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Review Article

Porphyromonas Gingivalis in the Pathogenesis of Alzheimer's Disease and Its Therapeutic Target

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Abstract

The leading cause of dementia is Alzheimer's disease (AD), which affects millions worldwide. Aging populations can foretell the worsening burden of the disease in the future. AD is characterised by the following hallmark pathologies: amyloid-β over-production and deposition, abnormal hyperphosphorylation of tau leading to the formation of neurofibrillary tangles, and neuroinflammation. Many potential treatments fail in clinical trials, suggesting that present theories are outdated or lead to therapeutic dead-ends. A gum disease-causing species of bacteria, *Porphyromonas gingivalis,* is being increasingly linked with AD, given the ubiquity of gum disease amongst older populations, and studies have revealed that the bacteria causes and exacerbates AD pathology both *in vitro* and *in vivo*. *P. gingivalis* produce many neurotoxic molecules, including gingipain enzymes, lipopolysaccharide and phosphoglycerol dihydroceramides, and all of these have been shown to affect AD pathologies. Numerous mechanisms by which these neurotoxic species reach the brain have been proposed, and one of these is the bacteria's use of outer membrane vesicles. This review presents the present evidence of the effects of *P. gingivalis* and its outer membrane vesicles, gingipains, lipopolysaccharide and phosphoglycerol dihydroceramides, on neurodegeneration in neuronal cultures, mice models and post-mortem studies, and determines how this evidence can be used to develop new treatments for AD.

Introduction

Alzheimer's Disease (AD) is a neurodegenerative disease, and the main cause of dementia, causing 60–70% of cases worldwide. Globally, there are approximately 50 million people with dementia.[1] At present, there are no treatment options for chronic AD.[2] Tanzi (2012) asserted that the two biggest risk factors for the development of AD are age and genetics,[3] although environmental factors, immune system dysfunction and infections are all closely linked to AD.[4] The two key AD pathologies are the presence of extracellular amyloid-β (Aβ) plaques and neurofibrillary tangles (NFTs) that form from abnormally hyperphosphorylated tau.[5]

The amyloid cascade hypothesis suggests that Aβ protein causes AD.[6] Aβ is produced from amyloid precursor protein (APP) using enzymes β-secretase and γ-secretase in the amyloidogenic pathway of APP processing.[5] Chen *et al*. (2017) stated that Aβ monomers form extracellular plaques,[6] However, the relationship of Aβ plaques to cognitive impairment and neurodegeneration remains unclear, since there is no strong correlation between these.[7] Nevertheless, small aggregations of Aβ, called oligomers, appear to be the most neurotoxic form of Aβ. These can easily spread throughout the brain, and cause mitochondrial dysfunction and oxidative stress in neurons, contributing to neuronal death (Fig. 1).[6,8] The tau protein is a microtubule-associated protein (MAP), and a key part of a neuron's cytoskeleton.[9] The phosphorylation of tau controls the function of tau, and is balanced by kinase and phosphatase activity.[10] The enzymes involved in tau phosphorylation involve

Keywords: Alzheimer's disease; Periodontitis; *Porphyromonas gingivalis*.

Abbreviations: AD, Alzheimer's disease; APP, amyloid precursor protein; Aβ, amyloid-β protein; BBB, blood-brain barrier; CNS, central nervous system; GSK3β, glycogen synthase kinase 3β; LPS, lipid lipopolysaccharide; LTD, long-term depression; LTP, long-term potentiation; NFTs, neurofibrillary tangles; OMVs, outer membrane vesicles; PGDHC, phosphoglycerol dihydroceramide; Pg-LPS, P. gingivalis-produced-lipid lipopolysaccharide; Pg-PGDHC, P. gingivalis-produced phosphoglycerol dihydroceramide; PKA, protein kinase A; PKB, protein kinase B; PP2A, protein phosphatase 2A; SASP, senescence-associated secretory phenotype; TLR, toll-like receptor; TNF-α, tumour-necrosis factor-alpha.

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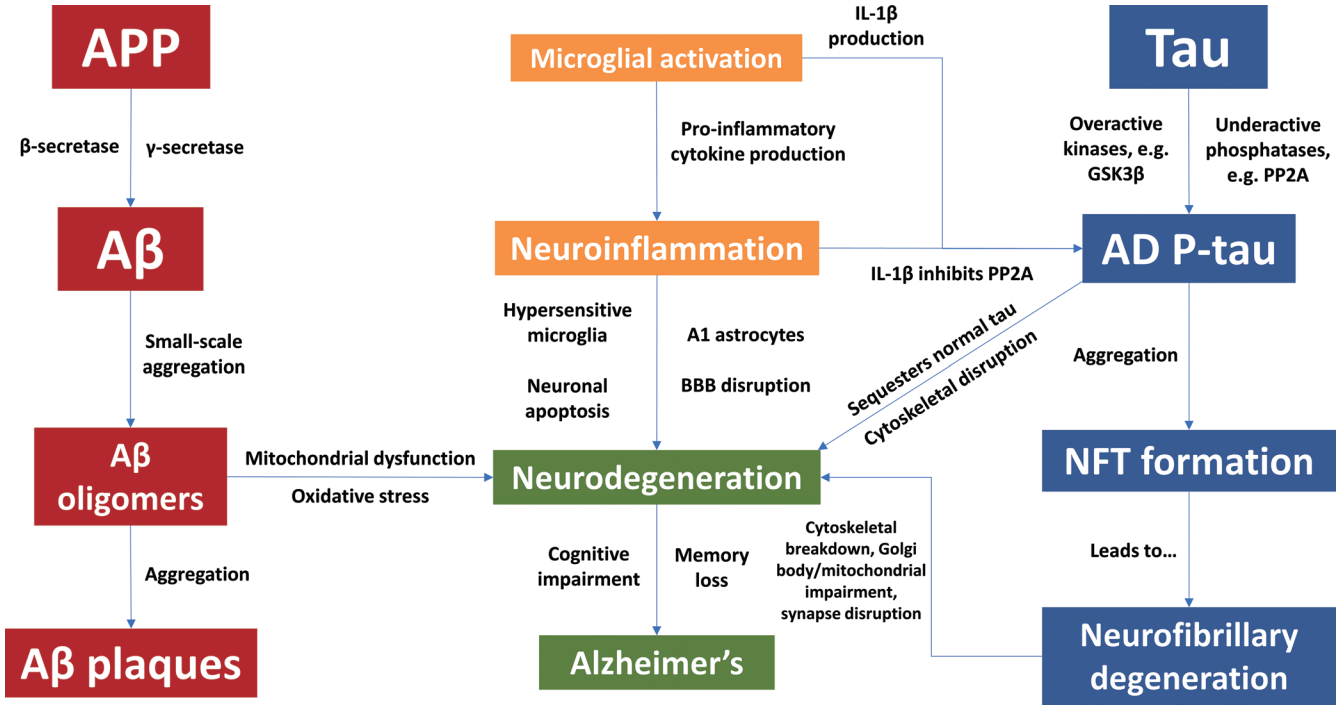

