Oral Treponema denticola Infection Induces Aβ1–40 and Aβ1–42 Accumulation in the Hippocampus of C57BL/6 Mice

Xinyi Su1 · Zhiqun Tang1 · Zhiyue Lu2 · Yuqiu Liu1 · Wanzhi He1 · Jiapei Jiang1 · Yifan Zhang1 · Hongkun Wu1

Received: 22 December 2020 / Accepted: 5 March 2021 / Published online: 24 March 2021 © The Author(s), under exclusive licence to Springer Science+Business Media, LLC, part of Springer Nature 2021

Abstract

Accumulation of amyloid-β (Aβ) in the brain is a central component of pathology in Alzheimer’s disease. A growing volume of evidence demonstrates close associations between periodontal pathogens including Porphyromonas gingivalis (P. gingivalis) and Treponema denticola (T. denticola) and AD. However, the effect and mechanisms of T. denticola on accumulation of Aβ remain to be unclear. In this study, we demonstrated that T. denticola was able to enter the brain and act directly on nerve cells resulting in intra- and extracellular Aβ1–40 and Aβ1–42 accumulation in the hippocampus of C57BL/6 mice by selectively activating both β-secretase and γ-secretase. Furthermore, both KMI1303, an inhibitor of β-secretase, as well as DAPT, an inhibitor of γ-secretase, were found to be able to inhibit the effect of T. denticola on Aβ accumulation in N2a neuronal cells. Overall, it is concluded that T. denticola increases the expression of Aβ1–42 and Aβ1–40 by its regulation on beta-site amyloid precursor protein cleaving enzyme-1 and presenilin 1.

Keywords Treponema denticola · Alzheimer’s disease · Amyloid-β · BACE1 · Presenilin 1

Introduction

Alzheimer’s disease (AD), the most common form of dementia, is characterized by a cerebral accumulation and aggregation of amyloid-β (Aβ) peptides and tau hyperphosphorylation, the main components of plaques and tangles, respectively (Scheltens et al. 2016). Aβ oligomers induce AD-like lesions, such as tau phosphorylation or synaptic loss, and accumulate in brain regions related to memory and cognitive function, resulting in related dysfunction (Viola et al. 2015), and recent evidence indicates a role for Aβ as an antimicrobial peptide (Gosztyla et al. 2018). Aβ1–42 and Aβ1–40 are the most common toxic subtypes in the human body (Wirths et al. 2019), which are believed to play a key role in neuronal loss and cognitive dysfunction in AD (Lesné et al. 2006). Aβ is a series of short peptides of 38–43 amino acids, produced by degradation of amyloid precursor protein (APP), which is highly expressed in the central nervous system. APP is first hydrolyzed into a β-N-terminal fragment and a β-C-terminal fragment under the action of β-secretase 1 (BACE1), and subsequently hydrolyzed by γ-secretase of which the catalytic active center is presenilin. Presenilin 1 (PS1) and presenilin 2 (PS2) are two subtypes of presenilin with similar biological functions. The abnormal expression of BACE1, PS1 and PS2 can affect the cleavage of APP, thus affecting the production of Aβ.

Chronic periodontitis (CP) has been identified as a significant risk factor for the development of AD (Kamer et al. 2015). The probability of cognitive impairment in the elderly with alveolar bone resorption is 2.4 times higher than that of those without alveolar bone resorption (Shin et al. 2016). It has been found that the degree of cognitive impairment in patients with severe periodontitis is three times higher than that of patients with mild periodontitis or without periodontitis (Gil-Montoya et al. 2017). The more alveolar bone absorption in the elderly with normal cognitive function, the greater the amount of Aβ deposition in brain tissue. Periodontal pathogens such as P. gingivalis or T. denticola can cause chronic periodontitis and possibly contribute to the clinical onset of AD (Sochocka et al. 2017),
with evidence revealing that oral *P. gingivalis* infection in mice leads to brain colonization and increased production of amyloid plaque component Aβ1–42 (Dominy et al. 2019). *T. denticola*, one of the important pathogenic bacteria of CP with a detection rate of 56.8% in CP patients (Sparks et al. 2012) and a member of spirochetes, was detected significantly more frequently in brain samples of AD patients (14 of 16) when compared with that of healthy controls (4 of 18) (Riviere et al. 2002, Poole et al. 2013). Thus, we speculate that *T. denticola* can promote Aβ deposition and the pathological process of AD similar to *P. gingivalis*. However, there is a lack of research about the effect and underlying mechanisms of *T. denticola* in AD.

