NEURAL REGENERATION RESEARCH
www.nrronline.org

Implication of salivary lactoferrin and periodontal-mediated infections in Alzheimer's disease

Cristina Municio*, Eva Carro*

Lactoferrin is an antimicrobial protein characterized by the exertion of many protective functions, including antibacterial, antifungal, antiviral, and antiparasitic properties, as well as anti-inflammatory and immunomodulatory activities (Kruzel et al., 2017). Lactoferrin is one of the major proteins present in exocrine secretions, including saliva, and is therefore associated with host defense against oral pathogens and control of the oral microbiome. In recent years, it has become clear that alterations in the oral microbiome may contribute to opportunistic pathogen infections in the brains of Alzheimer's disease (AD) patients and thus participate in or contribute to the development of this neurodegenerative disease (Sureda et al., 2020). Pathogenic oral microbes can affect neurological processes by entering brain tissue through various pathways and directly damaging the central nervous system. In the central nervous system, oral microbes may trigger an immune response that increases amyloid β (Aβ) production and may even trigger the Aβ cascade to promote the onset of AD, as we discuss in our previous study supporting the "infectious hypothesis" in AD (González-Sánchez et al., 2020).

Borsa et al. (2021) have identified an association between periodontal disease and AD. The incidence of periodontitis and caries, as well as the presence of antibodies in serum against periodontal diseases, are more prevalent in AD patients than in healthy individuals, suggesting a potential link between AD and a peripheral chronic inflammation (Sparks Stein et al., 2012). Increased *Porphyromonas gingivalis* (*P. gingivalis*) IgG antibodies in serum have been associated with problems in verbal function and short-term memory, and a relationship has been established between oral health, cognitive functions, AD incidence and mortality (Beydoun et al., 2020). In addition, postmortem studies in the neural tissue of AD patients have detected more virulence factors associated with peridontitis, such as lipopolysaccharide of *P. gingivalis*, than in healthy individuals (Poole et al., 2013). However, the nature of this relation remains unclear.

P. gingivalis is a Gram-negative bacteria component of the oral microbiome of healthy individuals, being one of the main causes of oral dysbiosis in order to its capacity for infection, formation of biofilms and chronic colonization. Recent data suggest that *P. gingivalis*, associated with chronic periodontitis and recently found in AD-diagnosed brain autopsy specimens, may be the link between those two pathological disorders (Dominy et al., 2019). Gram-negative bacteria naturally release outer membrane vesicles (OMV) that participate in signaling processes. OMV are lipid vesicles formed by components of the outer membrane and enrichment of cell signaling factors (RNAs, DNAs), proteins involve in iron acquisition (IhtB and HmuY) and virulence factors such as gingipains, lipopolysaccharides or muramic acid in the case of *P. gingivalis* (Nonaka et al., 2022). Consequently, OMVs may play a key role in the influence of *P. gingivalis* on the host.

P. gingivalis and bacterial OMV can enter the bloodstream during episodes of transient bacteremia and gain access to the brain via multiple routes including a leaky blood-brain barrier (BBB) (Olsen, 2021). A mechanistic pathway for bacterial entry into the brain is suggested to be via proteolytically active gingipains. Gingipains are the extracellular cysteine proteinases of *P. gingivalis* strain and can be classified as arginine-specific gingipain and lysine-specific gingipain. They are capable of degrading extracellular proteins such as fibronectin or collagen, increasing vascular permeability. They have also been described as disruptors of barriers through the degradation of tight junction proteins, as shown in **Figure 1**. In the case of the brain, gingipains are able to break down transmembrane proteins such as E-cadherin, β1 integrin, and occludin, thereby disrupting the tight junctions between capillary endothelial cells. These alterations in tight junction proteins cause an increase in the permeability of the BBB. More recently, it was confirmed that gingipains are involved in increasing permeability of human cerebral microvascular endothelial cells, through degradation of tight junction proteins, including zona occludens and occludin, reinforcing the role of gingipains as responsible for the damage of BBB (Nonaka et al., 2022). In AD, *P. gingivalis* may cause vascular endothelial barrier disruption and increased permeability destroying intercellular junctions (Olsen, 2021). This could promote the passage of this bacterium through the BBB to the AD brain where it has been found (Dominy et al., 2019).