Fig. 1. Outline of amyloid-β and tau's contributions to dementia. The amyloid precursor protein (APP) is converted to amyloid-β, which allows amyloid-β plaques and oligomers to form, causing neurodegeneration. Tau forms abnormally hyperphosphorylated tau (AD P-tau) that aggregates into neurofibrillary tangles, which cause neurofibrillary degeneration. Alzheimer's disease is a complicated disease, in which a number of key factors play a role. The figure presents a basic overview of the fundamentals. *Porphyromonas gingivalis* can influence the formation of amyloid-β and AD P-tau, and neuroinflammation, causing/exacerbating the AD pathology. AD, Alzheimer's disease; AD P-tau, abnormally-hyperphosphorylated tau; APP, amyloid precursor protein; Aβ, amyloid-β protein; BBB, blood-brain barrier; GSK3β, glycogen synthase kinase 3β; IL-1β, interleukin-1β; NFTs, neurofibrillary tangles.

a number of kinases, such as GSK3β, PKA, PKB, and various phosphatases. The main phosphatase of tau is protein phosphatase 2A (PP2A), although PP1, PP3 and PP5 can also act on tau.[10] In AD, both kinases and phosphatases become dysregulated, such that tau is abnormally hyperphosphorylated.[10] These reduce the normal function of tau and form abnormally hyperphosphorylated tau (AD P-tau).[9] AD P-tau self-assembles into NFTs.[9] NFTs cause neurofibrillary degeneration, which involves the impairment of function of normal tau, causing cytoskeletal breakdown,[11] and the impairment of Golgi bodies and mitochondria,[11,12] and disrupting synapses,[2] leading to the death of affected neurons, and subsequently, neurodegeneration (Fig. 1).[11] Glycogen synthase kinase 3β (GSK3β) is a widely expressed kinase in the central nervous system (CNS), which is overactive in AD, and plays a large role in the hyperphosphorylation of tau and the modulation of APP amyloidogenic processing, leading to Aβ formation.[13,14] Hippocampal GSK3β expression is high. This has been considered to play a vital role in hippocampal long-term potentiation (LTP), long-term depression (LTD) and neurogenesis, and all of which are key processes in memory formation/consolidation. In addition, it has been shown that GSK3β inhibition can promote the proliferation, migration and differentiation of neural stem cells.[14] When overactive, as observed in AD, GSK3β activation can impair LTP and promote LTD.[14] The production of pro-inflammatory cytokine, tumour necrosis factor-alpha (TNF-α), is dependent on GSK3β to some extent,[13] and contributes to neuroinflammation, alongside other cytokines, such as IL-1α/β and IL-6.[15] Microglia, which are immune cells of the brain, are responsible for much of the production of pro-inflammatory cytokines in the CNS, and often responds to pathogens/pathogenic insults, resulting in neuroinflammation.[15,16] The lack of hippocampal neurogenesis caused by aberrant GSK3β activity can also activate the pro-inflammatory state of microglia.[14] Microglia can adopt a number of different phenotypes that alter the cytokines they produce,[17] despite being once considered to able to adopt one of the two microglial phenotypes: M1 (pro-inflammatory state) or M2 (anti-inflammatory state).[17] However, this theory is becoming outdated, and a number of studies have suggested that there are more than two microglial phenotypes.[17] Neuroinflammation is highly common in AD patients,[15,18] and is strongly implicated in neurodegeneration in many ways. One example of how this occurs is the microglial production of pro-inflammatory cytokines.[15,17,18] The cytokines produced by microglia contribute to neuroinflammation in numerous ways, and have numerous effects. One of these is to induce astrocytes to adopt to their A1 phenotype. A1 astrocytes lose their regular homeostatic functions, such as the formation of synapses (synaptogenesis), and instead induce apoptosis in neurons and glia, causing neurodegeneration (Fig. 1).[15,18]

There is an urgent need to find effective treatments for AD. A number of present therapies treat psychotic symptoms/depression without affecting the causes.[2] AD treatments include β-secretase inhibition and the upregulation of phosphatases to lower tau phosphorylation,[2] but most of these are ineffective or even toxic.[19] Huang *et al*. (2020) listed nine different treatments for AD that failed in phase 3 trials between 2016 and 2019 alone.[20]

Porphyromonas gingivalis is a gram-negative gum-disease (periodontitis) causing bacteria linked to AD,[21] and there are multiple ways by which *P. gingivalis* has been shown to influence AD pathologies. The bacterial key neurotoxic secretions are gingipain enzymes, lipopolysaccharide (LPS) and phosphoglycerol dihydroceramide (PGDHC), which can be released in outer membrane

Table 1. Other pathogens associated with Alzheimer's disease[21]