To verify the aforementioned assumption, oral *T. denticola* infection was induced in C57BL/6 mice, and examined the effect of *T. denticola* on Aβ in those mice with *P. gingivalis* as positive control and PBS as negative control was examined. Our study demonstrated that oral *T. denticola* infection induces Aβ1–42 and Aβ1–40 accumulation in the hippocampus of C57BL/6 mice by upregulating BACE1 and PS1. Here, we also reported that both KMI1303 and t-butyl ester (DAPT) could inhibit the effect of *T. denticola* on Aβ in N2a neuronal cells.

**Materials and Methods**

**Bacterial Strains and Growth**

*P. gingivalis* ATCC 33277 cultures were routinely grown in brain heart infusion (BHI) medium containing 37 g/L brain heart infusion (Becton, Dickinson and Company), L-cysteine hydrochloride (0.5 mg/mL) and hemin (5 μg/mL) under anaerobic conditions; *T. denticola* ATCC 35405 was grown under anaerobic conditions in new oral spirochete (NOS) broth, as described previously (Fenno et al. 2005). All growth media were incubated in anaerobic conditions at least 18 h prior to use.

**Mice and Treatments**

The male mice used in this study (the animal experiment center of Sichuan University, Chengdu, China) were of a C57BL/6 background and at 8 weeks of age. All the mice were divided into three groups randomly, including the experimental group, the positive control group and the blank control group. The experimental group and the positive control group were separately treated with a mixture of 25U 2% CMC and 25U *T. denticola* or *P. gingivalis* fluid for 24 weeks at a frequency of three times a week (Chukkapalli et al. 2014; Ilievski et al. 2018), while the blank control group received an equal volume of phosphate-buffered saline (PBS) solution three times a week. One week after the final treatment, mice were anaesthetized. After the collection of blood, mice were quickly perfused intracardially with chilled PBS (0.1 M, pH 7.3), then hippocampi, trigeminal ganglions and aortas were removed, and the hippocampi were dissected. All trigeminal ganglions and aortas and half of the hippocampi were flash-frozen in liquid nitrogen and stored at −80 °C, and the other half of the hippocampi were fixed with 4% paraformaldehyde. Relevant guidelines were followed in our animal experiment.

**Polymerase Chain Reaction**

To confirm the spread of periodontal pathogens from the mouth to the brain of mice, genomic DNA was isolated from the tissues samples with the DNeasy Blood & Tissue Kit (Qiagen, Germany). DNA amplification was performed using a PCR amplification kit (TAKARA, Japan) according to the manufacturer’s instructions. Briefly, the PCR mixture contained 12.5 μL Taq PCR Master, 0.5 μL (10 μg/mL) DNA samples, 1 μL forward primer, 1 μL reverse primer and 10 μL sterilized ddH2O. The primers used for amplification were as follows: *P. gingivalis*, 5′-AGGCAGCTTTGCC ATACTGGCG-3′ (forward), 5′-ACTGTTAGCAACTACCGA TGT-3′ (reverse) and *T. denticola*, 5′-TAATACCGAATG TGCTCATTTACAT-3′ (forward), 5′-CTGCCATTCTC TATGTCATTGCTTT-3′ (reverse) (TSINGKE, China). The sequencing parameters consisted of an initial denaturation step at 94 °C for 4 min and 35 cycles as follows: 94 °C for 30 s, annealing at 55 °C for 5 s and elongation at 72 °C for 10 s. The PCR products were analyzed using agarose gel electrophoresis (1.5%) under 100 V for 23 min with *P. gingivalis* ATCC 33277 DNA and *T. denticola* ATCC 35405 DNA as the positive control and a blanket reaction system as the negative control. The specific bands of the samples were compared with the positive group to determine whether the samples contained *P. gingivalis* ATCC 33277 and *T. denticola* ATCC 35405.