Once reached by the brain, microbial invasion elicits the cerebral immune response resulting in neuroinflammation and in the formation of diagnostic lesions of AD. An increase in $A\beta_{42}$, production and accumulation in AD brains was observed after *P. gingivalis* infections (Dominy et al., 2019). Infection-induced BBB dysfunction can cause accumulation of Aβ leading to alterations and disruption of the neurovascular unit. It was showed that *P. gingivalis* significantly increased a time-dependent advanced glycation end products expression resulting in a dramatic increase in Aβ influx in the brain endothelial cells. Advanced glycation end products is accepted as the receptor for Aβ transport from the peripheral blood to the brain (Aβ influx) (Zeng et al., 2021). Furthermore, there was a significant decline in memory after 3 weeks of chronic systemic *P. gingivalis* infection in middle-aged mice (Zeng et al., 2021).

Previously, we have demonstrated that decreased salivary levels of lactoferrin are found as excellent early diagnostic performance markers to detect prodromal AD and AD dementia, even distinguishing them from other dementia as frontotemporal dementia (González-Sánchez et al., 2020). We found that reduced lactoferrin levels were associated to positive amyloid positron emission tomography result, one of the available pathophysiological validated AD biomarkers. Therefore, it is remarkable that salivary lactoferrin levels do not decrease in some other dementias-such as frontotemporal dementia. A possible explanation could be that the hypothalamic region is affected in the earliest prodromal stages of AD, but not in frontotemporal dementia. Typical AD hallmarks, including Aβ deposits, are observed in the hypothalamus of AD patients (Ishii and Iadecola, 2015). The apparently AD-specific salivary lactoferrin reduction may thus not only be useful in the differential diagnosis but could also provide important insights into selective immune vulnerability in neurodegenerative diseases. Cholinergic parasympathetic nerves, connected with the hypothalamus control the secretion of salivary proteins. Our hypothesis proposes that Aβ-induced hypothalamic dysfunction may lead dysregulation of salivary gland function resulting in decreased salivary lactoferrin secretion. It has been proposed that early hypothalamic Aβ accumulation is associated with gingipains deposition found in postmortem brain tissue (Nara et al., 2021). This could be an early switch that begins the loss of control and disrupt hypothalamic function affecting salivary gland regulation that ultimately results in reduced salivary lactoferrin secretion.

It is important to note that the neuroanatomical analysis of *P. gingivalis* mark cholinergic neurons, basal forebrain and anterior hypothalamic regions, and regions near ventricles and peripheral neurons, linking parts of the "gingipain" and "cholinergic hypotheses", as has been discussed by Nara et al. (2021). Extending this mechanism forward would mean ultimately interference with the cholinergic input to the salivary glands. Thus, lesions of the lateral or ventromedial hypothalamus impair salivary secretion, in AD, as recently discussed (Nara et al., 2021).

Antimicrobial peptides and proteins control the dissemination of oral microorganisms in the brain. Salivary lactoferrin plays an important role in regulating the oral microbiota and the inflammatory state of the oral mucosa, and contributes to the maintenance of symbiosis in the host-microbiome relationship (Kruzel et al., 2017). In this context, it was reported that lactoferrin displayed proteinase inhibitory activity against *P. gingivalis*, significantly inhibiting gingipain (Dashper et al., 2012). In addition, there are iron acquisition adhesive lipoproteins, presents both in *P. gingivalis* and OMV, on which lactoferrin may interferes by competing for iron uptake, essential nutrient for the bacteria development. Since the proteolytic activity of *P. gingivalis* is central to its pathogenicity, lactoferrin may have an important role in helping to prevent *P. gingivalis*-associated disease, including AD.