Pathogens			
Viruses	**Bacteria**	**Fungal**	**Protozoa**
Human herpesvirus 1 (HHV-1)	*Chlamydia pneumoniae*	*Candida albicans*	*Toxoplasma gondii*
Human herpesvirus 2 (HHV-2)	*Helicobacter pylori*		
Cytomegalovirus (CMV/HHV-3)	*Borelia burgdorferi*		
Epstein-Barr virus (EBV, HHV-4)	*Treponema pallidum*		
Varicella-zoster virus (VZV, HHV-5)	*Porphyromonas gingivalis*		
Human herpesvirus 6 (HHV-6)	*Fusobacterium nucleatum*		
Hepatitis C virus (HCV)	*Prevotella and other periodontal bacteria*		

Note: The table lists some of the different pathogens that have been associated with Alzheimer's disease, which range from viruses to protozoa. *Porphyromonas gingivalis* is merely one of the different varieties. Any of these pathogens can be involved in a patient's Alzheimer's disease. Hence, treatments that target each specific one would be the best, although there is evidence of *P. gingivalis* involvement, and that other gum disease (periodontal) bacteria are strong.

vesicles (OMVs) in the body, and reach distant areas from the original site of infection in the oral cavity through blood, such as the brain.[22–24] The bacteria, their OMVs and their virulence factors are able to enter the brain following infection of the oral cavity, and influence AD.[22,23] These have been confirmed by their presence in post-mortem AD brains, but not in non-AD brains.[24,25] Aβ is an antimicrobial peptide that causes neuroinflammation.[26] Implying Aβ deposition can initially be a response to infection in the brain. *P. gingivalis* are iron auxotrophs,[22,27] which is significant, given that cortical iron dysregulation and subsequent deposition are correlated with and theorised to accelerate Aβ deposition, tau aggregation and neurodegeneration.[28] Iron is toxic to neurons *via* the generation of reactive oxygen species (ROS).[22,27] In AD brains, iron depositions are often co-localised with Aβ accumulation, NFTs and gingipain accumulation,[22,27] and these areas present with neurodegeneration, oxidative stress and mitochondrial dysfunction.[22,28] *P. gingivalis*, which breaks down salivary lactoferrin in the oral cavity to receive the iron it needs, may seek for the AD brain when salivary lactoferrin levels are reduced. This is attributable to hypothalamic lesions (which dysregulate salivary secretions as a result), causing oral dysbiosis.[22,27] Although the levels are lowered in the saliva, lactoferrin is upregulated in AD brains, and has neuroprotective effects against iron, Aβ deposition and NFT formation, as well as against oxidative stress and mitochondrial dysfunction.[22] *P. gingivalis* is a powerful lactoferrin degrader, and this likely acts to reduce the neuroprotective effects that lactoferrin have in AD brains.[22]

There are several other pathogens linked to AD (Table 1),[21,29] although *P. gingivalis* is of special interest and given the prevalence of gum disease. Nearly 50% of the adult population were observed to have some form of periodontitis in a US study,[30] while another study revealed that 61.9% of participants had periodontitis, with age being the most significant risk factor.[31] Given that periodontitis is treatable, this offers potential targets for AD therapies.[29] The present review aims to discuss the role of *P. gingivalis* in neurodegeneration and AD, and determine how targeting this can lead to new AD treatments.

Porphyromonas gingivalis, periodontitis and AD

A growing body of evidence has shown that *P. gingivalis* is able to increase Aβ production, tau phosphorylation and neuroinflammation, contributing to AD (Fig. 2).[32–35] *P. gingivalis* causes periodontitis, elevates pro-inflammatory cytokine levels, and causes inflammation.[36] Furthermore, cytokines can increase blood-brain barrier (BBB) permeability, which can allow *P. gingivalis*, OMVs, or its secretions to cross.[21] The post-mortems of AD brains revealed the bacteria and virulence factors in both the brain and cerebrospinal fluid.[25] Noble *et al*. (2009) reported the correlation between increasing *P. gingivalis* antibody levels and cognitive impairment, suggesting that *P. gingivalis* causes cognitive decline,[37] although the study used secondary data in excess of 15 years old. Furthermore, the findings were solely correlational, but were significant nevertheless.

P. gingivalis induces AD pathology. The bacteria and/or its secretions invade the brain, significantly increasing Aβ deposits in the brain, and raising pro-inflammatory cytokine levels.[21] Furthermore, *P. gingivalis* activates microglia, degenerates neurons, and increases tau hyperphosphorylation and the subsequent formation of NFTs, leading to neurofibrillary degeneration.[23] Moreover, *P. gingivalis* has been shown to increase tau phosphorylation in wild-type mice models by inducing the neuroinflammation-mediated production of IL-1β that inhibits PP2A.[35] In studies that have shown this result, the effects developed within a reasonably short period of time, in only 22 weeks after initial infection in one study, and even in young adult wild-type mice.[23] This clearly demonstrates the link of *P. gingivalis* to AD, and the ability to accelerate AD pathogenesis.

Interestingly, *P. gingivalis* and its secretions are adept in evading and attenuating immune response in a wider body, as well as in the brain, with the latter's response being mediated by microglia and the complement system.[38,39] This allows for the persistent presence of bacteria and their secretions in the brain, exacerbating the effects these have on AD pathology over a longer period of time. Gingipains inhibit the complement system by breaking down the C3 protein, which is central to complement system activation, and gingipains can also degrade toll-like receptor (TLR) coreceptors, cytokines and antimicrobial peptides.[38] LPS resists complement-mediated lysis and upregulates negative regulator TLRs in monocytes/microglia.[38] This information is significant when considering the importance of an effective immune response to eliminate *P. gingivalis* and its virulence factors from the brain. The nuances of the expertise of *P. gingivalis* in evading immune response[38,39] must be considered when therapeutically targeting the bacteria and secretions.

In order to best understand the bacteria's role in AD, it is necessary to first discuss the effects of each key secretion on the neurons/brain, and the contributions to neurodegeneration and AD (Fig. 2).