**Aβ1–40 and Aβ1–42 ELISA**

Protein of mice hippocampi was extracted with a total protein extraction kit (PE001, Signalway Antibody, USA). The homogenate was centrifuged, and the supernatant was collected to detect the levels of Aβ1–40 and Aβ1–42, which were quantified with beta amyloid (1–42) monoclonal antibody (1:500, GT622, Thermo Fisher Scientific, USA) and human amyloid beta (1–40) ELISA Kit (ab193692, Abcam, Cambridge, MA, USA) according to the manufacturer’s specifications.
**Immunohistochemistry**

The tissues of the mice hippocampi fixed by 4% paraformaldehyde were embedded in paraffin and sliced coronally into 5-μm sections using a vibratome (Leica, Germany). The sections were performed for antigen repair at 99.9°C for 30 min after deparaffination and then incubated for 10 min in 3% H2O2 and incubated with primary rabbit polyclonal antibodies against Aβ1–40 (bs-0877R, 1:100, Boiss, China), and primary rabbit monoclonal antibodies against Aβ1–42 (ab224275, 1:200, Abcam, Cambridge, MA, USA) overnight at 4°C. Then, the sections were incubated with biotin-labeled secondary antibodies (1:300, 865,002, R&D Systems) was used to detect the positive staining area. The resultant cDNA was used for the template for quantitative PCR analyses with gene-specific primers (TSINGKE, China). The sequences of the primers were as follows: BACE-1, 5′-CAGTGGGACCACCCACCT TC -3′ (forward) and 5′-GCTGCTTGTGAGCTTTGAC -3′ (reverse); APP, 5′-TCCGAGAGGTGTGCTGAA-3′ (forward) and 5′-CCACATCCGGCCGTAAGAATG-3′ (reverse); PS-1, 5′-GTGCTTGTGTTGACTCCCAA-3′ (forward) and 5′-CAACCACACCATTGTTGAGA GAT -3′ (reverse); and PS-2, 5′-GAAGACTCTAGCAGTC TTGG-3′ (forward) and 5′-CACCGAGGCGTTGAGA GAT -3′ (reverse). Real-time PCR reactions were performed with the Applied Biosystems QuantStudio 6 Flex Real-Time PCR System in the presence of 0.8 μL for each primer, 2 μL of cDNA, 6μL nuclease-free water, 0.4 μL ROX Reference Dye II and 10 μL of TB Green Primix Ex Taq II (TAKARA, Japan) for a total volume of 20 μL. The following PCR conditions were used for all samples: 95°C for 30 sec, and then 40 cycles of 95°C for 5 s, and 60°C for 30 sec. The fluorescence intensity was monitored at the end of each amplification step. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) served as the internal reference. The method of was adopted to calculate the relative mRNA expression of the target genes.

**Western Blot Analysis**

Hippocampi were lysed using lysis buffer containing 20 mM Tris, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 50 mM NaF, 1 mM Na3VO4, 1 mM PMSF and a protease inhibitor (200612, Signalway Antibody, USA). The BCA method was used to qualify protein concentration. The supernatant of the hippocampus extract was mixed with SDS-PAGE protein loading buffer (5×) (Beyo) which was used as γ-secretase inhibitor or treated with 1 μmol/L KMI1303(WaKo, Japan) for 6 h, or cultured with no treatment. After that, cells were infected with T. denticola or P. gingivalis at a density of 105 CFU per well for 2 h.