Therefore, when salivary lactoferrin levels are low, as seen in AD patients, oral dysbiosis is expected to be triggered freely. Indeed, Olsen and Singhrao (2021) proposed that salivary lactoferrin deficiency may act as an unknown trigger of oral microbial dysbiosis, supporting the concept that low levels of lactoferrin might indicate oral dysbiosis. This process link with the question of how and to what extent do infections affect the development of AD pathology? AD is multifactorial and heterogeneous disorder with several different etiopathogenic mechanisms. In this complex interaction of risk factors, infection-mediated immune responses, including lactoferrin expression in saliva, seem to be important.

There are conflicting data on whether or not oral pathogens could degrade lactoferrin. While some studies pointed out that, other data showed that after incubation with *P. gingivalis* proteinases, lactoferrin retained its inhibitory activity (Dashper et al., 2012). Thus, it is highly plausible that low salivary lactoferrin levels could promote the dissemination of oral-related microorganisms and inflammatory tissue mediators to the brain by reducing innate immunity.

In summary, the possibility of an infectious AD etiology has been postulated over the last two decades. At least two possibilities can be envisaged to explain the association between microbial infections and AD. One is that patients with AD are particularly prone to microbial infections, as it has been demonstrated in coronavirus disease 2019 pandemic, as we recently revised (Bartolomé et al., 2022). The other possibility is that pathogens may have a causative or contributory role in AD onset and progression. Since clinical trials targeting Aβ in the brain have mostly modest effects, therapeutic trials with antimicrobial in AD cohorts could shed some light. We believe now is the time to break with the established norm and introduce new and groundbreaking tests to verify whether these therapies may be of benefit, as has been proposed by other authors. As it is not clear if AD is associated with a single specific pathogen, antipathogen agents, such as lactoferrin, should possess wide antibacterial and antiviral activity, including strains found in the gut and in the oral microbiomes. As currently, there are no truly effective drugs capable of reversing or at least slowing the development of AD, if a subgroup of

AD patients could benefit from a well-tolerated antimicrobial agent, it would have a huge impact on public health, social and economic burden associated to AD. Latterly, neuroscientists and clinicians are realizing that answers to the cause of AD do not reside solely in the hallmarks of brain pathology, but rather in the etiology of that pathology, for which infectious agents provide a fascinating hypothesis. In this context, we propose that lactoferrin and its salivary levels represent a disrupted dual element in AD, as a new non-invasive diagnostic biomarker and a potential therapeutic target.

Our hypothesis proposes a positive feedback loop. In this cycle, AD-related hypothalamic dysfunction may lead dysregulation of salivary gland resulting in decreased salivary lactoferrin secretion. Based on lactoferrin-mediated antimicrobial activities, low levels of lactoferrin in saliva may represent a disruption in the defensive line against pathogens, including *P. gingivalis* that would have more possibilities of proliferating. *P. gingivalis* seriously affects BBB permeability facilitating its brain entrance and the subsequent neurological impairments. In brain, *P. gingivalis* infections are able to induce pathological processes, including Aβ accumulation that induces hypothalamic-mediated salivary gland dysfunction (**Figure 2**).

Cristina Municio*, Eva Carro*
Group of Neurodegenerative Diseases, Hospital Universitario 12 de Octubre Research Institute (imas12), Madrid, Spain; Network Center for Biomedical Research in Neurodegenerative Diseases (CIBERNED), Instituto de Salud Carlos III, Madrid, Spain (Municio C)
Neurobiology of Alzheimer's disease Unit, Functional Unit for Research into Chronic Diseases, Instituto de Salud Carlos III, Madrid, Spain; Network Centre for Biomedical Research in Neurodegenerative Diseases (CIBERNED), Instituto de Salud Carlos III, Madrid, Spain (Carro E)
***Correspondence to:** Eva Carro, PhD, eva.carro@isciii.es; Cristina Municio, PhD, cmunicio.imas12@h12o.es.
https://orcid.org/0000-0002-6504-4579 (Eva Carro)
https://orcid.org/0000-0002-8552-8183 (Cristina Municio)
Date of submission: January 17, 2023
Date of decision: March 8, 2023
Date of acceptance: March 25, 2023
Date of web publication: May 12, 2023