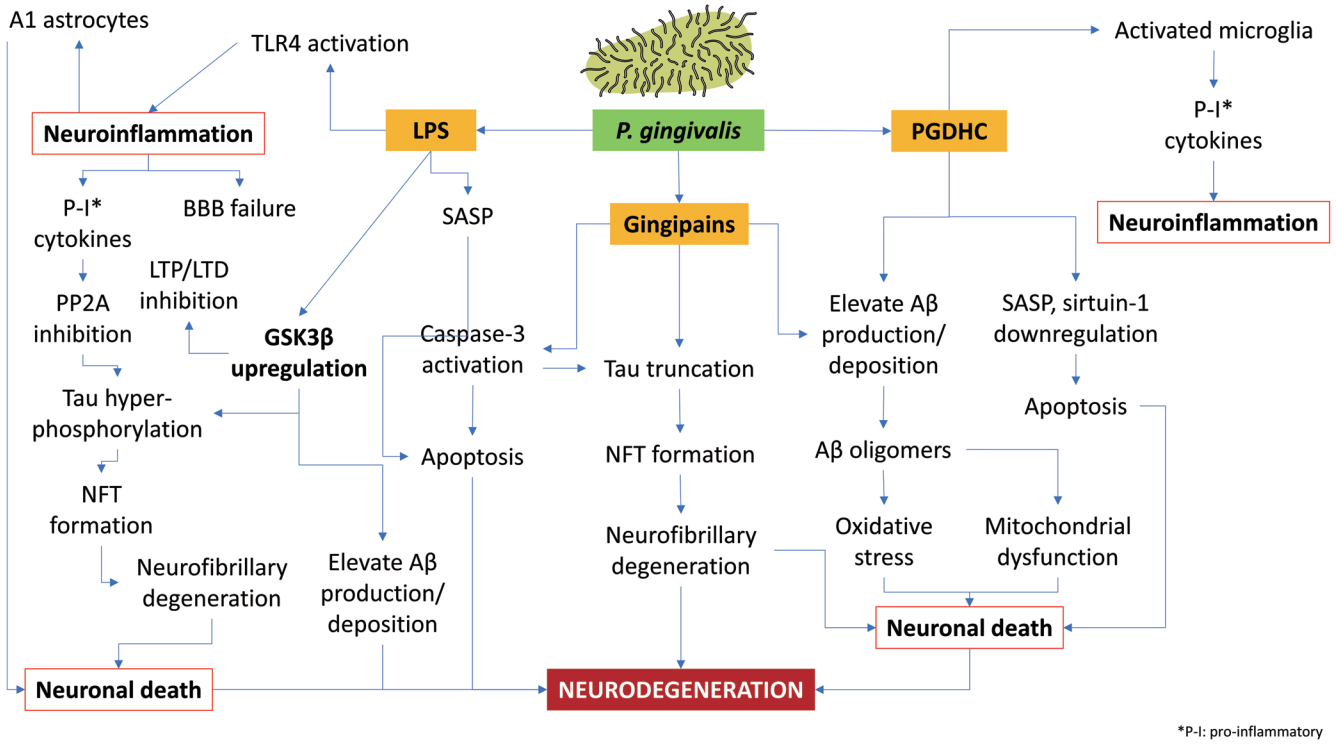

Fig. 2. Overview of the roles of *P. gingivalis* virulence factors in Alzheimer's disease. *P. gingivalis* produces the key neurotoxic secretions: LPS, gingipains and PGDHC. Some of the numerous effects were depicted, and all of these mainly cause neuroinflammation *via* various mechanisms, as well as NFT formation and Aβ deposition. These contribute to neuronal death, and ultimately, neurodegeneration and AD. It is likely that the effects of each virulence factor are more complex, and that there is interplay among a number of other factors, although it must be noted that in the likelihood of AD, *P. gingivalis* plays a part in the disease, and is not the sole initiator/effector of disease progression. AD, Alzheimer's disease; Aβ, amyloid-β; BBB, blood-brain barrier; GSK3β, glycogen synthase kinase 3β; LPS, lipopolysaccharide; LTD, long-term depression; LTP, long-term potentiation; NFT, neurofibrillary tangle; PGDHC, phosphoglycerol dihydroceramide; P-I, pro-inflammatory; PP2A, protein phosphatase 2A; SASP, senescence-associated secretory phenotype; TLR4, toll-like receptor 4.

The role of gingipain enzymes in neurodegeneration and AD

Gingipains are protease enzymes produced by *P. gingivalis* that are secreted by the bacteria, and can be contained in OMVs.[33,36] Gingipains are correlated with hyperphosphorylated tau and AD severity in the brain, and often co-localize with Aβ, NFTs and iron deposits.[22,36,40] Studies have confirmed its presence in the human brain, and one study found this in >90% of post-mortem AD brains that contained gingipains.[40] It has also been shown that gingipains target tau, and truncate it, increasing the self-assembly of tau into NFTs.[9] *P. gingivalis*-infected neurons express elevated, abnormally hyperphosphorylated tau levels and reduced normal tau levels, which is most likely the result of the gingipain proteolytic activity induced by the effect on tau.[40] APP-transgenic mice infected with *P. gingivalis* have been shown to have abundant gingipain levels in the brain, which often co-localizes with tau/Aβ, and causes a significant increase in Aβ deposition, while the activation of caspase-3, which is involved in apoptosis and tau truncation, results in a significant loss of hippocampal neurons.[36] These findings are consistent with the findings reported by Ishida *et al*. (2017) and Ilievski *et al*. (2018). However, through infecting mice with *P. gingivalis*, the specific cause of the observed results remains unclear,[21,23,36] although the presence of gingipains, and its ability to affect tau and Aβ makes this the likely culprit.

Gingipain inhibitors have been demonstrated to enter the brain of mice, reduce *P. gingivalis* load, prevent gingipain action on tau and gingipain-induced neurodegeneration, and recover degenerating neurons.[36] This makes a strong case for gingipains as a primary therapeutic target for *P. gingivalis* AD.