**Cell Culture and Treatment**

N2a neuronal cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum, 1 U/mL penicillin and 1 μg/mL streptomycin in a humidified (5% CO2, 37°C) incubator. After confirming the cell status, the N2a cells were first seeded in 24-well tissue culture plates at a density of 105 cells per well in DMEM culture medium with 10% fetal bovine serum. After cells adhesion, N2a cells were treated with 1 μmol/L DAPT (ApexBio Technology, USA) which was used as γ-secretase inhibitor or treated with 1 μmol/L KMI1303(WaKo, Japan) for 6 h, or cultured with no treatment. After that, cells were infected with T. denticola or P. gingivalis at a density of 107 CFU per well for 2 h.

**Immunofluorescence**

N2a neuronal cells fixed with 4% paraformaldehyde were permeated by 0.5% TritonX-100 in PBS, and then blocked in 2% BSA at 37°C for 1 h and then incubated with β-amyloid (1–40 Specific) (D8Q7I) Rabbit mAb (12990, 1: 600, Cell Signaling Technology, USA), rabbit monoclonal antibodies against Aβ1–40 (ab224275, 1: 200, Abcam, Cambridge, MA, USA) and Aβ1–42 (ab224275, 1: 200, Abcam, Cambridge, MA, USA) for 1 day at 4°C. After washing with PBS, the sections were incubated with goat anti-rabbit IgG (H&L) FITC (L30113, 1:200; Signalway Antibody, USA) at room temperature for 1 h in the dark. After washing with PBS, the sections were incubated with DAPI solution (Solarbio,
Beijing, China) at room temperature for 5 min and mounted in the Vectashield anti-fading medium (Solarbio, Beijing, China). Fluorescent images were captured by LEICA DMi8 and quantified by the ImageJ software (1.41v, US National Institutes of Health).

**Statistical Analysis**

Data were presented as the mean ± standard deviation and analyzed by SPSS 16.0 statistical software (SPSS Inc., Chicago, IL, USA). Student’s t test was applied to analyze statistical differences. The level of significance was set at \( p < 0.05 \) (*).

**Results**

**T. denticola 16S rDNA was Detected in the Aorta, Trigeminal Ganglion and Hippocampus of Mice**

Spirochetes like *Treponema pallidium* are capable of entering brain tissue, and pathologies suggest a relationship with AD (Miklossy et al. 2016). Besides, *P. gingivalis* was identified in the brain of Alzheimer’s disease patients (Dominy et al. 2019). Thus, after 24 weeks of oral injection, polymerase chain reaction was employed to detect whether *T. denticola* and *P. gingivalis* entered the hippocampus. As presented in Fig. 1, *T. denticola* and *P. gingivalis* were detected significantly more frequently in the hippocampus (7 of 10), compared with blank control samples (0 of 10). To detect how *T. denticola* and *P. gingivalis* entered the hippocampus, the aortas and trigeminal ganglion were subjected to detection. *T. denticola* was found in seven aorta samples, while *P. gingivalis* was found in nine samples. Further, three trigeminal ganglion samples were revealed to contain *T. denticola*.

**T. denticola induced Aβ1–40 and Aβ1–42 Accumulation in the Hippocampus**

We wondered whether *T. denticola* or *P. gingivalis*, which entered the hippocampus, had an effect on Aβ, so we subjected the samples to ELISA analysis and immunohistochemistry using the PBS group as blank control and the *P. gingivalis* group as positive control. Our data revealed that oral *T. denticola* or *P. gingivalis* infection induced Aβ1–40 and Aβ1–42 accumulation in the hippocampus of mice, and there was no significant difference between the two groups (Fig. 2b and c). Overexpressed Aβ1–40 and Aβ1–42 mainly accumulated in the cytoplasm and intercellular substance.

### Fig. 1
*T. denticola* and *P. gingivalis* are detected in the samples of mice following oral application. **a** From top to bottom, the hippocampus of the PBS group, hippocampus of the *P. gingivalis* group and aorta of the *P. gingivalis* group are shown in turn. **b** The hippocampus of the blank control group, hippocampus of the *T. denticola* group, aorta of the *T. denticola* group and trigeminal ganglion are shown in turn. All results above have been verified more than three times.
T. denticola induced Aβ1–40 and Aβ1–42 Accumulation by Directly Acting on Mouse Nerve Cells

