et al.*, 2006; Paradowski *et al.*, 2007), others have failed to confirm these findings (Gieffers *et al.*, 2000; Ring *et al.*, 2000). Unfortunately, a lack of suitable chlamydial infection models severely hampers research in the field (Shima *et al.*, 2010).

Helicobacter pylori

H. pylori is associated with stomach ulcers and gastric cancer. A study of 27 patients with Alzheimer's disease and 27 controls found both serum and CSF anti-*H. pylori* antibodies levels were significantly higher in patients with Alzheimer's disease than in controls, and correlated with Alzheimer's disease severity (Kountouras *et al.*, 2009*a*). Data from both epidemiologic studies and animal experiments suggest that *H. pylori* infection might influence the course of Alzheimer's disease, being in particular associated with poorer cognition (Franceschi *et al.*, 2019). Access to the brain may occur via the oral-nasal-olfactory pathway or by circulating monocytes carrying *H. pylori* through a disrupted blood–brain barrier (Doulberis *et al.*, 2018).

Porphyromonas gingivalis

P. gingivalis is the main pathogen in chronic periodontitis, which has been associated with several systemic diseases including Alzheimer's disease (Kamer *et al.*, 2008*a*) and increased systemic inflammation (Hayashi *et al.*, 2010), suggesting that it may have a role in Alzheimer's disease (Shoemark and Allen, 2015). It has been repeatedly identified in the brain of patients with Alzheimer's disease (Poole *et al.*, 2013; Singhrao *et al.*, 2015), and misregulated genes in infected macrophages matched those in the hippocampus of patients with Alzheimer's disease (Carter *et al.*, 2017). Exposure to lipopolysaccharide (LPS) from *P. gingivalis* induced neuronal amyloid-β accumulation and learning/memory deficits in normal mice (Wu *et al.*, 2017*a*), but failed to aggravate cognitive impairment in a transgenic mouse Alzheimer's disease model (Hayashi *et al.*, 2019). *P. gingivalis* and related toxic proteases (gingipains) were identified in the brain of patients with Alzheimer's disease and correlated with tau pathology (Dominy *et al.*, 2019).

Spirochetes

Spirochetes are gram-negative, helical bacteria that can cause pathological changes in the brain; an atrophic form of such chronic infection is caused by *Treponema pallidum* and presents with general paresis, slowly progressive dementia, cortical atrophy and brain amyloidosis over an average of 20 years. A meta-analysis of 25 primarily case-control studies found a 10-fold increased risk of Alzheimer's disease in subjects with evidence of spirochetal infection compared to controls (Maheshwari and Eslik, 2015). Spirochetes and their DNA have been found in the brain of patients with Alzheimer's disease (Riviere *et al.*, 2002). A study has found that the number of spirochetes in the brain correlates with dementia severity and degree of atrophy, with cortical spirochetal colonies appearing morphologically indistinguishable from senile plaques (Miklossy, 2015). *B. burgdorferi* can also cause dementia associated with cortical atrophy and microgliosis in advanced Lyme disease (borreliosis) and it has been isolated from the cortex of an Alzheimer's disease patient (MacDonald and Miranda, 1987). Interneuronal transmission is proposed to explain the spread of tau pathology in the Alzheimer's disease brain (MacDonald, 2007). However, other studies using specific antibody or western blot methods (Pappolla *et al.*, 1989) or sensitive PCR assays (Marques *et al.*, 2000) found no evidence of *Borrelia* in brains of patients with Alzheimer's disease.

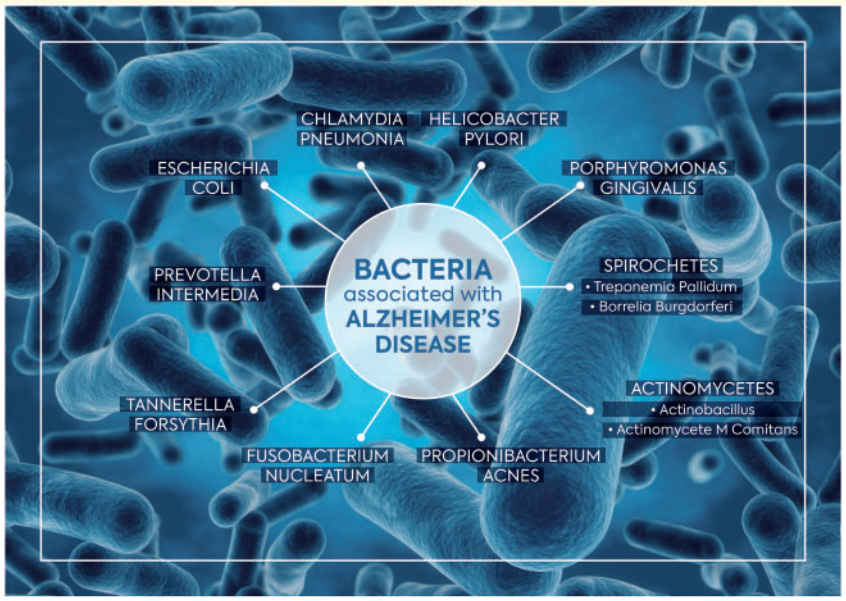

Figure 2 Main bacteria associated with Alzheimer's disease. Several bacteria have been associated with Alzheimer's disease. The most robust evidence relates to *P. gingivalis*, *H. pylori* and *C. pneumoniae*.

Actinomycetes

Actinobacteria are oral commensals and the most common cause of oral infection. They have a two to five times higher presence in Alzheimer's disease than in other pathological conditions and the presence in plaques of fibrillary lesions may also correspond to *Actinomycetes* (Howard and Pilkington, 1992; Emery *et al.*, 2017).

Propionibacterium acnes

Propionibacterium acnes is the causative agent of acne vulgaris. In a study involving nine patients undergoing cortical biopsy for cerebral tumour, *P. acnes* was identified in three of four patients with Alzheimer's disease, and in one of five control subjects (Kornhuber, 1996).

Escherichia coli

Escherichia coli is a Gram-negative bacterium commonly found in the lower intestine. Most strains are harmless, but some serotypes can cause serious food poisoning. *E. coli* secretes lipo-oligosaccharides and LPS that are strongly immunogenic and highly pro-inflammatory for human neurons (Hug *et al.*, 2016), with LPS being found to be significantly more abundant in Alzheimer's disease-affected brain parenchyma than controls (Zhan *et al.*, 2016).

Other bacteria

Other periodontal pathogens suck as *Prevotella intermedia*, *Tannerella forsythia* and *Fusobacterium nucleatum* have also been associated with Alzheimer's disease (Kamer *et al.*, 2009; Shoemark and Allen, 2015).

Fungi associated with Alzheimer's disease

There is evidence of fungal infection in patients with Alzheimer's disease, with proteins and DNA of fungal species (*Saccharomyces cerevisiae*, *Malassezia globose*, *Malassezia restricta*, *Penicillium* and *Phoma*) detected in brain samples of eight patients with Alzheimer's disease and five controls (Alonso *et al.*, 2014, 2018). Fungal DNA and proteins of several fungi including *Candida famata*, *Candida albicans*, *Candida glabrata*, *Phoma betae* and *Syncephalastrum racemosum* were also found in brain tissue from patients with Alzheimer's disease but not from controls (Pisa *et al.*, 2015). Bacterial infection with the *Proteobacteria*, *Firmicutes*, *Actinobacteria*, and *Bacteroides* can also accompany these mycoses (Alonso *et al.*, 2018).

Protozoa associated with Alzheimer's disease

Toxoplasma gondii is one of the most common parasitic infections in developed countries, with acute infection usually being asymptomatic in healthy adults; in vulnerable subjects, it may cause toxoplasmosis. Chronic infection, frequent in the elderly, has been suggested to cause neuroinflammation that may facilitate Alzheimer's disease. In a case-control study of 34 patients with Alzheimer's disease and 37 healthy individuals, seropositivity rates for anti-*T. gondii* IgG antibodies were 44% and 24%, respectively

(Kusbeci *et al.*, 2011). However, in another study of 75 patients with Alzheimer's disease and 75 healthy volunteers, rates were 61% and 63%, respectively (Mahami-Oskouei *et al.*, 2016). A larger study of 105 patients with Alzheimer's disease and 114 controls also found positivity rates did not differ significantly (41% versus 33%, respectively) (Perry *et al.*, 2016). In transgenic mouse models of Alzheimer's disease, chronic *Toxoplasma* infection ameliorated β-amyloidosis by activating immune-mediated clearance of soluble amyloid-β (Möhle *et al.*, 2016) and increased anti-inflammatory cytokines, lowered amyloid-β plaque deposition and reduced cognitive deficit compared with uninfected mice (Jung *et al.*, 2012). In contrast, a mouse model featuring hippocampal amyloid-β injection showed chronic *T. gondii* infection promoted neuroinflammation and aggravated cognitive impairment (Mahmoudvand *et al.*, 2016). However, a recent cross-sectional population-based study carried out in Central Africa in 1662 older participants showed *T. gondii* infection was not associated with dementia (Bouscaren *et al.*, 2018).