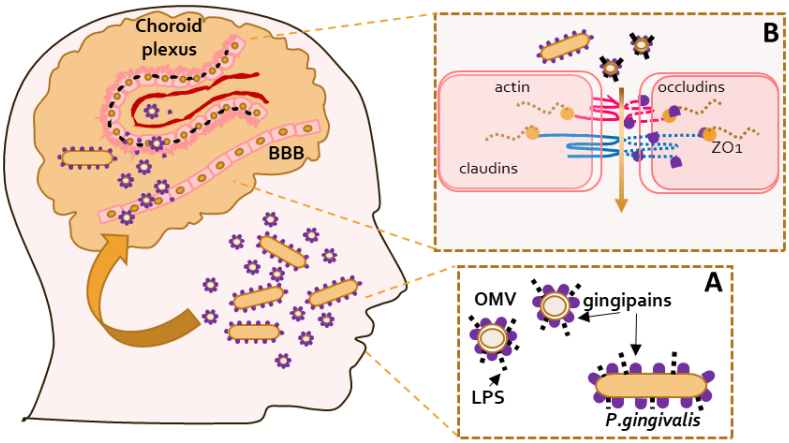

Figure 1 | **Schematic representation of the entry of *Porphyromonas gingivalis* (*P. gingivalis*) to the CNS.**
In healthy conditions, *P. gingivalis* population is under control in oral cavity. After dysregulation of the oral microbioma, *P. gingivalis* invades and colonizes the oral cavity. (A) Production of OMV after the bacterial cell wall is excised. OMV are enrichment in outer membrane proteins, lipoproteins, and extracellular components from *P. gingivalis*. (B) *P. gingivalis* and OMVs after entering in cerebral endothelial cells, through the blood circulation. Gingipains increase the permeability of the BBB and the choroid plexus epithelial cells due to degradation of tight junction proteins as occludin or ZO1. BBB: Brain-blood barrier; CNS: central nervous system; LPS: lipopolysaccharide; OMV: outer membrane vesicle; ZO1: zonula occludens. Created by the authors with PowerPoint and Canva.com.

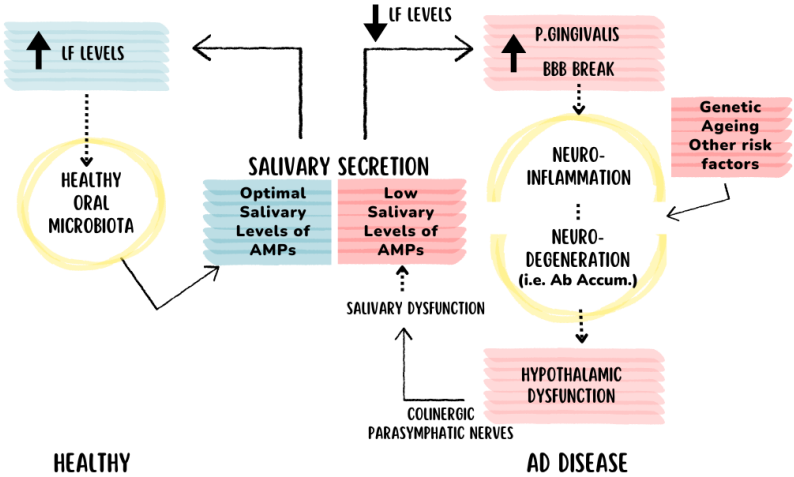

Figure 2 | **Flowchart showing lactoferrin-mediated antimicrobial activities in healthy and AD conditions.**
AD: Alzheimer's disease; AMPs: antimicrobial peptides; BBB: brain-blood barrier; LF: lactoferrin. Created by the authors with Canva.com.

https://doi.org/10.4103/1673-5374.373712
How to cite this article: *Municio C, Carro E (2023) Implication of salivary lactoferrin and periodontal-mediated infections in Alzheimer's disease. Neural Regen Res 18(12):2691-2692.*