The above experiments revealed that T. denticola and P. gingivalis entered the hippocampus and that the expression of Aβ1–40 and Aβ1–42 increased in the hippocampus of mice. We speculated that T. denticola and P. gingivalis entering the hippocampus could act directly on mouse nerve cells and cause Aβ1–40 and Aβ1–42 accumulation. To test our hypothesis, we stimulated N2a neurons with T. denticola and P. gingivalis in vitro. The immunofluorescence results confirmed our hypothesis. As shown in Fig. 3, the expression of Aβ1–40 and Aβ1–42 in N2a cells cocultured with T. denticola or P. gingivalis was significantly increased compared with the blank control group.

T. denticola Induced Aβ Accumulation by Upregulating BACE1, and PS1, KMI‑1303 and DAPT Inhibited the Effect of T. denticola on Aβ

For the purpose of further exploring the mechanism of T. denticola on Aβ, total RNA extracted from the brain tissues was used for real-time PCR. It was shown that the expressions of BACE1, PS1 and PS2 increased in T. denticola and P. gingivalis groups. The expression level of BACE1 and PS1 in the T. denticola group was significantly higher than that in the blank control group. Next, we conducted Western blot analysis. The results of Western blot showed that the expression levels of BACE1 and PS1 in the T. denticola and P. gingivalis groups were significantly higher than those in the blank control group. In addition to the effect on β-secretase and PS1, P. gingivalis had an effect on PS2, a subtype of presenilin with similar biological functions to PS1. In order to further confirm the mechanism of T. denticola inducing Aβ accumulation, β-secretase inhibitor and γ-secretase inhibitor were used to test and verify the results above in vitro. KMI-1303, the inhibitor with an IC50 value of 9 nM, has the same effect and mechanisms as KMI429 (Asai et al. 2006) on inhibiting β-secretase activity according to the manufacturer. PS1 and PS2 are two subtypes of presenilin and have similar biological functions, and the effect of T. denticola on PS1 was more significant. Thus, we choose DAPT, a potent γ-secretase inhibitor which can lead to a decreased flexibility of key PS1 regions related to the recognition and internalization of γ-secretase substrates (Aguayo-Ortiz et al. 2019), to confirm the mechanisms of T. denticola on Aβ. As presented in Fig. 4, the expression of Aβ1–42 in the inhibitor group is significantly lower than that in the coculture group.
Discussion

CP is known to trigger several human diseases including AD (Kamer et al. 2015) and atherosclerosis (Arabi et al. 2018). Periodontal pathogens such as *P. gingivalis* and *T. denticola* have been found in human postmortem brain tissues of AD patients (Riviere et al. 2002, Poole et al. 2013). Many attempts have been made to clarify the role of periodontal pathogens in systemic diseases. Some studies have shown that *P. gingivalis* infection induces Aβ accumulation (Dominy et al. 2019), tau hyperphosphorylation (Tang et al. 2020) and neuroinflammation (Tang et al. 2020; Liu et al. 2020). The viewpoint that spirochetes may be involved in the etiology of AD was first proposed by Miklossy (Miklossy et al. 1993). In a more recent meta-analysis of 495 brain and blood samples from AD patients, 91% were positive for spirochetes compared to 0% for 185 controls (Miklossy et al. 2011). However, the effect and mechanisms of *T. denticola*, a periodontal pathogen as well as a spirochete in AD, was not clearly understood. Here, we used a *P. gingivalis* orally infected mouse model (Dominy et al. 2019; Liu et al. 2020) as a positive control to study the effect of *T. denticola*. Our experiment demonstrated that *P. gingivalis* and *T. denticola* could enter the brain mainly through the blood and cause Aβ aggregation to the same extent. Besides, *T. denticola* 16S rDNA was also detected in a small number of trigeminal ganglion samples. Hence, we speculate *T. denticola* may directly

Fig. 3 *T. denticola* directly induces Aβ$_{1-40}$ and Aβ$_{1-42}$ accumulation in mouse nerve cells. a, c Images represent the results of three repeated experiments. N2a cells were cocultured with *T. denticola* or *P. gingivalis* for 2 h, and used for immunofluorescence with anti-Aβ$_{1-40}$ antibody and anti-Aβ$_{1-42}$ antibody. Green fluorescence shows Aβ$_{1-40}$ and Aβ$_{1-42}$. Counterstaining with DAPI, blue color, suggests cell nuclei. b, d Results of IF are expressed as means±std. dev., ***p<0.001
enter the hippocampus via blood and trigeminal nerves to induce Aβ 1–40 and Aβ1–42 accumulation.