Oral and gut microbiota and Alzheimer's disease

Among possible reversible risk factors for Alzheimer's disease, one of the most intriguing is the association of specific dietary patterns and foods with dementia and Alzheimer's disease (Solfrizzi *et al.*, 2017). There has been increasing recent interest in the role that oral (Shoemark and Allen, 2015; Aguayo *et al.*, 2018) and gut microbiota (Tremlett *et al.*, 2017; Sherwin *et al.*, 2018) may play in the microbiota-gut-brain axis. The trillions of microorganisms (predominantly non-pathogenic) colonizing humans from birth have been conventionally examined by anatomical location, notably skin, mouth (oral microbiota), respiratory, urogenital, and gastrointestinal tract (gut microbiota) (Ley *et al.*, 2006; Turnbaugh *et al.*, 2007). A healthy gut microbiota is stable through most of the adult life, with about 1000 different species predominantly from six major bacterial phyla (Rajilic-Stojanovic and de Vos, 2014), dominated by *Bacteroidetes* and *Firmicutes* (90%), with *Actinobacteria*, *Proteobacteria*, *Fusobacteria and Verrucomicrobia* comprising the remainder (The Human Microbiome Project Consortium, 2012). A significant number of species in the human gut remain uncultured (Lagier *et al.*, 2015), an obstacle to understanding their biological roles. A recent study uncovered 1952 uncultured bacterial species (Almeida *et al.*, 2019), expanding the known species with a 281% increase in phylogenetic diversity, and confirming the complexity of gut microbiota.

The microbiota-gut-brain axis is a bidirectional communication system that is not fully understood, with neural, immune, endocrine and metabolic pathways (Pellegrini *et al.*, 2018) that may exert profound influence on key brain processes, such as the stress response (O'Mahony *et al.*, 2009), neurogenesis (Heiss and Olofsson, 2019), neuroinflammation (Lin *et al.*, 2018), and neurotransmission (Strandwitz, 2018). Gut enteroendocrine cells may excite sensory nerves through release of glutamate (Kaelberer *et al.*, 2018), and the microbiota-gut-brain axis can be considered to include other discrete pathways such as the vagus pathway (Sherwin *et al.*, 2016) and humoral circulation of dietary amino acids (i.e. tryptophan) (O'Mahony *et al.*, 2015). This axis may also modulate behaviours such as anxiety (Lach *et al.*, 2018) and sociability (Warda *et al.*, 2019) through the ability of gut bacteria to synthesize neurotransmitters such as serotonin (O'Mahony *et al.*, 2015), γ-amino butyric acid (GABA) (Barrett *et al.*, 2012), noradrenaline (Diaz Heijtz *et al.*, 2011), and dopamine (Asano *et al.*, 2012). They can also modulate the immune system and produce metabolites with neuroactive properties such as short chain fatty acids (Miller and Wolin, 1996) and fermentation products of carbohydrates (Bourassa *et al.*, 2016). Evidence from animal models links gut dysbiosis not only to various gut disorders but also to depression (Yu *et al.*, 2017), neurodegenerative conditions such as Alzheimer's disease (Jiang *et al.*, 2017), Parkinson's disease (Sampson *et al.*, 2016), Huntington's disease (Rosas *et al.*, 2015) and amyotrophic lateral sclerosis (Wu *et al.*, 2015), and neurodevelopmental conditions such as Down syndrome (Biagi *et al.*, 2014).

Gut microbiota and Alzheimer's disease

Some preclinical and epidemiological evidence links gut microbiota alterations with the onset and development of Alzheimer's disease (Tables 1 and 2). Although no Alzheimer's disease epidemiological studies have explored the gut microbiota directly, patients with irritable bowel syndrome (which features hyperactivation of the gut immune system and dysbiosis) have an increased risk of developing both non-Alzheimer's disease dementia and Alzheimer's disease (Chen *et al.*, 2016). A group of 178 older subjects in various care settings had reduced gut microbiota diversity, frailty, and markers of inflammation of potential relevance to Alzheimer's disease (Claesson *et al.*, 2012). *Escherichia/Shigella* bacterial genera, which are associated with mediating inflammation, were found to be increased in faecal samples from patients with cognitive impairment and brain β-amyloidosis in comparison with controls. Moreover, there was a positive correlation between that increase and the elevated expression of the proinflammatory cytokines IL-1β and CXCL2 in whole blood (Cattaneo *et al.*, 2017). With advancing ageing, the blood–brain barrier and the gut become more permeable and exacerbation of this by dysbiosis may also impact Alzheimer's disease pathogenesis. Neuropathological findings suggested that levels of LPS and *E. coli* K99 pili protein were higher in Alzheimer's disease brain parenchyma compared to control brains. Moreover, LPS was co-

Table 1 Principal clinical studies evaluating the direct or indirect association of gut microbiota and related diseases with Alzheimer's disease

Reference	Study design and sample, animal model	Duration	Measurements	Main findings
Claesson *et al.* (2012)	Observational study 178 older subjects residing in the community, day-hospital, rehabilitation, or long-term residential care Age: >65 y	Cross-sectional analysis	Metabolomic analysis (NMR spectroscopy) of faecal water from 29 subjects Shotgun metagenomic sequencing to investigate microbial SCFA production FFQ Markers of inflammation (serum TNF-α, IL-6 and IL-8 and CRP), CCI, GDT, the Barthel index, FIM, MMSE and MNA	Lower gut microbiota diversity significantly correlated with measures of frailty, comorbidity, nutritional status, markers of inflammation and with metabolites in faecal water
Zhan *et al.* (2016)	Case-control study 24 AD patients and 18 non-demented age-matched controls	Cross-sectional analysis	Brain samples from grey and white matter LPS and Ec K99 pili protein Human brain samples were assessed for Ec DNA followed by DNA sequencing	Ec K99 and LPS levels were greater in AD compared to control brains. LPS co-localized with $A\beta_{1\text{-}40/42}$ in amyloid plaques and around vessels in AD brain
Chen *et al.* (2016)	Nationwide, retrospective, matched-cohort study 32 298 patients, with IBS selected from the National Health Insurance Research Database of Taiwan along with 129 192 controls matched for sex, age, and baseline year	12 y	IBS Incident dementia, non-AD dementia, and AD	IBS was associated with an increased risk of dementia in patients older than 50 y. Patients with IBS were also more likely to develop either non-AD dementia or AD
Cattaneo *et al.* (2017)	Case-control study 40 cognitively impaired patients with brain amyloidosis (mean age: 71 y), 33 without brain amyloidosis (mean age: 70 y), and a group of 10 controls without brain amyloidosis and cognitive impairment (mean age: 68 y)	Cross-sectional analysis	Selected bacterial taxa of gut microbiota and blood expression levels of cytokines	In patients with cognitive impairment and brain amyloidosis, there was an increase in the abundance of the pro-inflammatory taxon *Escherichia/Shigella*, and a reduction in the abundance of the anti-inflammatory taxon Er, possibly associated with a peripheral inflammatory state
Vogt *et al.* (2017)	Case-control study 24 AD patients and 25 nondemented age- and sex-matched controls Mean age AD patients: 71.3 y Mean age controls: 69.3 y	Cross-sectional analysis	Bacterial 16S ribosomal RNA gene sequencing of DNA isolated from fecal samples CSF AD biomarkers	In the gut microbiome of AD participants, there was differences in bacterial abundance including decreased *Firmicutes*, increased *Bacteroidetes*, and decreased *Bifidobacterium*, with correlations between levels of differentially abundant genera and CSF AD biomarkers
Vogt *et al.* (2018)	Case-control study 40 AD patients, 35 MCI subjects, and 335 cognitively healthy individuals Mean age AD patients: 63.8 y Mean age MCI subjects: 73.2 y Mean age controls: 61.9 y	Cross-sectional analysis	TMAO levels in CSF CSF AD biomarkers	CSF TMAO levels were higher in individuals with MCI and AD compared to cognitively-unimpaired subjects, and elevated CSF TMAO was associated with biomarkers of AD pathology (phosphorylated tau and phosphorylated tau/$A\beta_{1\text{–}42}$) and neuronal degeneration (total tau and neurofilament light chain protein)
Zhuang *et al.* (2018)	Case-control study 43 AD patients and 43 non-demented age- and sex-matched controls Mean age AD patients: 71.3 y Mean age controls: 69.3 y	Cross-sectional analysis	Bacterial 16S ribosomal RNA gene sequencing of DNA isolated from faecal samples	Several bacteria taxa in AD patients were different from those in controls at taxonomic levels, such as *Bacteroides*, *Actinobacteria*, *Ruminococcus*, *Lachnospiraceae*, and *Selenomonadales*
Nho *et al.* (2019)	1562 subjects from the ADNI 305 AD patients, 505 late MCI subjects, 284 early MCI subjects, 98 SMC subjects, and 370 cognitively healthy individuals	Cross-sectional analysis	Serum levels of 20 primary and secondary bile acid metabolitesCSF biomarkers, neurodegenration (MRI), and brain glucose metabolism (^{18}F-FDG PET)	Three bile acid signatures were associated with CSF $A\beta_{1\text{-}42}$ and three with CSF phospho-tau181. Furthermore, 3, 12, and 14 bile acid signatures were associated with CSF t-tau, glucose metabolism, and atrophy, respectively
MahmoudianDehkordi *et al.* (2019)	1464 subjects from the ADNI 305 AD patients, 505 late MCI subjects, 284 early MCI subjects, and 370 cognitively healthy individuals	Cross-sectional analysis	Serum levels of 15 primary and secondary bile acid metabolites AD-related genetic variants and cognitive tests	In AD patients compared to cognitively healthy older adults, there was a significantly lower serum concentrations of cholic acid and increased levels of the bacterially produced deoxycholic acid, and its glycine and taurine conjugated forms. An increased ratio of deoxycholic acid/cholic acid was also strongly associated with cognitive decline, a finding replicated in serum and brain samples in other two population-based studies (Rush Religious Orders and Memory and Aging Project)
Haran *et al.* (2019)	108 nursing home elders 51 (47.2%) had no dementia, while 24 elders (22.2%) had AD and 33 elders (30.6%) had other dementia types	5 months	Metagenomic sequencing and *in vitro* T84 intestinal epithelial cell functional assays for P-glycoprotein performed on stool samples	Stool samples from elders with AD can induce lower P-glycoprotein expression levels than those seen with samples from elders with either no dementia or other types of dementia. A loss of P-glycoprotein expression or a reduction in its function correlates with inflammation in the gastrointestinal tract in mice and humans

Aβ = amyloid-β; AD = Alzheimer's disease; ADNI = Alzheimer's disease Neuroimaging Initiative; CCI = Charlson comorbidity; CRP = C-reactive protein; Ec = *Escherichia coli*; Er = *Eubacterium rectale*; FFQ = food-frequency questionnaire; FIM = functional independence measure; GDT = geriatric depression test; IBS = irritable bowel syndrome; IL = interleukin; LPS = lipopolysaccharides; MMSE = Mini Mental State Examination; MNA = mini nutritional assessment; NMR = nuclear magnetic resonance; SCFA = short-chain fatty acid; SMC = subjective memory complaint; TMAO = trimethylamine *N*-oxide; TNF-α = tumor necrosis factor-α.