Lipopolysaccharide and AD

P. gingivalis produces lipid lipopolysaccharide (Pg-LPS), which is correlated with neuroinflammation/degeneration.[41] Wu *et al*. (2017) and Zhang *et al*. (2018) demonstrated that Pg-LPS impairs spatial learning/memory and significantly increases pro-inflammatory cytokine production, which in turn, activates astrocytes/microglia in mice models.[41,42] However, there were inconsistencies between these two studies. One study observed these effects in wild-type mice,[42] while the other study only observed the same effects in cathepsin-B deficient mice, but not in wild-type controls.[41] Zhang *et al*. (2018) did not identify or mention cathepsin B-dependency,[41] or cited Wu *et al.* (2017), complicating the interpretation. Despite these, both studies revealed that Pg-LPS can cause Alzheimer's disease pathology. Given that both studies injected mice solely with Pg-LPS, and not the bacteria, it is clear that the observed differences are due to Pg-LPS alone.

A similar and more recent study used Pg-LPS to induce the periodontitis mice model that echoed and expanded on these earlier findings.[43] Pg-LPS-induced neuroinflammation significantly impaired spatial learning and memory, as assessed using the Morris water maze.[43] The levels of pro-inflammatory cytokines, such as IL-1β and IL-6, were observed to be significantly elevated due to

Pg-LPS, and there was a significant increase in activated microglia.[43] Overall, these show that Pg-LPS is adept in initiating neuroinflammation. Furthermore, Pg-LPS has been shown to increase the APP and BACE1 expression, as well as the levels of tau,[43] suggesting that Pg-LPS has a significant influence over key AD pathologies. Toll-like receptor-4 (TLR4) recognises LPS, and activates microglia and other inflammatory processes upon binding,[43] perhaps providing a therapeutic target to prevent Pg-LPS-mediated neuroinflammation and Aβ/tau elevation.

Furthermore, Pg-LPS has been reported to induce microglia-mediated neuroinflammation at the leptomeninges in the blood-cerebrospinal fluid barrier (BCSFB), which increases pro-inflammatory cytokine production.[44]

Phosphoglycerol dihydroceramides in neurodegeneration and AD

The lipid PGDHC is produced by *P. gingivalis*. Yamada *et al.* (2020) produced compelling evidence suggesting that PGDHC has a role in AD.[24] In hamster and human neuron cultures exposed to *P. gingivalis* PGDHC (Pg-PGDHC) and *P. gingivalis* lipopolysaccharide (Pg-LPS), it was found that Pg-PGDHC, but not Pg-LPS, increased the production of APP and Aβ in hamster neurons, while tau phosphorylation increased in human neurons. However, both Pg-PGDHC and Pg-LPS downregulated sirtuin-1, which is a marker for protecting cells from cellular senescence. Furthermore, Pg-PGDHC and Pg-LPS increased the senescence-associated secretory phenotype (SASP) in neurons.[24] The SASP marks cells for apoptosis, and contains pro-inflammatory cytokines and cathepsin B.[24] There was no explanation for the different effects in hamster and human cells. These could be anomalous results, although this suggests that the effects of Pg-PGDHC are not universal. Thus, further research is required. An earlier study revealed that Pg-PGDHC can cause apoptosis in human endothelial cells.[45] Hence, *P. gingivalis* can use PGDHCs to breach the BBB endothelium and enter the brain, although OMVs also provide the mechanism for crossing the BBB.[46] Further research on the effect of Pg-PGDHC on the BBB endothelium is specifically required.

Outer membrane vesicles are potential vehicles for *P. gingivalis* to enter into the brain

A point of contention in the literature is to determine whether *P. gingivalis* enters the brain itself in the pathogenesis of AD, or if the entry of bacteria into the brain comes at a later stage when *P. gingivalis* secretions, such as Pg-LPS and gingipains, accesses the brain using a method, such as OMVs. A number of studies have 'detected' the presence *P. gingivalis* in the brain, but did not distinguish between bacterial cells or the secreted virulence factors.[22] This suggests that the presence of *P. gingivalis* virulence factors does not necessarily equate to bacterial presence in the brain, which would complicate therapies that target the prevention of bacterial access to the brain. However, it is irrefutable that *P. gingivalis* virulence factors and/or OMVs can reach and enter the brain.[22]

P. gingivalis produces OMVs that contain a number of bacteria virulence factors, including gingipains, LPS and phospholipids.[33] These OMVs are released by *P. gingivalis* in the oral cavity, allowing these to access the blood, and travel throughout the body, including the brain.[22,33] OMVs fulfil a number of roles, such as nutrient acquisition (often iron),[27] communication and host immune system evasion, while delivering and spreading virulence factors throughout the host and host organs, which are accessed using the blood.[34] These are mainly produced during bacterial growth, with an approximate ratio of 1 bacterium to 2,000 OMVs.[34]

Nara *et al.* (2021) reported compelling evidence that suggests that OMVs are the key to the ability of *P. gingivalis* to target anatomically distant organs from the oral cavity, such as the brain.[22] The brain does not appear to be a suitable environment for *P. gingivalis*, that is, the bacteria appears better suited to anaerobic polymicrobial films.[22] The mechanism and reason(s) by which *P. gingivalis* and/or their virulence factors initially reach the brain to influence AD pathologies remain unclear. However, OMVs provide a solution. That is, there are a number of proposed mechanisms by which OMVs can initially access the brain, and subsequently release *P. gingivalis* virulence factors within the CNS.[22,46] The role of OMVs in the nutrient acquisition of iron can imply that *P. gingivalis* OMVs actively seek the brain as a source of iron, given the elevated levels of iron in AD brains.[27] A larger number of OMVs would likely seek the brain after the initial OMVs have already reached the brain, and begun to influence the AD pathologies.[22] Nara *et al*. (2021) suggested that initial *P. gingivalis* virulence factors that reach the brain in OMVs would localise to areas, such as the anterior hypothalamus. The damage caused by the *P. gingivalis* virulence factors and the neuroinflammation caused by its presence would begin causing AD pathologies that would, in turn, dysregulate lactoferrin release into the oral cavity. Then, this would induce the *P. gingivalis* in the oral cavity to release more OMVs that seek iron. As the AD progresses, this would be drawn to the brain, given the iron surplus.[22,27] Furthermore, the *P. gingivalis* entry into the brain may be made easier through tauopathies, which can be observed in AD, and are correlated to BBB dysfunction.[44] Given the ability of *P. gingivalis* to exacerbate tauopathy in the brain,[35] the development of tauopathy may worsen the presence of *P. gingivalis* and *P. gingivalis* virulence factors in the brain through the induction of BBB dysfunction.

