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Editorial

Porphyromonas gingivalis infection may contribute to systemic and intracerebral amyloid-beta: Implications for Alzheimer's disease onset

Abstract

The microbiota of "chronic" periodontitis, particularly *Porphyromonas gingivalis*, have been implicated in Alzheimer's disease (AD) because this bacterium has a range of enzymes (cathepsin B and gingipains) that are shown to interact with the amyloid precursor protein (APP) and neuronal tau resulting in the formation of amyloid-beta (Aβ) and neurofibrillary tangles (NFTs). These two lesions remain pivotal to explaining AD pathogenesis alongside of clinical symptoms. Deposits of Aβ in the brain can start 10-20 years before the clinical symptoms of cognitive decline and the diagnosis of AD is established. It is rarely mentioned that the AD risk doubles if the individual has received a diagnosis of periodontitis for around 10 years. This editorial is a review of recent but salient literature supporting the idea that periodontal disease can contribute to a systemic Aβ pool that may enter the brain over time. In addition, intracerebral production of Aβ can be initiated by *P. gingivalis*, which occurs via host and bacterially derived cathepsin B acting as β-secretase to process the APP via the amyloidogenic pathway yielding $A\beta_{3\text{-}42}$. These findings support a systemic and an intracerebral Aβ contribution from "chronic" periodontitis in subsequent AD development.

Keywords Inflammation; microbiota; periodontitis; systemic; amyloid; $A\beta_{3\text{-}42}$; cathepsin B;

Introduction

Generalized ("chronic") periodontitis, a common inflammatory disease affecting the supporting tissues of teeth, has been associated with several systemic diseases, e.g. cardiovascular diseases, diabetes, adverse pregnancy outcomes, rheumatoid arthritis, respiratory diseases, and Alzheimer's disease (AD).[1-7] Bacteria of the periodontal pocket can spread through the blood stream, which is the common but not the only way of systemic bacterial dissemination in periodontitis.[8] Dental treatment, tooth brushing, flossing, chewing, and use of tooth-picks in a patient with periodontitis will release a bacteremia.[9] This can occur several times during the day and has been estimated to last for up to 3 hours.[10] Tooth-related bacteremia contains a wide spectrum of bacteria[11] among which the Gram-negative anaerobic rod *Porphyromonas gingivalis* seems to have a key role in the adult form of generalized periodontitis.[12,13]

A plethora of studies firmly place *P. gingivalis* but not its companion species (for example *Tannerella forsythia* and *Treponema denticola* in the red complex[13]) as a risk factor for AD. This is because *P. gingivalis* is adept at modifying the peripheral and intracerebral immune responses.[14-16] Furthermore, this bacterium has a range of enzymes including cathepsin B[17] and gingipains[18] that are respectively shown to interact with the amyloid precursor protein (APP) and neuronal tau resulting in the formation of amyloid-beta (Aβ) and neurofibrillary tangles (NFTs),[19,20] which are the cardinal hallmarks of AD. Prospective, retrospective population-based and nested control studies have shown that the risk of developing the sporadic form of AD doubles when periodontal disease persists for about ten years.[21-23] This is evident from the fact that a large section of individuals who go on to developing clinical AD also suffers from periodontitis.

Brain inflammation, characterized by increased activation of microglia and astrocytes, increases during aging and is a key feature of AD.[24] This has been explained in terms of the hallmark lesions of AD, which are $A\beta_{40/42}$ extracellular deposits in the form of plaques and hyperphosphorylated tau protein associating with intraneuronal lesions called NFTs. Accumulation of Aβ plaques results from the proteolytic cleavage of the APP by β- and γ-secretase enzymes.[25,26] These secretases are different in AD driven by bacterial infections compared to the classically described site-specific secretases in the mutated APP of AD.[27, 28] Similarly, toxic proteases from *P. gingivalis* called gingipains have been identified in the brain of AD patients, and the levels correlated with tau and ubiquitin pathology.[15]