Table 2 Principal preclinical studies evaluating the direct or indirect association of gut microbiota and related diseases with Alzheimer's disease

Reference	Study design and sample, animal model	Duration	Measurements	Main findings
Minter *et al.* (2016)	Life-long antibiotic-treated transgenic $APP_{SWE}/PS1_{\Delta E9}$ mouse model of AD	5–6 months	Bacterial 16S ribosomal RNA gene sequencing of DNA isolated from cecal and faecal samples Aβ plaque quantification and soluble and insoluble Aβ in brain tissues Aβ plaque-localized microglial populations Serum circulating chemokines and cytokines	There was a striking reduction in Aβ plaque deposition and elevated levels of soluble Aβ in male mice. Antibiotic-induced perturbations in gut microbial diversity also influenced neuroinflammatory responses by conferring reduced Aβ plaque-localized gliosis and altered microglial morphology
Minter *et al.* (2017)	Early post-natal antibiotic-treated transgenic $APP_{SWE}/PS1_{\Delta E9}$ mouse model of AD	6.5 months	Bacterial 16S ribosomal RNA gene sequencing of DNA isolated from cecal and faecal samples Aβ plaque quantification and soluble and insoluble Aβ in brain tissues Aβ plaque-localized microglial populations Peripheral and central immune cell assessments Serum and CSF circulating chemokines and cytokines	Early post-natal antibiotic treatment resulted in long-term alterations of gut microbial genera (predominantly *Lachnospiraceae* and S24–7) and reduction in brain Aβ deposition. There were elevated levels of blood- and brain-resident Foxp3+ T-regulatory cells and an alteration in the inflammatory serum and CSF milieu, with reduction of Aβ plaque-localized microglia and astrocytes
Scott *et al.* (2017)	12 young (2-month-old) and 10 aged (18-month-old) male C57BL/6J mice	5 weeks	Bacterial 16S ribosomal RNA gene sequencing of DNA isolated from cecal samples Intestinal barrier function assessment Corticosterone response to acute stress Serum circulating cytokines	Older mice exhibited increased gut permeability compared to younger mice, which was directly correlated with elevations in peripheral pro-inflammatory cytokines. Furthermore, stress exacerbated the gut permeability of aged mice. Examination of the cecal microbiota revealed significant increases in phylum TM7, family *Porphyromonadaceae* and genus *Odoribacter* of aged mice
Harach *et al.* (2017)	APPPS1 transgenic mice, age-matched control wild-type littermates, and APPPS1 germ-free mice	1.5 and 8 months	Bacterial 16S ribosomal RNA gene sequencing of DNA isolated from faecal samples Aβ deposition and Aβ plaque-localized microglial reaction in brain tissues	Presence of a remarkable shift in the gut microbiota in transgenic mice as compared to non-transgenic wild-type mice. There was also a drastic reduction of cerebral Aβ pathology in APPPS1 germ-free mice when compared to control mice with gut microbiota.
Wu et al. (2017*b*)	*Drosophila* AD model with enterobacteria infection	13 days	Lifespan and locomotion activity Brain histology Brain ROS stress measurement Brain and circulating haemocytes	Enterobacteria infection exacerbated progression of AD in this model by promoting immune haemocyte recruitment to the brain, thereby provoking TNF-JNK mediated neurodegeneration

Aβ = amyloid-β; AD = Alzheimer's disease; JNK = c-Jun N-terminal kinase; ROS = reactive oxygen species.

localized with amyloid-β in amyloid plaques and blood vessels in Alzheimer's disease brains, suggesting that gut bacterial components may translocate in patients with Alzheimer's disease (Zhan *et al.*, 2016). A study involving 25 patients with Alzheimer's disease and 24 normal controls, found decreased amounts of *Firmicutes* and *Bifidobacterium* and increased *Bacteroidetes* in the faecal microbiome of patients with Alzheimer's disease (Vogt *et al.*, 2017). In contrast, a study of 43 patients with Alzheimer's disease and 43 controls, found differences between the two groups in several bacteria taxa with lower numbers of *Bacteroides*, *Ruminococcus*, *Lachnospiraceae* and *Selenomonadales*, and more *Actinobacteria* in patients with Alzheimer's disease (Zhuang *et al.*, 2018). Xu and Wang (2016) identified common genetic pathways underlying Alzheimer's disease biomarkers and trimethylamine *N*-oxide (TMAO), a gut microbial metabolite of dietary meat and fat. A study in 410 subjects found that CSF levels of TMAO were higher in individuals with MCI and Alzheimer's disease dementia compared to controls, which correlated to biomarkers of Alzheimer's disease pathology (phosphorylated tau and phosphorylated tau/amyloid-$\beta_{1–42}$) and neuronal degeneration (total tau and neurofilament light chain protein) (Vogt *et al.*, 2018). Bile acids are the end products of cholesterol metabolism produced by human and gut microbiome co-metabolism. Significant relationships between CSF bile acid profile and CSF amyloid-$\beta_{1–42}$, CSF tau and brain atrophy imaging biomarkers were found in a large cohort study involving 1562 Alzheimer's disease, MCI and normal subjects (Nho *et al.*, 2019). Another large study involving 1464 subjects reported reduced primary bile acid (cholic acid) and elevated bacterial-derived secondary bile acid (deoxycholic acid) in serum samples from patients with Alzheimer's disease (MahmoudianDehkordi *et al.*, 2019). It has been proposed that in patients with Alzheimer's disease, gut *Bacteroides fragilis* and HHV-1 activate NF-κB, with induction and stimulation of innate-immune and neuroinflammatory