OMVs are capable of increasing BBB permeability, and there is evidence that suggests that these can cross the BBB, although the latter awaits complete resolution.[22,46] Gingipains are contained within OMVs, and have been implicated in the process of increasing endothelial permeability.[33] OMVs can infect monocytes prior to migration to the brain,[22] or directly infect cranial nerves and spread along these to gain access to the brain.[22] Once inside the brain, OMVs release *P. gingivalis* virulence factors, allowing these to spread throughout the brain, and cause/contribute to neurodegeneration and AD.[22] Microglia that encounter *P. gingivalis* virulence factors, such as gingipains and LPS, adopt a hypersensitive phenotype, which upregulate pro-inflammatory cytokines (such as IL-1β, IL-6 and TNF-α) and reactive oxygen species (ROS), and contribute to neuroinflammation and degeneration.[47] Nonetheless, as previously mentioned, *P. gingivalis* and *P. gingivalis* secretions are adept in evading immune response to an extent that permits persistence within the brain, without preventing neuroinflammation.[38,39]

OMVs may adopt one of a range of phenotypes.[22] Initially, these may express high levels of adhesion molecules that assist in gaining access into tissues, such as the brain, before adopting an 'iron-laden' phenotype that would cause the greater deposition of iron in the brain upon entry.[22]

GSK3β, *Porphyromonas gingivalis* and lipopolysaccharide in AD

The serine-threonine kinase, glycogen synthase kinase-3β (GSK3β), is an isoform of GSK that is widely expressed through-

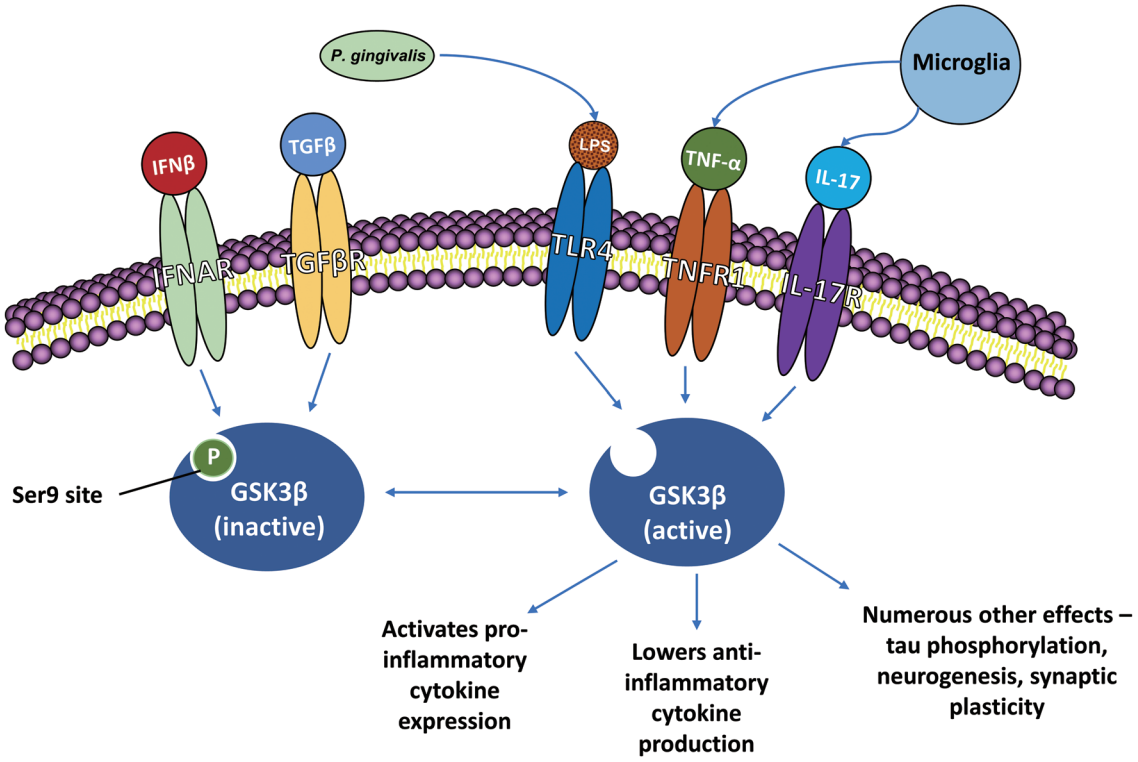

Fig. 3. GSK3β signalling overview. GSK3β is a kinase enzyme regulated by phosphorylation at the Ser9 site. Numerous factors are controlled by phosphorylation regulation. The activating signals include LPS in activating TLR4. In this example, the LPS was produced by *P. gingivalis*. The microglia produced TNF-α and IL-17, and both have pro-inflammatory effects, in which one of the effects is the activation of GSK3β. The activation of GSK3β allows the enzyme to produce numerous effects, which are mainly pro-inflammatory cytokine modulation and tau phosphorylation. Although correctly regulated, GSK3β remains vital for synaptic plasticity and neurogenesis. Conversely, the inhibition of GSK3β can be stimulated by IFNβ and TGFβ binding to their respective effects. In AD, GSK3β is hyperactive due to the microglia-produced pro-inflammatory cytokines and *P. gingivalis*-LPS that stimulate its dephosphorylation. AD, Alzheimer's disease; GSK3β, glycogen synthase kinase 3β; IFNβ, beta-interferon; IFNAR, interferon-alpha/beta receptor; IL-17, interleukin-17; IL-17R, interleukin-17 receptor; LPS, lipopolysaccharide; P, phosphate; TGFβ, transforming growth factor-beta; TGFβR, transforming growth factor-beta receptor; TLR4, toll-like receptor 4; TNF-α, tumor necrosis factor-alpha; TNFR1, tumor necrosis factor receptor 1.