Cerebral accumulation and aggregation of Aβ peptides is a characteristic pathological marker of AD patients. Soluble Aβ oligomers are believed to represent key structures that produce cytotoxicity, contribute to synaptic deficits and initiate the detrimental cascade involved in the pathology of AD (Salahuddin et al. 2016; Larson et al. 2012; Klein et al. 2006). BACE1 cleavage of APP is the rate-limiting step along the amyloidogenic processing pathway (O’Brien et al. 2011), and PS1 is involved in γ-secretase activity and influences Aβ1–40 and Aβ1–42 production. Knockout of BACE1 completely blocks the generation of Aβ (Cai et al. 2001). About 90% of mutations in human PS1 individually lead to reduced production of Aβ1–40 and Aβ1–42 (Sun et al. 2017). Our data indicate that T. denticola increases Aβ1–40 and Aβ1–42 accumulation in the hippocampus of C57BL/6 mice by upregulating BACE1 and PS1. Besides, we have verified the above results with β-secretase inhibitor and γ-secretase inhibitor in N2a neuronal cells.

In summary, we present data obtained by PCR, ELISA, IHC, qPCR, Western blot and IF to support the hypothesis

**Fig. 4** *T. denticola* induces Aβ accumulation by up regulating BACE1 and PS1. KMI-1303 and DAPT can inhibit the effect of *T. denticola* on Aβ. a RNA was extracted from mouse hippocampus, and the expression of genes related to Aβ accumulation was detected by qPCR. The values are shown as the mean ± SD of three independent experiments. *p < 0.05, **p < 0.01, ***p < 0.001, N = 4 mice per group. b, c, d, e Protein expression levels of APP, BACE1, PS1 and PS2 in the hippocampus were examined by western blotting. The results of the quantitative analysis of the blots; results are presented as means ± std. dev., *p < 0.05, **p < 0.01, ***p < 0.001, N = 4 mice per group. f Images represent the results of three repeated experiments. N2a cells were treated with 107 CFU *T. denticola* or 1 μmol/L DAPT and 107 CFU *T. denticola* or 1 μmol/L KMI1303 and 107 CFU *T. denticola*. *T. denticola* -infected N2a cells were stained by an anti-Aβ1–42 protein antibody and viewed under a fluorescence microscope. g The results of IF are expressed as means ± std. dev., *p < 0.05, ***p < 0.001.
that \textit{T. denticola} in the oral cavity can enter the hippocampus via the blood and trigeminal ganglion and act directly on nerve cells resulting in intra- and extracellular Aβ\textsubscript{1-40} and Aβ\textsubscript{1-42} accumulation in the hippocampus of C57BL/6 mice. The upregulation of BACE1 and PS1 is the mechanism of Aβ\textsubscript{1-42} accumulation in the hippocampus of C57BL/6 mice.

Acknowledgments We thank M.D. Jia-Jia Wang Yubin Cao and Yuan-Yuan Yin for their help with our experiments and M.M. Rui-Ting Peng for the \textit{T. denticola} strain.

Author Contributions All authors contributed to the study. Hongkun Wu was responsible for conceiving, designing, and supervising the present study, and guiding the revision of the manuscript. Zhiyue Lu was responsible for manuscript revision and professional editing. Material preparation, data collection, and analysis were performed by Xinyi Su, Zhiqun Tang, Yuqiu Liu, Wanzhi He, Jiepei Jiang and Yifan Zhang. The first draft of the manuscript was written by Xinyi Su. All authors commented on previous versions of the manuscript and approved the final manuscript.