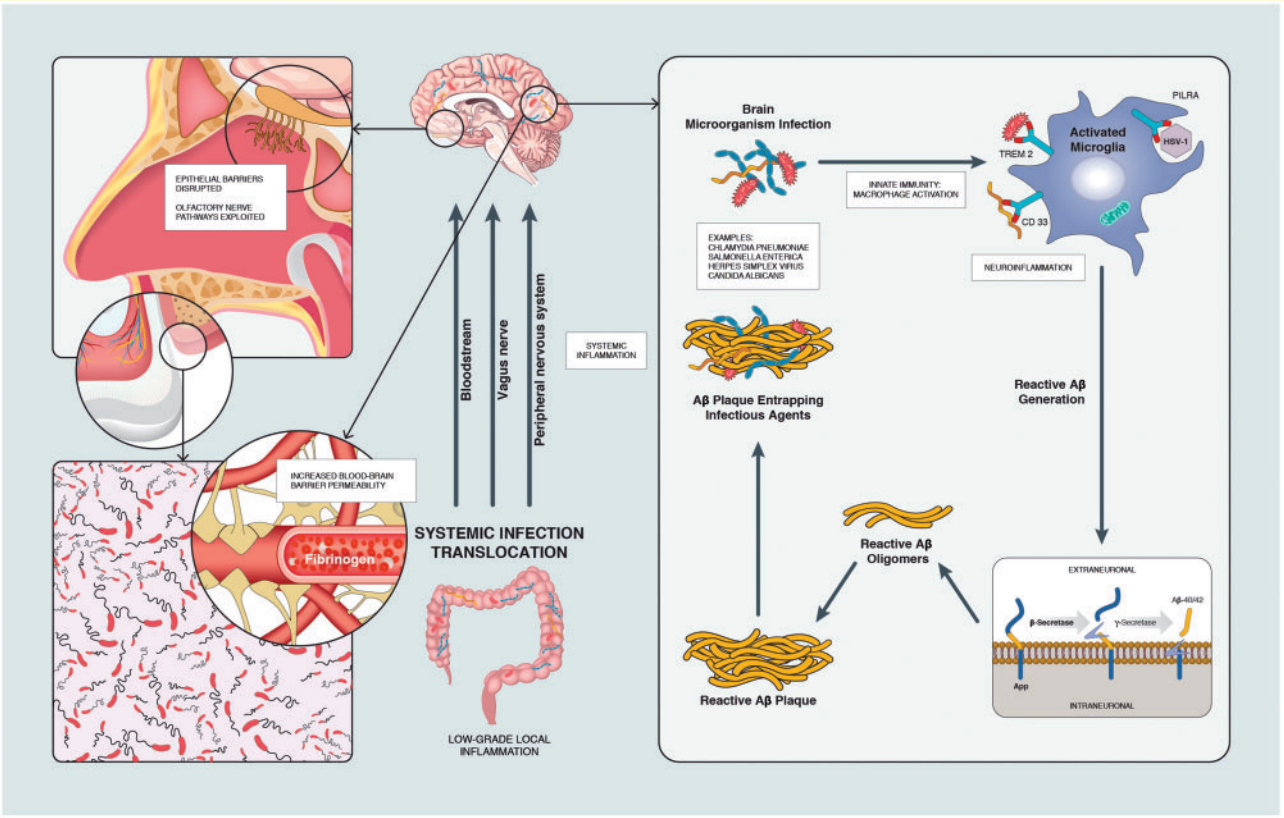

Figure 3 Scheme of the hypothetical process by which brain infection may lead to pathological amyloid-β plaque deposition in the Alzheimer's disease brain. The amyloid precursor protein (APP) is processed by β- and γ-secretases generating amyloid-β (Aβ). Amyloid-β functions as an antimicrobial peptide via oligomerization and plaque formation, trapping invading microorganisms, including bacteria (such as *Salmonella enterica* or *C. pneumonia*), fungi (such as *C. albicans*) and viruses (such as herpes simplex virus 1). Systemic infection and inflammation could weaken the blood–brain barrier (BBB), facilitate brain infection and trigger a neuroinflammatory response via activation of microglia with specific receptors triggering receptor expressed on myeloid cells 2 (TREM2), cluster of differentiation 33 (CD33, and human paired immunoglobulin-like type-2 receptor-α (PILR). A healthy microbiota could contribute to preventing systemic infection by limiting pathogen growth, maintaining blood–brain barrier function and training the host immune system, including microglia. Rifaximin treatment could limit overgrowth of pathogenic bacteria in the gastrointestinal tract while amoxicillin/clavulanic acid treatment could reduce bacterial load in the systemic circulation.

pathways (Zhao and Lukiw, 2018). Functional studies using stool samples from patients with Alzheimer's disease indicated that gut microbiome can affect intestinal health via dysregulation of the P-glycoprotein pathway (Haran *et al.*, 2019).

These clinical findings confirm preclinical data suggesting age-related alterations in the microbiota-gut-brain axis (including increased permeability and increased circulating inflammatory cytokines) seen in mice are associated with inflammation (Scott *et al.*, 2017) (Table 2). In the $APPS_{WE}/PS1\Delta_{E9}$ mouse model of Alzheimer's disease, the gut microbiota diversity was shown to regulate host innate immunity and impact amyloidosis in adult (Minter *et al.*, 2016) and newborn pathogen-free animals (Minter *et al.*, 2017), while fewer amyloid-β plaques developed when the latter were raised in a germ-free environment without gut microbiota (Harach *et al.*, 2017). Other findings suggest that microbiota may influence Alzheimer's disease neurodegeneration through molecular mimicry (Friedland, 2015), while a *Drosophila* Alzheimer's disease model supports a role for gut microbiota in modulating the progression of Alzheimer's disease (Wu *et al.*, 2017*b*). These *in vivo* experimental studies support the notion that human gut microbiota can contribute to Alzheimer's disease pathology by producing toxic metabolites, altering brain function, and contributing to local and systemic inflammation (Sherwin *et al.*, 2018). The relationship between gut microbiota and Alzheimer's disease is presented in Fig. 3.

Oral microbiota and Alzheimer's disease

Human oral bacteria accumulate on both hard and soft oral tissues in biofilms. A dynamic equilibrium exists between dental plaque bacteria and the innate host defence system and its perturbation may lead to dental caries and periodontal disease. Several clinical and preclinical studies

have associated poor oral health with increased ability of oral microbiota to reach the brain, affect cognitive function and increase risk of Alzheimer's disease (Tables 3 and 4). Periodontitis can challenge the brain with intact bacteria and inflammatory mediators due to daily, transient bacteraemias, and chronic periodontitis of 10-year duration is reported to double the risk of Alzheimer's disease (Tzeng *et al.*, 2016; Chen *et al.*, 2017). In three studies, raised serum IgG antibodies to periodontal pathogens were associated with established Alzheimer's disease (Kamer *et al.*, 2009; Sparks Stein *et al.*, 2012) or future Alzheimer's disease (Noble *et al.*, 2014). In one of these studies, plasma samples from 13 of 18 Alzheimer's disease subjects (73%) were serum-positive for at least one of the pathogens tested (*Aggregatibacter actinomycetemcomitans*, *P. gingivalis*, *and T. forsythia*) compared to only 6 of 16 controls (38%) (Kamer *et al.*, 2009). Tooth loss due to periodontal disease can double the risk for Alzheimer's disease onset (Gatz *et al.*, 2006; Stein *et al.*, 2007; Fang *et al.*, 2018). Irregular tooth brushing was also associated with higher dementia risk in a prospective study of 5468 residents of a Californian retirement community (Paganini-Hill *et al.*, 2012). In an observational cohort study of 60 mild-to-moderate patients with Alzheimer's disease, periodontitis at baseline was not related to baseline cognitive state but was associated with a 6-fold increase in the rate of cognitive decline in Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-Cog) score and an increased pro-inflammatory state over a 6-month follow-up period (Ide *et al.*, 2016). A study in 80 patients with Alzheimer's disease showed mean serum tumour necrosis factor-α (TNF-α) levels ~3-fold higher in patients with Alzheimer's disease with periodontitis compared to those without periodontitis (Farhad *et al.*, 2014). *P. gingivalis* (the principal pathogen in chronic periodontitis) and toxic proteases from the bacterium (gingipains) were identified in the brain of patients with Alzheimer's disease, with levels correlating with tau and ubiquitin pathology (Dominy *et al.*, 2019). During chronic periodontitis, leptomeningeal cells transmit systemic inflammatory signals from macrophages to brain-resident microglia (Olsen *et al.*, 2015). It has been hypothesized that oral pathogens can affect brain structures by two mechanisms. One possibility is proinflammatory cytokines travelling via the systemic circulation, the other is that periodontal bacteria or their products penetrate the CNS via the glossopharyngeal and/or trigeminal nerves (Kamer *et al.*, 2008*b*). A study in 38 cognitively normal elderly subjects found that clinical attachment loss ($\geqslant$3 mm), representing a history of periodontitis, was associated with increased amyloid-β load, as determined by ^{11}C-PIB PET imaging, in vulnerable brain regions (Kamer *et al.*, 2015). This observation was confirmed in mouse Alzheimer's disease models where periodontitis evoked by *P. gingivalis* increased brain amyloid-β deposition, elevated inflammatory markers (IL-1β and TNF-α) and impaired cognitive performance in affected mice compared to controls (Ishida *et al.*, 2017; Singhrao *et al.*, 2019) (Table 4). Of note, 10 wild-type 8-week-old C57BL/6 mice in which an experimental chronic periodontitis was induced by repeated oral application of *P. gingivalis*/gingipain for 22 weeks, showed neurodegeneration consistent with that of Alzheimer's disease and the formation of extracellular amyloid-β_{1-42} (Ilievski *et al.*, 2018). Moreover, Singhrao and colleagues (2017) found diffuse punctuate staining suggesting tissue damage and appearance of age-related granules in *APOE* gene knockout mice administered *P. gingivalis*. Notwithstanding, in another transgenic mouse model of Alzheimer's disease, continuous brain exposure of *P. gingivalis* LPS failed to enhance cognitive impairment (Hayashi *et al.*, 2019). It should be noted that while *P. gingivalis* and other oral bacteria may represent the primary cause of Alzheimer's disease or a co-factor in Alzheimer's disease pathogenesis, older subjects with dementia are more sensitive to oral bacterial and more prone to develop dental infections and therefore this putative causal association could be inverted. The relationship between periodontal disease and Alzheimer's disease is presented in Fig. 3.

Mechanisms of microbial infection of the brain

Viruses

Viruses can directly infect endothelial cells, cross the blood–brain barrier and enter the CNS. Paired immunoglobulin-like type 2 receptor alpha (PILRA) is a cell surface inhibitory receptor expressed on various innate immune cell types, including microglia. It has been shown that a common missense variant in PILRA (G78R) significantly reduces binding of the HHV-1 glycoprotein B (Rathore *et al.*, 2018). Macrophages derived from R78 homozygous donors showed significantly decreased levels of HHV-1 infection compared to homozygous G78 macrophages. Thus, it has been proposed that HHV-1 could infect the brain through PILRA receptors on microglia. PILRA G78R mutant may protect individuals from Alzheimer's disease risk via reduced inhibitory signalling in microglia and reduced microglial infection during HXV-1 recurrence (Rathore *et al.*, 2018). PILRA is also an entry receptor for HHV1, the pathogen most widely linked to Alzheimer's disease, which shows a preference for infecting the hippocampus, the brain region most severely affected by β-amyloidosis.