References

Bartolomé F, Rosa L, Valenti P, Lopera F, Hernández-Gallego J, Cantero JL, Orive G, Carro E (2022) Lactoferrin as immune-enhancement strategy for SARS-CoV-2 infection in Alzheimer's disease patients. Front Immunol 13:878201.
Beydoun MA, Beydoun HA, Hossain S, El-Hajj ZW, Weiss J, Zonderman AB (2020) Clinical and bacterial markers of periodontitis and their association with incident all-cause and Alzheimer's disease dementia in a large national survey. J Alzheimers Dis 75:157-172.
Borsa L, Dubois M, Sacco G, Lupi L (2021) Analysis the link between periodontal diseases and Alzheimer's disease: a systematic review. Int J Environ Res Public Health 18:9312.
Dashper SG, Pan Y, Veith PD, Chen YY, Toh EC, Liu SW, Cross KJ, Reynolds EC (2012) Lactoferrin inhibits Porphyromonas gingivalis proteinases and has sustained biofilm inhibitory activity. Antimicrob Agents Chemother 56:1548-1556.
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, et al. (2019) Porphyromonas gingivalis in Alzheimer's disease brains: evidence for disease causation and treatment with small-molecule inhibitors. Sci Adv 5:eaau3333.
González-Sánchez M, Bartolome F, Antequera D, Puertas-Martín V, González P, Gómez-Grande A, Llamas-Velasco S, Herrero-San Martín A, Pérez-Martínez D, Villarejo-Galende A, Atienza M, Palomar-Bonet M, Cantero JL, Perry G, Orive G, Ibañez B, Bueno H, Fuster V, Carro E (2020) Decreased salivary lactoferrin levels are specific to Alzheimer's disease. EBioMedicine 57:102834.
Kruzel ML, Zimecki M, Actor JK (2017) Lactoferrin in a context of inflammation-induced pathology. Front Immunol 8:1438.
Nara PL, Sindelar D, Penn MS, Potempa J, Griffin WST (2021) Porphyromonas gingivalis outer membrane vesicles as the major driver of and explanation for neuropathogenesis, the cholinergic hypothesis, iron dyshomeostasis, and salivary lactoferrin in Alzheimer's disease. J Alzheimers Dis 82:1417-1450.
Nonaka S, Kadowaki T, Nakanishi H (2022) Secreted gingipains from Porphyromonas gingivalis increase permeability in human cerebral microvascular endothelial cells through intracellular degradation of tight junction proteins. Neurochem Int 154:105282.
Olsen I (2021) Possible effects of Porphyromonas gingivalis on the blood-brain barrier in Alzheimer's disease. Expert Rev Anti Infect Ther 19:1367-1371.
Olsen I, Singhrao SK (2021) Low levels of salivary lactoferrin may affect oral dysbiosis and contribute to Alzheimer's disease: A hypothesis. Med Hypotheses 146:110393.
Poole S, Singhrao SK, Kesavalu L, Curtis MA, Crean S (2013) Determining the presence of periodontopathic virulence factors in short-term postmortem Alzheimer's disease brain tissue. J Alzheimers Dis 36:665-677.
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.
Sureda A, Daglia M, Argüelles Castilla S, Sanadgol N, Fazel Nabavi S, Khan H, Belwal T, Jeandet P, Marchese A, Pistollato F, Forbes-Hernandez T, Battino M, Berindan-Neagoe I, D'Onofrio G, Nabavi SM (2020) Oral microbiota and Alzheimer's disease: do all roads lead to Rome? Pharmacol Res 151:104582.
Zeng F, Liu Y, Huang W, Qing H, Kadowaki T, Kashiwazaki H, Ni J, Wu Z (2021) Receptor for advanced glycation end products up-regulation in cerebral endothelial cells mediates cerebrovascular-related amyloid β accumulation after Porphyromonas gingivalis infection. J Neurochem 158:724-736.

C-Editors: Zhao M, Sun Y, Yu J; T-Editor: Jia Y