Funding This work was supported by a research grant from the Sichuan Province Science and Technology Key Research and Development Program, Chengdu, China (grant no. 2018ZS0163), and the Geriatric Health Care and Medical Research Center, Sichuan University, Chengdu, Sichuan Province, China.

Data Availability The data sets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Research Involving Human and Animal All animal experiments were conducted at the State Key Laboratory of Oral Diseases and were licensed by the Research Ethics Committee of West China Hospital of Stomatology (no. WCHSIRB-D2019-013).

Conflict of Interest The authors declare that they have no competing interests.

References

Aarabi G, Heydecke G, Seedorf U (2018) Roles of Oral Infections in the Pathomechanism of Atherosclerosis. Int J Mol Sci 19:1978

Aguayo-Ortiz R, Guzmán-Ocampo DC, Dominguez L (2019) Toward the Characterization of DAPT Interactions with γ-Secretase. Chem Med Chem 14:1005–1010

Asai M, Hattori C, Iwata N, Saito TC, Sasagawa N, Szabó B, Hashimoto Y, Maruyama K, Kiso Y, Ishiura S (2006) The novel beta-secretase inhibitor KMI-429 reduces amyloid beta peptide production in amyloid precursor protein transgenic and wild-type mice. J Neurochem 96:533–540

Cai H, Wang Y, McCarthy D, Wen H, Borchelt DR, Price DL, Wong PC (2001) BACE1 is the major beta-secretase for generation of Abeta peptides by neurons. Nat Neurosci 4:233–234

Chakkapalli SS, Rivera MF, Velsko IM, Lee JY, Chen H, Zheng D, Bhattacharyya I, Gangula PR, Lucas AR, Kesavalu L (2014) Invasion of oral and aortic tissues by oral spirochete Treponema denticola in ApoE(-/-) mice causally links periodontal disease and atherosclerosis. Infect Immun 82:1959–1967

Dominy SS, Lynch C, Ermini F, Benedyk M, Marczyk A, Konradi A, Nguyen M, Haditsch U, Raha D, Griffin C, Holsinger LJ, Arastu-Kapur S, Kaba S, Lee A, Ryder MI, Potempa B, Mydel P, Hellvard A, Adamowicz K, Hasturk H, Walker GD, Reynolds EC, Faull RLM, Curtis MA, Dragonow M, Potempa J (2019) \textit{Porphyromonas gingivalis}&nbsp;in Alzheimer’s disease brains: Evidence for disease cause and treatment with small-molecule inhibitors. Sci Adv 23:eaaan3333.

Fenno JC (2005) Laboratory maintenance of Treponema denticola. Curr Protoc Microbiol Chapter 12 Unit 12B.1.

Gil-Montoya JA, Sanchez-Lara I, Carnero-Pardo C, Fornies F, Montes J, Vilchez R, Burgos JS, Gonzalez-Moles MA, Barrios R, Bravo M (2017) Is periodontitis a risk factor for cognitive impairment and dementia? A case-control study. J Periodontol 86:244–253

Gosztyla ML, Brothers HM, Robinson SR (2018) Alzheimer’s Amyloid-β is an Antimicrobial Peptide: A Review of the Evidence. J Alzheimers Dis 62:1495–1506

Ilievski V, Zuchowska PK, Green SJ, Toth PT, Ragozzin ME, Le K, Aljewari HW, O’Brien-Simpson NM, Reynolds EC, Watanabe K (2018) Chronic oral application of a periodontal pathogen results in brain inflammation, neurodegeneration and amyloid beta production in wild type mice. PLoS One 13:0204941

Kamer AR, Pirraglia E, Tsui W, Ruisene H, Vallabhajosula S, Mosconi L, Yi L, McHugh P, Craig RG, Svetcov S, Linker R, Shi C, Glodzik L, Williams S, Corby P, Saxena D, de Leon MJ (2015) Periodontal disease associates with higher brain amyloid load in normal elderly. Neurobiol Aging 36:627–633