Bacteria

Microvascular endothelial cells (pericytes and astrocytes) of the blood–brain barrier protect the CNS by selectively controlling the flux of molecules in and out of the brain (Gloor *et al.*, 2001). Outside the CNS, the close contact between

Table 3 Principal clinical studies evaluating the direct or indirect association of oral microbiota and related diseases with Alzheimer's disease

Reference	Study design and sample/animal model	Duration	Measurements	Main findings
Gatz *et al.* (2006)	Case-control and a co-twin control from the Swedish Twin Registry 310 dementia cases and 3063 non-demented controls Mean age dementia cases: 79 years Mean age controls: 79.2 years	Risk factors were assessed independently 3 decades previously	AD and total dementia	Case-control findings showed that history of tooth loss before age 35 and low educational attainment were significant AD risk factors
Kamer *et al.* (2009)	Case-control study 18 AD patients and 16 non-demented controls	Cross-sectional analysis	IgG antibodies against Aa serotype b, Tf, and Pg and cytokine plasma assessment (TNF-α, IL-1β and IL-6) AD	Plasma TNF-α level and the number of positive tests for antibodies against periodontal bacteria were elevated in AD and independently associated with AD
Sparks Stein *et al.* (2012)	Longitudinal population-based study 158 cognitively healthy participants	12.5 years	IgG antibody levels to Aa, Tf, Pg, Cr, Td, Fn and Pi Incident MCI and AD	Serum antibody levels to Fn and Pi, were significantly increased at baseline in AD patients compared to controls
Paganini-Hill *et al.* (2012)	Longitudinal population-based study 5468 cognitively healthy participants Age range: 52–105 years (median 81 years)	18 years	Questions regarding dental health Incident dementia	Dentate individuals who reported not brushing their teeth daily had a 22% to 65% greater risk of dementia than those who brushed three times daily
Noble *et al.* (2014)	Case-cohort study design 219 subjects (110 incident AD cases and 109 controls without incident cognitive impairment at last follow-up) Mean age AD cases: 79 years Mean age controls: 72 years	5 years	Serum IgG levels to Pg, Tf, *Actinobacillus actinomycetemcomitans* Y4, Td, Cr, En, and An genospecies-2) Incident AD	High serum An IgG antibody was associated with increased risk of AD
Farhad *et al.* (2014)	Case-control study 40 AD patients with chronic periodontitis and 40 AD patients without chronic periodontitis Age range: 40–70 years	Cross-sectional analysis	Serum assessment of TNF-α	Mean serum TNF-α levels was ~3-fold higher in AD patients with chronic periodontitis compared to those without chronic periodontitis
Kamer *et al.* (2015)	Community-based sample of 38 cognitively healthy participants Mean age: 69.2 years	Cross-sectional analysis	Periodontal disease assessed by clinical attachment loss Brain Aβ load using ^{11}C-PIB PET	Clinical measures of periodontal disease in cognitively normal healthy older subjects were positively associated with the magnitude of brain amyloid accumulation
Tzeng *et al.* (2016)	Nationwide, retrospective, matched-cohort study 2207 patients, with newly-diagnosed chronic periodontitis and gingivitis selected from the National Health Insurance Research Database of Taiwan along with 6621 controls matched for sex and age	10 years	Chronic periodontitis and gingivitis Incident dementia	Patients with chronic periodontitis and gingivitis had a higher risk of developing dementia
Ide *et al.* (2016)	560 community-dwelling participants with mild-to-moderate AD	6 months	Periodontitis ADAS-cog	The presence of periodontitis at baseline was associated with a 6-fold increase in the rate of cognitive decline and a relative increase in the pro-inflammatory state over a 6-month follow-up period
Chen *et al.* (2017)	Nationwide, retrospective, matched-cohort study 9921 patients, with newly diagnosed chronic periodontitis selected from the National Health Insurance Research Database of Taiwan along with 18672 controls matched for sex, age, index year, comorbidity and urbanization level	10 years	Chronic periodontitis Incident AD	Patients with chronic periodontitis had a 1.707-fold increase in the risk of developing AD
Dominy *et al.* (2019)	Human post-mortem brain tissue microarrays from 29 dementia-free control individuals and 29 AD cases Specific pathogen-free female BALB/c mice	6–10 weeks	Presence of Pg DNA and gingipain antigens in AD brains Effects of oral administration of small-molecule gingipain inhibitors	This study demonstrated the presence of Pg DNA and gingipain antigens in AD brains. Gingipain levels also correlated with tau and ubiquitin pathology in AD brains. Blocking gingipain-induced neurodegeneration with small-molecule gingipain inhibitors significantly reduced Pg load in the mouse brain, and significantly decreased the host Aβ_{1-42} response to Pg brain infection

Aa = *Aggregatibacter actinomycetemcomitans*; AD = Alzheimer's disease; ADAS-cog = Alzheimer's Disease Assessment Scale-Cognition; An = *Actinomyces naeslundii*; Aβ = amyloid-β; Cr = *Campylobacter rectus*; En = *Eubacterium nodatum*; Fn = *Fusobacterium nucleatum*; Pi = *Prevotella intermedia*; IgG = *immunoglobulin G*; IL = interleukin; Pg = *Porphyromonas gingivalis*; Td = *Treponema denticola*; Tf = *Tannerella forsythia*; TNF-α = tumor necrosis factor-α.

Table 4 Principal preclinical studies evaluating the direct or indirect association of oral microbiota and related diseases with Alzheimer's disease

Reference	Study design and sample, animal model	Duration	Measurements	Main findings
Ishida *et al.* (2017)	Transgenic APP-Tg mice model of AD with or without infection with Pg	5 weeks	Levels of TNFα, IL1β, Aβ deposition, $A\beta_{1-40}$, $A\beta_{1-42}$, and Aβ oligomers in brain tissues Levels of LPS in serum and brain tissues	Levels of Aβ deposition, $A\beta_{1-40}$, and $A\beta_{1-42}$, IL-1β and TNF-α were higher in the brain of inoculated APP-Tg mice than in control APP-Tg mice. The levels of LPS were increased in the serum and brain of Pg-inoculated mice suggesting that periodontitis evoked by Pg may exacerbate brain Aβ deposition
Singhrao *et al.* (2017)	$Apoe^{-/-}$ B6 background mice with or without chronic infection with Pg	24 weeks	Presence of Pg/gingipain in brain tissues Presence of age-related granules in brain tissues	The findings suggested clusters of granules in mice brains infected with Pg and areas of diffuse punctate staining supporting the possibility of early appearance of age-related granules in $Apoe^{-/-}$ mice following inflammation-mediated tissue injury
Ilievski *et al.* (2018)	20 6-week-old male C57BL/6 mice with or without repeated oral application of Pg/gingipain	22 weeks	Presence of Pg/gingipain in brain tissues Signs of AD neuropathology in hippocampi TNFα, IL1β, and IL6 expression, intact and degrading neurons, and $A\beta_{1-42}$ production and phosphorylation of tau protein Gene expression of APP, BACE1, ADAM10, and PSEN1 Microgliosis and astrogliosis	Presence of neurodegeneration and the formation of extracellular $A\beta_{1-42}$ in young adult wild type mice after repeated oral application of Pg, suggesting that low grade chronic periodontal pathogen infection may result in the development of AD neuropathology
Hayashi *et al.* (2019)	6- (young) and 13- (middle-aged) month-old 5XFAD mouse model of AD and 6-month-old littermate mice and treated with intracerebroventricular injection of Pg-LPS or saline	28 days	Assessment of cognitive functions, motor functions, and physical condition Brain immunohistochemical findings $A\beta_{1-40/42}$ brain deposition	Continuous intracerebroventricular injection of Pg-LPS increased ionized calcium binding adapter molecule-1 and cluster of differentiation 3 positive cells in periventricular area of 5XFAD mice without enhancement of cognitive impairment and Aβ protein deposition

AD = Alzheimer's disease; $Apoe^{-/-}$ = apolipoprotein ε gene knockout; Aβ = amyloid-β; IL = interleukin; LPS = lipopolysaccharides; Pg = *Porphyromonas gingivalis*; TNF-α = tumor necrosis factor-α.

nerve endings and immune cells is enhanced during inflammatory responses, particularly at interfaces with the external environment, i.e. mucosal sites (Kraneveld *et al.*, 2014). The identification of bacteria in the CNS of patients with Alzheimer's disease raises the question as to how they reach neuronal tissue. One possibility is that gut microbiota and highly pro-inflammatory neurotoxins (such as LPS) cross the gastrointestinal mucosa and reach the circulation, underscoring the critical role of cellular adhesion structures in allowing passage of such noxious entities (Montagne *et al.*, 2015; van de Haar *et al.*, 2016). Subsequently, these entities reach other organs and tissues, including the nervous system. Bacterial DNA has been identified in blood from healthy individuals, mostly from the *Proteobacteria* phylum (>80%) but also from *Actinobacteria*, *Firmicutes*, and *Bacteroidetes* species. Such DNA is located in the buffy coat (93.7%), erythrocytes (6.2%) and plasma (0.03%) (Paisse *et al.*, 2016). The acute inflammatory response to pathogens may be impaired during ageing, thus promoting infection sustainability (Pawelec *et al.*, 2014). The chronic presence of diverse microorganisms in the bloodstream could lead to both remote infection and/or a systemic and local pro-inflammatory state, thereby facilitating the development of Alzheimer's disease (Licastro *et al.*, 2014). Consistent with this model is the finding that some peripheral inflammatory markers are associated with Alzheimer's disease (Lai *et al.*, 2017) and may increase steadily in blood and CSF during disease progression or temporarily at the time of MCI to Alzheimer's disease conversion (Brosseron *et al.*, 2014). Another route of entry for microbes into the CNS could be the oral cavity (as mentioned above) and the nasopharyngeal region, with transmission via the olfactory nerve, olfactory bulb and entorhinal area (Olsen and Singhrao, 2015). Repeated assault weakens the blood–brain barrier, the brain's resilience is increasingly compromised and, with endotoxin intolerance and further inflammation, the brain tips into disease (Pritchard *et al.*, 2017). Recent evidence shows that blood–brain barrier breakdown is an early biomarker of human cognitive dysfunction independent of amyloid-β and tau (Nation *et al.*, 2019). Although adaptive immunity is constrained in the brain, the innate immune system is highly active (Lampron *et al.*, 2013). Infective damage to the CNS triggers the release of inflammatory mediators and

activation of innate immunity (Franceschi and Campisi, 2014), and accumulation/activation of microglia and astrocytes via cellular and molecular immune factors, which in turn stimulate production of amyloid-β (Schwab and McGeer, 2008). Replicating pathogens release component molecules that can be identified by pattern-recognition receptors on antigen presenting cells, principally microglia (Barichello *et al.*, 2015). Vascular pathology is a key feature of Alzheimer's disease, and fibrinogen induces microglia-mediated spine elimination and cognitive decline, effects that are mediated by reactive oxygen species and are independent of amyloid-β plaques (Merlini *et al.*, 2019). Unsurprisingly, an acute inflammatory response in the brain is beneficial and leads to repair the affected area and restoration of brain homeostasis, while a self-perpetuating, progressive inflammation leads to the chronic activation of harmful molecular pathways and neurodegeneration (Ashraf *et al.*, 2019).