Aβ is classically believed to be produced by neurons within the AD brain irrespective of the trigger that causes its release. However, this view is changing, as some researchers believe the peripheral/systemic Aβ pool is also a contribution from platelets, skeletal muscle cells, skin fibroblasts, and monocyte/macrophages[29-31] and this has implications for AD pathogenesis over time. Production of inflammagens such as gingipains and lipopolysaccharide (LPS) secreted by *P. gingivalis* also occurs in the periodontal pocket where inflammatory macrophages are reported to bear Aβ.[32] Gil-Montoya et al.[33] have reported increased plasma $A\beta_{1-42}$ levels in individuals who have severe periodontal disease. Thus Leira et al.[34] found when experimental periodontitis was induced in Sprague-Dawley rats, a strong positive correlation between alveolar bone loss and $A\beta_{1-40}$ serum levels at 7 days ($r = 0.695$, $P = 0.012$) and with serum $A\beta_{1-42}$ concentrations at 21 days ($r = 0.968$, $P = 0.002$). Taken together, Aβ also being generated peripherally in platelets, skin fibroblasts and skeletal muscles [29, 30] may enter the circulating blood.[31] The present editorial aims to discuss whether *P. gingivalis* can contribute to systemic and intracerebral pools of Aβ.

P. gingivalis induces systemic Aβ production in infected mice

Nie et al.[32] recently reported that chronic, systemic *P. gingivalis* infection increased the inflammatory responses and proteins associated with Aβ-production in the liver of mice. The liver was chosen for the peripheral Aβ source in macrophages because of the general abundance of these cells.[32] Nie et al.[32] observed that *P. gingivalis* infection in mouse liver macrophages, caused a rapid production of interleukin 1-beta (IL-1β) and thereafter an intracellular accumulation of Aβ through activation of Toll like receptor 2 /nuclear factor kappaB (TLR2/NF-κB) signaling. NF-κB-dependent cathepsin B appeared crucial for cleaving pro-IL-1β and processing APP to induce the accumulation of pathogenic $A\beta_{3-42}$, which was significantly increased in liver macrophages of the *P. gingivalis*-infected mice. This original study demonstrated peripheral pools of Aβ due to periodontitis in macrophages within the periodontal tissue and in mice hepatic macrophages following *P. gingivalis* infection. In a follow-up study, Zeng et al.[17] induced systemic *P. gingivalis* infection in mice by intraperitoneal injections containing (1×10^8 CFU/mouse every three days) for three weeks. This significantly increased the expression of the advanced glycation end products (RAGE) receptor in the cluster of differentiation 31 (CD31)-positive endothelial cells. This implied that *P. gingivalis* systemic infection up-regulated RAGE expression in cerebral endothelial cells and facilitated Aβ entry into the mouse brain. Cathepsin B was suggested to be a contribution from the bacterium and the host with a critical role in regulating the NF-

κB/RAGE expression and in the processing of APP. This study further supported the Nie et al.[32] concept for the potential in systemic spread of peripheral Aβ to the brain from *P. gingivalis* infection. In a proof of concept study, Bu et al.[31] had demonstrated the plausibility of peripheral Aβ entry to the brain being facilitated by the RAGE receptor within cerebral endothelial cells.[17] An alternative mode of peripheral Aβ entry into the brain is via macrophages of the lymphatic system.[35]

Another focus of Nie and colleagues[32] was $A\beta_{1-42}$, which is classically considered as the toxic form of Aβ. They observed that $A\beta_{3-42}$ (Fig. 1) not only occurred earlier but was also two-fold higher than $A\beta_{1-42}$ in the AD brain.[32] In AD, Cathepsin B stimulated intracellular production of Aβ in the brain, including the $A\beta_{3-42}$. Interestingly, $A\beta_{3-42}$ following *P. gingivalis*-infection in mice generated IL-1β, which is a proinflammatory cytokine.[32] IL-1β, participated in increasing the *in vivo* levels of $A\beta_{3-42}$ in the hepatic macrophages of *P. gingivalis*-infected mice and *in vitro P. gingivalis*-infected macrophages. Furthermore, $A\beta_{3-42}$ was induced by *P. gingivalis* infection, which had caused significant death of macrophages and reduced their phagocytic capacity compared to that of $A\beta_{1-42}$, suggesting $A\beta_{3-42}$ is very toxic. $A\beta_{3-42}$ was also detected exclusively in the AD brain, and this corroborates with the significantly more toxic form than $A\beta_{1-42}$.[32] This study agreed with that of Leira et al.[34] who reported that LPS from *P. gingivalis* increased Aβ protofibrils in the serum of rats. After experimental periodontitis had been induced in male Sprague-Dawley rats it caused an acute elevation of $A\beta_{1-40}$ in serum that lasted during the whole experiment. $A\beta_{1-42}$ peptide levels however, peaked at the end of the study.