Klein WL (2006) Synaptic targeting by A beta oligomers (ADDLS) as a basis for memory loss in early Alzheimer’s disease. Alzheimers Dement 2:43–55

Larson ME, Lesně SE (2012) Soluble Aβ oligomer production and toxicity. J Neurochem 120:125–139

Lesně S, Koh MT, Kotilinek L, Kayed R, Glabe CG, Yang A, Gallagher M, Ashe KH (2006) A specific amyloid-beta protein assembly in the brain impairs memory. Nature 440:352–357

Liu J, Wang Y, Guo J, Sun J, Sun Q (2020) Salvianolic Acid B improves cognitive impairment by inhibiting neuroinflammation and decreasing Aβ level in \textit{Porphyromonas gingivalis}-infected mice. Aging (Albany NY) 12:10117–10128

Miklossy J (1993) Alzheimer’s disease—a spirochetiosis? Neuro Report 4:841–848

Miklossy J (2011) Alzheimer’s disease—a neurospirochetosis. Analysis of the evidence following Koch’s and Hill’s criteria. J Neuroinflammation. 8 : 90.

Miklossy J (2016) Bacterial Amyloid and DNA are Important Constituents of Senile Plaques: Further Evidence of the Spirochetal and Biofilm Nature of Senile Plaques. J Alzheimers Dis 53:1459–1473

O’Brien RJ, Wong PC (2011) Amyloid precursor protein processing and Alzheimer’s disease. Annu Rev Neurosci 34:185–204

Poole S, Singhrao SK, Kesavalu L, Curtis MA, Crean S (2013) Detecting the presence of periodontopathic virulence factors in short-term postmortem Alzheimer’s disease brain tissue. J Alzheimers Dis 36:665–677

Riviere GR, Riviere KH, Smith KS (2002) Molecular and immunological evidence of oral Treponema in the human brain and their association with Alzheimer’s disease. Oral Microbiol Immunol 17:113–118

Salahuddin P, Fatima MT, Abdelhameed AS, Nusrat S, Khan RH (2016) Structure of amyloid oligomers and their mechanisms of toxicities: Targeting amyloid oligomers using novel therapeutic approaches. Eur J Med Chem 114:41–58
Scheltens P, Blennow K, Breteler MM, de Strooper B, Frisoni GB, Salloway S, Van der Flier WM (2016) Alzheimer’s disease. Lancet 388:505–517
Shin HS, Shin MS, Ahn YB, Choi BY, Nam JH, Kim HD (2016) Peri-odontitis Is Associated with Cognitive Impairment in Elderly Koreans: Results from the Yangpyeong Cohort Study. J Am Geriatr Soc 64:162–167
Sochocka M, Zwolińska K, Leszek J (2017) The Infectious Etiology of Alzheimer’s Disease. Curr Neuropharmacol 15:996–1009
Sparks Stein P, Steffen MJ, Smith C, Jicha G, Ebersole JL, Abner E, Dawson D 3rd (2012) Serum antibodies to periodontal pathogens are a risk factor for Alzheimer’s disease. Alzheimers Dement 8:196–203
Sun L, Zhou R, Yang G, Shi Y (2017) Analysis of 138 pathogenic mutations in presenilin-1 on the in vitro production of Aβ42 and Aβ40 peptides by γ-secretase. Proc Natl Acad Sci USA 114:476–485
Tang Z, Liang D, Cheng M, Su X, Liu R, Zhang Y, Wu H (2020) Effects of Porphyromonas gingivalis and Its Underlying Mechanisms on Alzheimer-Like Tau Hyperphosphorylation in Sprague-Dawley Rats. J Mol Neurosci doi: https://doi.org/10.1007/s12031-020-01629-1. Epub ahead of print.
Viola KL, Klein WL (2015) Amyloid β oligomers in Alzheimer’s disease pathogenesis, treatment, and diagnosis. Acta Neuropathol 129:183–206
Wirths O, Zampar S (2019) Emerging roles of N- and C-terminally truncated Aβ species in Alzheimer’s disease. Expert Opin Ther Targets 23:991–1004
Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.