Antibacterial activity of amyloid-β and the antimicrobial protection hypothesis of Alzheimer's disease

Several *in vitro* and *in vivo* studies have shown that amyloid-β displays antimicrobial properties. amyloid-$\beta_{1–42}$ exerts antimicrobial activity against eight (*C. albicans*, *E. coli*, *Staphylococcus epidermidis*, *Staphylococcus pneumoniae*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Enterococcus faecalis*, *Streptococcus agalactiae*) of 12 common and clinically relevant microorganisms with a potency equivalent to (and in some cases greater than) LL-37, an archetypical human antimicrobial peptide (Soscia *et al.*, 2010). Synthetic amyloid-β has also been shown to protect cultured cells from H3N2 and H1N1 influenza A viruses, with amyloid-$\beta_{1–42}$ showing greater activity than amyloid-$\beta_{1–40}$. amyloid-$\beta_{1–42}$ caused aggregation of virus particles and reduced virus-induced IL-6 production in monocytes (White *et al.*, 2014). Amyloid-β also has antiviral activity against HHV-1 replication in human lung fibroblasts, epithelial, neuroglioma and glioblastoma cell lines (Bourgade *et al.*, 2015, 2016). These observations were followed by *in vivo* testing of amyloid-β against fungal (*C. albicans*) and bacterial (*Salmonella typhimurium)* infections in transgenic mice, in the nematode *Caenorhabditis elegans*, and in human brain neuroglioma cell line (H4) models of Alzheimer's disease (Kumar *et al.*, 2016). Surprisingly, this activity was mediated by amyloid-β oligomerization, a phenomenon traditionally viewed as intrinsically pathological. Transgenic mice overexpressing human Alzheimer's disease genes (5XFAD) intracerebrally infected with *S. typhimurium* showed longer survival, better clinical scores and less body weight loss compared to wild-type mice (Kumar *et al.*, 2016). These data suggested a protective role for amyloid-β, in particular the amyloid-β oligomeric species. Amyloid-β oligomers protect against HSV infections by binding herpesvirus surface glycoproteins, accelerating amyloid-β deposition and promoting protective viral entrapment activity in 5XFAD mouse and 3D human neural cell culture infection models against HHV-1 and HHV-6A and HHV-6B (Eimer *et al.*, 2018).

These studies of the antimicrobial properties of amyloid-β have revealed a physiological protective role against viral, bacterial and fungal infections. This, and recent findings on inflammation-mediated neurodegeneration and the role of amyloid-β in immunity, have engendered the 'Antimicrobial Protection Hypothesis' of Alzheimer's disease (Moir *et al.*, 2018) in which the over-production of amyloid-β in the Alzheimer's disease brain results from neuronal efforts to neutralize microbial infection (Fulop *et al.*, 2018*a*). In this model, amyloid-β deposition is an early innate immune response to microbial challenge. Initially, amyloid-β entraps and neutralizes invading pathogens in amyloid-β oligomers, with amyloid-β fibrillization driving neuroinflammatory pathways to help fight the infection and clear amyloid-β-pathogen deposits. These beneficial effects would become progressively detrimental as infection continues due to sustained inflammation and neurodegeneration (Fulop *et al.*, 2018*a*). In the Alzheimer's disease brain, the ability to remove excess amyloid-β would decrease over time because of microglial senescence and formation of microbe-amyloid-β aggregates. The triggering receptor expressed on myeloid cells 2 (*TREM2*) variants associated with Alzheimer's disease induce partial loss of function of the TREM2 protein and alter the behaviour of microglial cells, including their response to amyloid plaques (Carmona *et al.*, 2018). A single nucleotide polymorphism that modulates CD33 splicing to favour CD33m is associated with enhanced microglial activity, and individuals expressing the more protective isoform accumulate less brain amyloid-β and have a reduced Alzheimer's disease risk (Siddiqui *et al.*, 2017). Clinical trials testing anti-amyloid-β drugs in patients with Alzheimer's disease may indirectly support the hypothesis of an antimicrobial role for amyloid-β, in that an increase in infections (specifically orolabial herpes relapse) was reported in clinical trials of anti-amyloid-β drugs, such as β- and γ-secretase inhibitors, the γ-secretase modulator tarenflurbil, and the amyloid-β-binding compound ELND005 (Gosztyla *et al.*, 2018).

Amyloid-β is a physiological, ubiquitously expressed peptide involved in synaptic function, long-term potentiation, and memory function (Bishop *et al.*, 2004). Increases in amyloid-β secretion have been described in several other clinical conditions other than Alzheimer's disease implicating acute (sleep deprivation, traumatic brain injury, general anaesthesia or acute cerebral ischaemia) or chronic (chronic cerebral ischaemia, chronic traumatic encephalopathy, depression or amyotrophic lateral sclerosis) brain injury (Panza *et al.*, 2019). These amyloid-β increases might

represent an attempt by the brain to mitigate or repair neuronal damage or insult (Brothers *et al.*, 2018). Similarly, in Alzheimer's disease, amyloid-β overproduction could represent an attempt to ameliorate the loss of neuronal functioning (Kokjohn *et al.*, 2012). The negative clinical results of anti-amyloid-β therapies and the detrimental cognitive and behavioural effects of γ- and β-secretase inhibitors seem to confirm that in patients with sporadic Alzheimer's disease, amyloid-β accumulation could be a reactive compensatory response to neuronal damage of unknown cause, one of which could be microbial invasion.

Antiviral agents and Alzheimer's disease

The reported association between viruses and Alzheimer's disease suggest that antiviral agents could be beneficial in slowing down the rate of decline of patients with Alzheimer's disease. A population-based study has found that 5.8% of HSV-infected patients treated with anti-herpetic agents developed senile dementia in 10 years compared to 28.3% of untreated HSV-infected patients (Tzeng *et al.*, 2018). Another population-based cohort study of 78 410 subjects identified 39 205 with herpes zoster (HHV-5) infection/exposure and 39 205 matching subjects, of whom 4204 (5.4%) were diagnosed as having dementia during a mean follow-up period of 6.2 years. Use of antiviral therapy in subjects with HHV-5 was associated with a 45% lower risk of developing dementia compared to untreated infected subjects (Chen *et al.*, 2018). An 18-week, double-blind, placebo-controlled study of valacyclovir in 24 HSV-1-seropositive schizophrenia subjects showed that patients on the antiviral drug improved in verbal memory, working memory, and visual object learning compared with those on placebo, although psychotic symptoms did not improve. Both groups were taking antipsychotic medication (Prasad *et al.*, 2013). As a result of these findings, it has been suggested that vaccination against HSV-1 should be considered to prevent the development of Alzheimer's disease (Harris and Harris, 2018; Itzhaki, 2018; Ashraf *et al.*, 2019). Although an anti-HSV-1 vaccine is not yet available, a vaccine of mixed HSV-1 glycoprotein has been shown to be effective in reducing HSV-1 in mouse brain after peripheral infection (Lin *et al.*, 2001). Below, we briefly review the main antiviral agents that are being or could be evaluated in patients with Alzheimer's disease.

Acyclovir and valacyclovir

Acyclovir is an antiviral medication primarily used for HSV infections, chickenpox, and shingles. Other uses include prevention of HCMV infections following transplant and severe complications of EBV infection. Acyclovir is a nucleoside analogue and interferes with HHV-1 DNA replication by integrating into viral DNA to induce premature chain termination (Elion, 1982). After oral administration, valacyclovir is rapidly hydrolysed to acyclovir in the intestine and liver, which crosses the blood–brain barrier to reach the CNS (Smith *et al.*, 2010). In an *in vitro* study in HSV-1-infected kidney epithelial cells, acyclovir inhibited amyloid-β and phosphorylated-tau accumulation, as well as HSV-1 proteins in a concentration-dependent fashion (Wozniak *et al.*, 2011). Phosphorylated-tau accumulation was dependent on HSV-1 DNA replication, whereas amyloid-β accumulation was not. The antiviral-induced decrease in amyloid-β was ascribed to reduced viral replication (Wozniak *et al.*, 2019).

A 4-week open label study is evaluating the effects of valacyclovir in 36 Alzheimer's disease/MCI subjects with HSV IgG-positivity and bearing the *APOE* ε4 allele (ClinicalTrials.gov Identifier:NCT02997982). Valaciclovir is being administered at 500 mg thrice daily the first week and 1000 mg thrice daily for Weeks 2–4. Efficacy variables include Mini Mental State Examination (MMSE), CSF biomarkers and ^{18}F-FHBG-PET as a biomarker of active HSV infection within the brain. The study should be completed in April 2019. An 18-month, double-blind, placebo-controlled study of valacyclovir (2–4 g/day) in 130 patients with mild Alzheimer's disease positive for HSV-1 or HSV-2 is presently underway. The primary efficacy measure is the ADAS-Cog, while secondary efficacy variables are the Alzheimer's Disease Cooperative Study-Activities of Daily Living (ADCS-ADL) scale, ^{18}F-florbetapir amyloid-β PET, and ^{18}F-MK-6240 tau PET; completion is expected in August 2022.