P. gingivalis also generates Aβ in the periodontium and within the brain

Systemically produced Aβ probably occurs in addition to locally generated Aβ in the periodontium and in the brain induced by *P. gingivalis*. As mentioned, Leira et al.[34] found a strong positive correlation between alveolar bone loss and $A\beta_{1-40}$ serum levels at 7 days ($r = 0.695$, $P = 0.012$) and with serum $A\beta_{1-42}$ concentrations at 21 days ($r = 0.968$, $P = 0.002$). Intracerebral production of Aβ generated by *P. gingivalis* has been seen in the brain of experimental wild type animals and with AD transgenes.[19, 30-32] Ilievski et al.[19] found that chronic oral application of *P. gingivalis* to wild type mice resulted in deposition of extracellular $A\beta_{1-42}$ together with neurodegeneration and intracerebral inflammation, as demonstrated previously by Poole et al.[36] Similarly, Wu et al.[37] found that chronic exposure to

LPS from *P. gingivalis* for five consecutive weeks caused learning and memory deficits together with intracellular accumulation of Aβ in neurons of middle-aged wild-type mice. Taken together, these reports suggest that *P. gingivalis* can induce both a local periodontal and a systemic Aβ production, thereby contributing to a pool of Aβ that can enter the brain facilitated by the endothelial RAGE receptor.

P. gingivalis interferes with components of the peripheral immune system aimed to defend the brain

Unexpectedly, recent research has shown that even components of the peripheral immune system, such as macrophages can participate in defending the brain from insults occurring outside the brain.[38] However, *P. gingivalis* has the ability to abolish the anaphylatoxin complement component 5a (C5a) in macrophages thereby undermining TLR2/4 immunity and degrade some of the complement receptor 1 (CR1) molecules that help clear amyloid via the spleen.[39] Whether this affects other macrophages in a similar way is not known. Further immune evasion strategies of *P. gingivalis* in relation to AD are discussed elsewhere.[40]

Concluding remarks

We have communicated that monocytes/macrophages from the periodontium and the liver may provide an additional circulating pool of unique $A\beta_{3\text{-}42}$ fragments in patients with periodontitis. Entry of *P. gingivalis* and/or its gingipains and LPS into the brain due to a defective blood-brain barrier can lead to intracerebral deposition of Aβ plaques. These findings support the notion that the adult form of generalized periodontitis via *P. gingivalis*, contributes to both an oral and hepatic cellular source of cells that add to the systemic pool of Aβ. This peptide can also be a contribution of other cell sources of peripheral organs like skin smooth cells and platelets which have the potential to transport Aβ to the brain and over time may play a role in AD pathogenesis. Deposits of Aβ in the brain can start 10-20 years before cognitive decline and the diagnosis of AD. This agrees with the timeline of at least 10 years required for periodontitis to initiate AD and emphasizes the need for meticulous dental hygiene as a feasible prophylaxis for AD.

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Figure legend

Fig. 1 summarizes the Nie et al.[32] vision as interpreted by Olsen and Singhrao for the contribution to AD of peripheral pools of Aβ, specifically $A\beta_{3\text{-}42}$. It is generated by *P. gingivalis* (*Pg*) oral infection that eventually reaches the liver and the brain. The proposed signaling pathway (TLR2,4/NF-κB) is also indicated where it is likely to act liberating interleukin-1β (IL-1β) cytokine that facilitates the amyloid precursor protein cleavage of Aβ via secretase enzymes, one of which is cathepsin B. The low-density lipoprotein receptor-related protein 1 (LRP1) is the receptor for Aβ transport from the brain to the peripheral blood. The Aβ from the systemic circulation can enter the brain using the advanced glycation end products (RAGE) receptor. Nie et al.[32] have shown Aβ within the gingival tissues of periodontitis patients and in the liver of middle-aged mice after chronic systemic *P. gingivalis* infection, thereby contributing to the peripheral pools of Aβ. Some researchers believe the peripheral Aβ also comes from platelets, skeletal muscle cells, skin fibroblasts, and monocyte/macrophages. The implications of the peripheral Aβ is that it can also enter the brain and contribute to AD pathology as shown by Bu et al.[31]