out the CNS.[13] GSK3β targets a variety of substrates, which include metabolic proteins, transcription and translation factors, and cytoskeletal proteins.[16] Furthermore, GSK3β is involved in various processes, including synaptic plasticity, memory, neurogenesis, and microtubule morphology. All of these become dysregulated in AD, and are implicated in inflammation and tau phosphorylation (Fig. 3).[13,14,16] GSK3β activity is negatively correlated with tau phosphorylation.[13] The exact mechanisms by which GSK3β is involved in these processes remain to be fully elucidated, although the role of GSK3β in AD is supported by a wide body of evidence.[13,14,16] The activity level of GSK3β is regulated by phosphorylation at an inhibitory Ser9 site, which is controlled by protein kinase A and B (PKA and PKB, respectively). The extent of the GSK3β phosphorylation/inactivation affects a number of these functions.[13,14] GSK3β activity increases as age increases, and as inhibitory phosphorylation decreases, activating more of the enzyme, and contributing to neurodegeneration through a wide variety of mechanisms.[13,16] GSK3β is the key, due to its involvement in AD, and its susceptibility to being affected by *P. gingivalis*, particularly Pg-LPS.[13]

GSK3β is implicated in a number of AD neurodegenerative processes. The enzyme is hyperactive in AD, enabling it to phosphorylate many targets, mainly tau, and disrupt key processes that contribute to neurodegeneration and AD.[14] Furthermore, as a major kinase of tau protein, this increases the level of tau phosphorylation, contributing to further NFT formation, and ultimately, neurodegeneration.[13,14] GSK3β is a key contributor to hippocampal LTP/LTD, and processes that are vital for memory formation.[14] The elevated GSK3β activity levels observed in AD would impair LTP and promote NMDA receptor-mediated LTD, while reducing the pre-synaptic glutamate release.[14,16] GSK3β further contributes to AD by increasing Aβ formation and accumulation through the modulation of amyloidogenic APP processing *via* BACE1 and γ-secretase.[14]

Jiang *et al.* (2021) reported that there are interactions between GSK3β and Pg-LPS, which can make numerous contributions to AD through microglia-mediated neuroinflammation and tau hyperphosphorylation.[13] The Pg-LPS itself is capable of causing neuroinflammation mediated by IL-1β and TNF-α.[13,41,42] Furthermore, Pg-LPS elevates GSK3β activity in neurons, microglia and astrocytes.[13] The study revealed that Pg-LPS can induce GSK3β and TNF-α to interact and modulate the production of one another, raising microglial pro-inflammatory cytokine production, and furthering the neuroinflammation.[13] This cycle perpetuates as Pg-LPS contributes to neuroinflammation, and further raises the inflammation while elevating GSK3β activity, leading to increased tau hyperphosphorylation, NFT formation and Aβ formation, and ultimately leading to neurodegeneration.[13] The inhibition of GSK3β lowers TNF-α production from the Pg-LPS-activated microglia, while the inhibition of TNF-α lowers the *in vitro* activity of GSK3β,[13] suggesting that these are potential therapeutic targets.

Table 2. The range of Alzheimer's disease therapies presently in phase 3 clinical trials in 2019[15]

Agent	Anti-/Non-anti-amyloid	Mechanism of action
ANAVEX2-73	Anti-amyloid	Anti-tau/-amyloid
Gantenerumab	Anti-amyloid	Monoclonal antibody
AC-1204	Non-anti-amyloid	Induction of ketosis
COR388	Non-anti-amyloid	Bacterial protease inhibitor
Escitalopram	Non-anti-amyloid	Serotonin reuptake inhibitor
Methylphenidate	Non-anti-amyloid	Dopamine reuptake inhibitor

Note: The above table lists some of the Alzheimer's disease treatments in phase 3 clinical trials as of 2019. Anti-amyloid denotes those based on the amyloid cascade hypothesis, while non-anti-amyloid treatments were based on different hypotheses, such as the Alzheimer's disease was caused by *P. gingivalis*. The mechanisms of action of the wide range of potential treatments should be explored. AC-1204, tricaprilin; ANAVEX2-73, blarcamesine; COR388, atuzaginstat.

Overall mechanism of *Porphyromonas gingivalis* in AD

It must be initially noted that AD is very complex, and is far from being fully understood, particularly when introducing a pathogen, such as *P. gingivalis*, into the proposed mechanism. A very large number of variables can affect the onset, development, progression and overall timescale of the disease, and the majority of these may be unique for each patient. Nara *et al.* (2021) proposed a compelling hypothesis on how *P. gingivalis* OMVs can be the major driving factor in *P. gingivalis*-influenced AD,[22] and this counters the proposal of Dominy *et al*. (2019),[36] in which gingipains is the key factor.