Penciclovir and foscarnet

Penciclovir is a guanosine analogue antiviral drug used for various herpesvirus infections. Foscarnet is a DNA polymerase inhibitor primarily used to treat herpesviridae infections. *In vitro* studies show that both drugs concentration-dependently inhibit amyloid-β and phosphorylated-tau accumulation induced by HSV-1 infection, foscarnet being less potent than penciclovir (Wozniak *et al.*, 2011).

Pleconaril and ribavirin

Pleconaril is an antiviral drug administered either orally or intranasally, is active against viruses in the *Picornaviridae* family, including *Enterovirus* and *Rhinovirus*. Ribavirin is an antiviral medication used to treat human respiratory syncytial virus infection, hepatitis C, and viral haemorrhagic fever. A double-blind, placebo-controlled study of combined pleconaril and ribavirin in 69 subjects with mild Alzheimer's disease found that the combination was not well tolerated; the drop-out rate was ~50% because of ribavirin side effects. At 9 months, only 18 subjects remained in the active group, with 31 taking placebo. Nevertheless, cognition (ADAS-Cog) and clinical global status (Clinical Dementia Rating - Sum of Boxes, CDR-SB) worsened steadily in the placebo group over time,

while the treated group improved by three ADAS-Cog points, with CDR-SB unchanged (Wahlund *et al.*, 2018). A new, 1-year, double-blind, placebo-controlled study of 600 mg/day pleconaril alone in 120 patients with Alzheimer's disease, started in July 2018.

Anti-HIV drugs

HIV therapies may help stop genes linked to inflammation associated with ageing. The reverse transcriptase inhibitor antiretroviral therapies block an enzyme essential for viral replication and may offer a new way of treating age-related disorders such as Alzheimer's disease, as recently evidenced in a study on aged mice treated with lamivudine (De Cecco *et al.*, 2019). Lamivudine has also been shown to reduce age-associated chronic inflammation in multiple tissues. Sterile inflammation is a hallmark of ageing and a contributing factor to many age-related diseases such as Alzheimer's disease (Franceschi and Campisi, 2014).

Antimicrobial agents and Alzheimer's disease

In vivo studies have shown that long-term broad-spectrum antibiotic treatment decreases amyloid-β plaque deposition, attenuates plaque-localized glial reactivity and alters microglial morphology in the $APP_{SWE}/PS1_{\Delta E9}$ mouse model of Alzheimer's disease (Minter *et al.*, 2016). A 34-month, controlled study of (unspecified) antibiotics versus palliative care in 104 institutionalized patients with Alzheimer's disease found that average survival rates for the patients with more severe disease were 38% for palliative care versus 47% for the antibiotic approach. In less severely affected patients, survival was significantly higher for antibiotic (100%) than the palliative (60%) recipients (Fabiszewski *et al.*, 1990). Another study in 68 patients with advanced Alzheimer's disease showed that antibiotic use was significantly associated with prolonged survival. Of patients surviving for more than 6 months, 31% were on antibiotic care versus 14% on palliative care ($P = 0.003$) (Volicer *et al.*, 1993). However, we did not find epidemiological data suggesting the hypothesis that antibiotic use is associated to decrease risk of Alzheimer's disease onset. Below, we briefly review preclinical studies of antibacterials in animal models of Alzheimer's disease and controlled studies of antibiotics in patients with Alzheimer's disease.

Cycloserine

Cycloserine is an oral antibiotic used to treat tuberculosis, which was tested in the past in patients with Alzheimer's disease based on its NMDA subtype glutamate receptor modulating properties. A 10-week, double-blind, placebo-controlled trial in 108 patients with mild-to-moderate Alzheimer's disease compared cycloserine (5, 15, or 50 mg, twice daily) with placebo. Implicit memory performance of words repeated across trials was improved compared to placebo for all three cycloserine doses, the effect of the 15 mg dose being significant (Schwartz *et al.*, 1996). In another 4-week, double-blind, placebo-controlled study in 17 patients with Alzheimer's disease, cycloserine 100 mg/day produced significant improvement in ADAS-Cog compared to placebo (Tsai *et al.*, 1999).

Doxycycline and rifampicin

Doxycycline is a tetracycline antibiotic that is used in the treatment of bacterial pneumonia, acne, chlamydia infections, early Lyme disease, cholera and syphilis. Rifampicin, also known as rifampin, is an antibiotic used to treat tuberculosis, Mycobacterium avium complex, leprosy, and Legionnaires' disease.

In a neuroblastoma cell line, doxycycline prevented amyloid-β fibrillization and favoured the generation of smaller, non-amyloid structures that were non-toxic, while administration of doxycycline to amyloid-β_{1-42}-expressing flies did not improve their lifespan but was able to slow the progression of their locomotor deficits (Costa *et al.*, 2011). A study in 15-month-old APP/PS1 transgenic mice showed that doxycycline, administered with different treatment regimens, was able to attenuate memory deficit without brain plaque reduction (Balducci *et al.*, 2018). An acute doxycycline treatment was also sufficient to improve APP/PS1 mouse memory, suggesting an action against soluble amyloid-β oligomers. This was confirmed in an amyloid-β oligomers-induced mouse model, where the amyloid-β oligomers-mediated memory impairment was abolished by doxycycline pretreatment. In both the amyloid-β oligomers-treated and APP/PS1 mice, the memory recovery was associated with lower neuroinflammation (Balducci *et al.*, 2018).

In vitro, rifampicin inhibited oligomer formation of amyloid-β, tau, and α-synuclein (Umeda *et al.*, 2016). In 13-month-old Tg2576 mice, oral rifampicin at 0.5 mg/day for 1 month decreased amyloid-β oligomer accumulation, tau hyperphosphorylation, synapse loss, and microglial activation, but not amyloid deposition. Rifampicin treatment of 14–15-month-old tau609 mice at 0.5 and 1 mg/day for 1 month also reduced tau oligomer accumulation, tau hyperphosphorylation, synapse loss, and microglial activation in a dose-dependent fashion, and improved the memory almost completely at 1 mg/day (Umeda *et al.*, 2016). Doxycycline was shown to disassemble amyloid-β fibrils (Forloni *et al.*, 2001) and suppresses mutant tau production in transgenic mice (SantaCruz *et al.*, 2005).

Doxycycline and rifampin were studied in patients with Alzheimer's disease based on their anti-amyloid-β and anti-tau properties. An initial 12-month study in 101 patients with mild-to-moderate Alzheimer's disease on the combined antibiotics (doxycycline 200 mg/day + rifampin 300 mg/day) for 3 months showed beneficial effects on ADAS-Cog at 6 months in the antibiotic group compared to the placebo group. At 12 months, the difference between

groups was not significant. There were no differences in *C. pneumoniae* detection using PCR or antibodies between groups (Loeb *et al.*, 2004). However, a subsequent 12-month, double-blind, placebo-controlled study in 406 patients with mild-to-moderate Alzheimer's disease of doxycycline (200 mg/day) alone or in combination with rifampin (300 mg/day) failed to show any beneficial effects on cognition or function with either regimen (Molloy *et al.*, 2013).

Ceftriaxone

Ceftriaxone, a third-generation cephalosporin, is an antibiotic useful for the treatment of a number of bacterial infections. Tg2576 transgenic mice intraperitoneally infected with *Streptococcus pneumoniae* and treated with ceftriaxone (100 mg/kg, s.c.) did not show altered motor or cognitive performance or brain amyloid-β plaque load compared to uninfected mice (Ebert *et al.*, 2010). In the 3xTg-Alzheimer's disease transgenic mouse model of Alzheimer's disease, ceftriaxone [200 mg/kg intraperitoneally (i.p.) for 2 months] has been shown to ameliorate tau accumulation, restore synaptic proteins, and rescue cognitive decline with minimal effects on amyloid-β pathology (Zumkeher *et al.*, 2015). In accelerated senescent OXYS rats, a 5-week treatment with ceftriaxone (50 or 100 mg/kg/day, i.p.) partially inhibited impairments of movement and restored the deficit in the novel object recognition test. Both doses of ceftriaxone increased the density of pyramidal neurons in the hippocampal CA1 area (Tikhonova *et al.*, 2017). Based on these encouraging results in murine models of Alzheimer's disease, ceftriaxone has been recently proposed for the treatment of neurodegenerative diseases (Tai *et al.*, 2019).

Erythromycin

Erythromycin is a macrolide antibiotic used for the treatment of a number of bacterial infections. A pilot study in the TgCRND8 transgenic mouse model of Alzheimer's disease, has shown that 3 months treatment with erythromycin in the drinking water (0.1 g/l) reduced the amyloid-$\beta_{1–42}$ levels in the cortex by 54% compared to vehicle-treated animals (Tucker *et al.*, 2005). These results were replicated in a further study in TgCRND8 mice (Tucker *et al.*, 2006).

Minocycline

Minocycline is a broad-spectrum tetracycline antibiotic used for the treatment of acne vulgaris and other skin infections, and Lyme disease. Several studies suggest that minocycline has neuroprotective and anti-neuroinflammatory actions in a number of animal models. In microglial cell cultures, minocycline was able to attenuate oligomeric amyloid-β-induced neuroinflammatory response and enhance of fibrillar amyloid-β phagocytosis (El-Shimy *et al.*, 2015). These effects appear linked to the ability of minocycline to selectively inhibit microglial polarization to a proinflammatory state (Kobayashi *et al.*, 2013). Minocycline has also been shown to protect against NMDA-induced cell death by inhibiting 5-lipoxygenase activation (Song *et al.*, 2006). In the early stages of the Alzheimer's disease-like amyloid-β pathology, minocycline treatment (50 mg/kg for 4 weeks) attenuated behavioural abnormalities, neuroinflammatory markers, and amyloid-β in a transgenic hAPP mouse model of Alzheimer's disease (Cuello *et al.*, 2010). In 3xTg-Alzheimer's disease mice, 4 months treatment with minocycline (55 mg/kg/day in food), reduced brain levels of insoluble amyloid-β, decreased neuroinflammatory markers (GFAP, TNF-α and IL-6) and reversed cognitive deficit (Parachikova *et al.*, 2010). In another mouse model of Alzheimer's disease featuring intracerebroventricular administration of amyloid-$\beta_{1–42}$ oligomers, minocycline (50 mg/kg for 17 days) was able to reduce brain inflammatory parameters (IL-1β, TNF-α and IL-10) and reverse spatial memory impairment (Garcez *et al.*, 2017). Based on its anti-inflammatory and neuroprotective properties, minocycline has been proposed for the treatment of patients with Alzheimer's disease (Hashimoto, 2011; Budni *et al.*, 2016). A 2-year, double-blind, placebo-controlled study of minocycline (200 and 400 mg/day) in 480 patients with early Alzheimer's disease recently completed in the UK, but is not yet published.

Amoxicillin and clarithromycin

Amoxicillin is a broad-spectrum antibiotic used for the treatment of numerous bacterial infections. Clarithromycin is a macrolide antibiotic used to treat various bacterial infections, including Lyme disease. In a 2-year study involving 56 histologically *H. pylori*-positive patients with Alzheimer's disease, 33 patients underwent bacteria eradication with triple therapy (omeprazole, clarithromycin and amoxicillin) and 23 controls did not. *H. pylori* eradication was successful in 28 patients with Alzheimer's disease (85%) of treated patients. After 2 years, cognitive (MMSE and Cambridge Cognitive Examination for the Elderly) and functional (Functional Rating Scale for Symptoms of Dementia) performance significantly improved in the subgroup of patients with *H. pylori* eradication but not in the other patients (Kountouras *et al.*, 2009*a*).

Gingipains inhibitors

Toxic proteases from *P. gingivalis*, called gingipains, have been identified in the brain of Alzheimer's disease, and were neurotoxic *in vivo* and *in vitro*, exerting detrimental effects on tau. A number of small-molecule inhibitors targeting gingipains have been identified recently (Dominy *et al.*, 2019). Gingipain inhibitors reduced the bacterial load of an established *P. gingivalis* brain infection model, blocked amyloid-$\beta_{1–42}$ production, reduced neuroinflammation, and

rescued neurons in the hippocampus (Dominy *et al.*, 2019). A small 4-week, double-blind, placebo-controlled phase 1 study with an oral antibacterial (COR388) that targets gingipains, in nine patients with mild-to-moderate Alzheimer's disease who all had fragments of DNA from *P. gingivalis* in their CSF at baseline, has completed recently. There were no withdrawals because of adverse events, the pharmacokinetic profile of COR388 was similar to that in healthy volunteers, and CSF levels of COR388 were detected at levels similar to that seen in nonclinical studies, indicating high brain penetration (Mackins, 2018). A 48-week, double-blind, placebo-controlled trial (GAIN) in 573 patients with mild-to-moderate Alzheimer's disease was recently started in 90 sites in the USA and Europe (ClinicalTrials.gov Identifier:NCT03823404).

Probiotics

Dietary probiotic bacteria, which are live microorganisms and considered to be beneficial for health, are often prescribed to patients after a course of antibiotics in order to rebuild their gut bacteria. It has been shown that treatment with probiotics increases brain performance, as measured by a maze test and an altered microbiome environment (Athari Nik Azm *et al.*, 2018; Leblhuber *et al.*, 2018). Although the precise mechanisms of the effects of probiotics on memory and learning have not been fully established, some studies have suggested mechanisms including changing hypothalamic–pituitary–adrenal activity, increasing the expression of brain neurotrophic factor (a protective agent and the main modulator of synaptogenesis in the hippocampus) and increasing GABA (O'Hagan *et al.*, 2017). However, it seems that probiotics can affect memory and plaque formation by influencing pathological mechanisms involved in Alzheimer's disease, such as oxidative stress. Treatment with probiotics might serve as a potential tool to retard the progress of Alzheimer's disease. Furthermore, chronic neurodegenerative diseases, including Alzheimer's disease, have a high rate of gastrointestinal comorbidities and it has been proposed that management of the gut microbiota by probiotics may prevent or alleviate the symptoms of these chronic diseases (Westfall *et al.*, 2017). However, studies that evaluate the potential benefit of probiotic supplements in the course of Alzheimer's disease are still scarce.

Among others functions, probiotic supplementation is reported to cause a significant increase of serum kynurenine, which could indicate a stimulation of the immune system, leading to the modulation of the tryptophan pathway involving indoleamine 2,3-dioxygenase-1 (Widner *et al.*, 2000). The progression of Alzheimer's disease is associated with an increase of immune activation, which is reflected by increasing neopterin (Parker *et al.*, 2013) and kynurenine concentrations (Giil *et al.*, 2017).This immunobiological response could represent a means through which the Th1-type immune system tries to compensate for the mechanism driving the pathogenesis of Alzheimer's disease, involving events that relate to Th2 type immunity. However, although the reported changes indicate activation of immunological processes, and the stimulation of anergic immune cells for triggering mechanisms that are helpful in removing amyloid aggregates and damaged cells, overly intensive activation could negatively impact gut barrier function and further stimulate neurodegenerative processes (Leblhuber *et al.*, 2018).

Furthermore, evidence for probiotic impact on post-antibiotic reconstitution of the gut mucosal host-microbiome niche remains elusive. A recent study warns that probiotics should be treated as a drug, not as a food supplement (Rao *et al.*, 2018). The use of probiotics can result in an accumulation of bacteria in the small intestine, producing D-lactic acidosis that may be temporarily toxic to brain cells, interfering with cognition and thinking and leading to gastrointestinal bloating. Typically, D-lactic acidosis is caused by the fermentation of ingested carbohydrate by D-lactic acid-producing bacteria such as lactobacillus and bifidobacterium in the bowel (Oh *et al.*, 1979; Uribarri *et al.*, 1998). Thus, the presumed probiotic-induced protection from antibiotic-associated adverse effects may not be risk-free. Probiotics could perturb rather than aid in microbiota recovery after antibiotic treatment in humans (Suez *et al.*, 2018).

Future perspectives and conclusions

A pathogenetic role for viral or bacterial infection in Alzheimer's disease onset has long been suspected and has recently received further support (Balin and Hudson, 2018; Moir *et al.*, 2018). At least two possibilities can be envisaged to explain the association between microbial infections and Alzheimer's disease. One is that patients with Alzheimer's disease are particularly prone to microbial infections. The other possibility is that viruses, bacteria or both may have a causative role or a contributory role in Alzheimer's disease onset and progression. Therapeutic trials with antivirals and/or antibacterials in selected Alzheimer's disease cohorts (*APOE* ε4 allele-carriers with serum IgG positivity for one of more suspected pathogens) could shed light on this dilemma. While antiviral treatments in Alzheimer's disease are already being actively investigated, we believe now is the time to conduct placebo-controlled trials with a widely used and well tolerated antibacterial to verify whether this may be of benefit. The study should monitor Alzheimer's disease-associated inflammation markers (for example, fibrinogen and TNF-α) and bacterial IgG-positivity for the main suspected Alzheimer's disease-associated bacteria (i.e. *P. gingivalis*, *H. pylori*, *C. pneumoniae*). It is not clear if microbial dysregulation in patients with Alzheimer's disease would best be corrected at the gastrointestinal or systemic level. To answer this question antimicrobials trials in Alzheimer's

disease should compare agents poorly absorbed in the gastrointestinal tract (as for example rifaximin) with agents that are well absorbed and are able to enter the CNS (as for example amoxicillin/clavulanic acid). As it is not clear if Alzheimer's disease is associated with a single specific pathogen, antibacterial agents should possess wide antibacterial activity, including strains found in the gut and in the oral microbiomes. Because the risk of antibiotic-related adverse events, including resistance, increases with longer treatment courses, a study with antibacterials should be conducted with short-term course of antibiotics (for example 2 weeks) spaced by wash-out periods (e.g. 6 weeks). Indeed, it has been shown that 'short' antibiotic courses are as effective as 'long' courses for most infections treated in primary care (Dawson-Hahn *et al.*, 2017). This approach mimics what it currently done in anti-cancer therapy where chemotherapy is used in short courses spanned by wash-out periods. While anticancer drugs target neoplastic cells, antibacterials target microbes.

As no disease-modifying drugs are presently available for Alzheimer's disease, if a subgroup of patients with Alzheimer's disease could benefit from short-term, multiple cycles of a well-tolerated antimicrobial agent, it would have a huge impact on public health, social and economic burden associated to Alzheimer's disease. The recent studies on herpesviruses and periodontal bacteria and their apparent association with Alzheimer's disease support a conceptual shift in understanding the Alzheimer's disease neuropathogenesis. In the past 28 years, attention has been focused on amyloid-β and tau. Latterly, neuroscientists and clinicians are realizing that answers to the cause of Alzheimer's disease do not reside solely in the hallmarks of brain pathology, but rather in the aetiology of that pathology, for which infectious agents provide a fascinating hypothesis. While further studies on infection and Alzheimer's disease pathogenesis are required, it will be quite difficult to know if patients with Alzheimer's disease are sick because of one or more pathogens or if they are simply more vulnerable to infective agents. The initiation of clinical trials of patients with Alzheimer's disease who have been exposed to suspected pathogens associated to Alzheimer's disease is a viable way to resolve this enigma and most importantly, to determine whether antimicrobial agents could be an effective treatment strategy for Alzheimer's disease.

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