In summary, *P. gingivalis* initially infects the oral cavity as a small part of a polymicrobial film. In this stage, the bacteria's iron requirement is met through the degradation of salivary lactoferrin. Through multiple mechanisms of action, *P. gingivalis* damages the tissue that supports the tooth, in order to allow the OMVs to penetrate/bypass the superficial supporting tissue, and reach the blood stream. After reaching the blood stream, these OMVs would circulate and reach the brain. Using one or more yet to be elucidated mechanism(s), the OMVs will cross the BBB and enter the brain. Furthermore, tauopathy has been considered a normal characteristic of brain aging, to a certain extent,[48] which develops before the OMVs that reach the brain damage the BBB to permit OMVs to cross.[44] Nara *et al.* proposed that OMVs that access the brain will localise to areas, such as the basal forebrain, hippocampus, and the anterior hypothalamus.[22] The content of the OMVs released at these sites would induce neuroinflammation to develop, and lead to numerous effects, such as the induction of hypersensitive microglia that modulate pro-inflammatory response. Gingipains will cleave tau to increase the formation of NFTs. In addition, iron deposition would increase due to disruptions in metabolism. At this point, sufficient hypothalamic damage may be caused to disrupt the salivary lactoferrin levels, since this is upregulated in the brain for its neuroprotective effects, causing oral dysbiosis that benefits *P. gingivalis*, and increasing the OMV release with the 'iron-laden' phenotype.[22] These OMVs will reach and access the brain in greater numbers, releasing more Pg-LPS, gingipains and Pg-PGDHC, among other secretions, more globally in the brain, and to areas such as the frontal lobe, temporal lobes and neocortex.[22] Subsequently, neuroinflammation would further increase, since the microglia are activated in larger numbers, thereby producing greater quantities of pro-inflammatory cytokines, and elevating gingipain, LPS and PGDHC activity and influence. The combined effect of these would increase the iron deposition, activating more tau kinases such as GSK3β, inhibiting tau phosphatases (PP2A), contributing to NFT formation, and ultimately, leading to neurofibrillary degeneration, while modulating the amyloidogenic processing of Aβ increases the Aβ formation and deposition throughout the brain. Neurons would die due to various mechanisms, including Aβ oligomers/deposits, NFTs, aberrant neuroinflammation (microglia, A1 astrocytes), and reactive oxygen species that cause oxidative stress and mitochondrial dysfunction. Furthermore, this would cause aggressive cognitive decline. Moreover, BBB integrity would fail at some point, exacerbating these problems, thereby allowing more *P. gingivalis* to access the brain, and other pathogens and neurotoxic species.

Future directions

All things considered, the contribution of *P. gingivalis* to AD remains irrefutable. It remains unclear exactly how the bacteria initially enter the brain, and to what extent the bacteria cause, accelerate and/or exacerbate the AD pathology. However, the evidence for their contribution cannot be ignored. The bacteria are highly accomplished at entering the brain, causing chronic neuroinflammation and influencing the AD pathology. A disease that is as pervasive as gum disease and has clear links to AD is of great concern. The possibilities presented in the present review show that further research on *P. gingivalis*, each of its secretions, its OMVs and potential therapeutic targets must be performed and expedited. Given the complexity of AD, specific therapies for each patient would be the best approach, and effective *P. gingivalis*-based therapies would help a number of patients.

Conclusions

The influence of AD and *P. gingivalis* on the disease are both highly complex, although this presents numerous therapeutic opportunities. Evidence for the role of periodontal pathogens such as *P. gingivalis* continues to grow.

One of the most prominent therapeutic targets presented by *P. gingivalis* is the inhibition of gingipains. Dominy *et al.* (2019) reported the promise of this with COR388, a gingipain inhibitor, which has been shown to stop *P. gingivalis* growth and prevent gingipain action on tau, while recovering degenerating neurons.[36] COR388 passed the double-blind phase I clinical trial in 2018,[49] and is presently in the double-blind phase II/III clinical trial (to be completed by 2023), in order to assess the effect of COR388 on cognitive decline and *P. gingivalis* levels.[50] At present, COR388 appears to be the only *P. gingivalis*-based therapy in clinical trials (Table 2).[15] Further research on therapies based on OMVs, Pg-LPS and Pg-PGDHC is required, particularly given the apparent

importance of the initial entry of bacteria into the brain, causing neuroinflammation/degeneration.[22,45]

Given that *P. gingivalis*-induced neuroinflammation causes PP2A inhibition through IL-1β, modulating PP2A and/or preventing its inhibition may present a promising therapeutic target.[10] Martin *et al.* (2013) listed two PP2A-targeting drugs, memantine and homocysteine, and both of these lower tau phosphorylation, although these require further research in animal models.[10] GSK3β, which is a major tau kinase modulated by *P. gingivalis*, is another promising therapeutic target, given that it is highly overactive in AD. Phosphorylation at its Ser9 residue inactivates the enzyme, offering a clear potential therapy that can lower the levels of tau phosphorylation. SAR502250 is a selective GSK3 inhibitor that has exhibited promising neuroprotective effects in mice models, that is, it lowers tau hyperphosphorylation.[51]

Pg-LPS can be therapeutically targeted *via* the inhibition of microglial TLR4. TLR4 recognises Pg-LPS, and the binding of this produces a microglia-mediated pro-inflammatory response. Inhibiting TLR4 can prevent neuroinflammation, and the associated damage this causes and exacerbates. Zhou *et al.* (2020) provided a good overview of TLR4 in AD, and listed different therapies for targeting TLR4, which have been shown to ameliorate cognitive impairment and AD-like pathologies in animal models.[52]

The testing levels of salivary lactoferrin may provide a biomarker of Aβ deposition in the brain and general AD progression.[53] Decreased levels of salivary lactoferrin appear to be unique to AD, and contribute to oral dysbiosis, while the levels of lactoferrin are elevated in AD.[53] It may be considered to test the levels of salivary lactoferrin as an early, non-invasive biomarker for AD.

Given that the primary pathogenic effect of *P. gingivalis* is the causation of periodontitis, improvements in oral hygiene at an early age would undoubtedly serve to improve dental health, reduce the incidence of highly-prevalent periodontitis,[30,31] and potentially slow the development of AD.

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Conflict of interest

Dr. Jinwei Zhang has been an editorial board member of the *Journal of Exploratory Research in Pharmacology* since May 2021. The authors have no other conflicts of interest related to this publication.

Author contributions

Conceptualization (TS and JZ), writing-original draft preparation (TS and JZ), writing-review and editing (TS and JZ), supervision (JZ), project administration (JZ), and funding acquisition (JZ). All authors have read and agreed to the published version of the manuscript.

Data sharing statement

No additional data are available.

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