Unfolded p53 in non-neuronal cells supports bacterial etiology of Alzheimer’s disease

Peter W. French

Abstract
Alzheimer’s disease has proven to be largely intractable to treatment, despite years of research, and numerous trials of therapies that target the hallmarks of the disease – amyloid plaques and neurofibrillary tangles. The etiology of Alzheimer’s disease remains elusive. There is a growing body of evidence for an infectious trigger of Alzheimer’s disease, and, in particular, the focus has been on the oral pathogen Porphyromonas gingivalis (P. gingivalis). Reports of the expression of a misfolded form of p53 in non-neuronal cells (fibroblasts, peripheral blood mononuclear cells, and B cells) and serum, which appears several years before clinical symptoms manifest, may provide further support for the role of bacteria in general, and P. gingivalis in particular, in the initiation of the disease. This review presents a model of the pathway from initial oral infection with P. gingivalis to amyloid plaque formation and neuronal degeneration, via the steps of chronic periodontitis; secretion of the inflammasomes lipopolysaccharide and gingipains into the bloodstream; induction of an inflammatory response in both peripheral cells and tissues; disruption of the blood-brain barrier, and entry into the central nervous system of the inflammasomes and the P. gingivalis bacteria themselves. In this model, the misfolded p53 (or “unfolded p53”; up53) is induced in non-neuronal cells and upregulated in serum as a result of oxidative stress due to lipopolysaccharide from P. gingivalis. up53 is therefore a potential biomarker for early diagnosis of the presence of a causative agent of Alzheimer’s disease. Fastidious dental hygiene and aggressive antibiotic treatment may prevent the patient progressing to clinical Alzheimer’s disease if serum up53 is detected at this pre-symptomatic stage.

Key Words: Alzheimer’s disease; gingipains; lipopolysaccharide; P. gingivalis; periodontitis; unfolded p53

Introduction
Alzheimer’s disease (AD) is a severe neurodegenerative disease. It is well-defined and clearly distinguishable from other dementias and neurodegenerative disorders. The initial cognitive symptoms manifest as memory problems, from which the disease progresses toward the total loss of the patient’s cognitive functions and identity (Castellani et al., 2010). As Western society ages, AD has increasingly become a significant public health issue.

Amyloid plaques that accumulate in the brain are pathological hallmarks of AD. Amyloid plaques are composed of amyloid-beta (Aβ) peptides. These peptides are generated by β- and γ-secretases, which sequentially cleave amyloid-beta precursor protein on the surface of neurons to release Aβ fragments of varying lengths. The production of these fragments is elevated in patients with mutations predisposing them to early-onset AD.

The role of amyloid in AD is controversial. A multitude of attempts over decades has been made to treat or prevent AD using drugs that target the amyloid plaques, but almost all of these drugs have proven to be either ineffective or highly toxic in humans. There is now extensive evidence of the ineffectiveness of any anti-amyloid agents to protect patients from cognitive and functional decline (Richard et al., 2021). Despite this, pharmaceutical companies continue to develop therapies based on reducing amyloid plaques. Aducamab is the most recent example. Aducamab is a monoclonal antibody that removes amyloid plaques (Sevigny et al., 2016), but its clinical benefit has been described as “uncertain” by the USFDA (USFDA, 2021). Despite this, in July 2021 the USFDA used the Accelerated Approval pathway to approve the drug. This decision has been met with concern from many quarters. Writing in the British Medical Journal in July 2021, Walsh et al. stated, “Aducamab’s approval does little to resolve the amyloid controversy, while creating unhelpful uncertainties for patients, clinicians, and researchers. Some see aducamab as proof of concept for the amyloid cascade theory, justifying decades of unsuccessful research costing billions of pounds. Others fear it will simply encourage futile investment in anti-amyloid therapies, diverting funds away from effective preventive measures and better support after diagnosis (Walsh et al., 2021).”

Diagnosis of AD is currently based on clinical symptoms, although several blood-based biomarkers have been suggested as candidates for an early, pre-symptomatic, pathological test. Biomarkers of AD that have been proposed include Aβ and pTau levels in the cerebrospinal fluid (Reiman et al., 2011; Blennow and Zetterberg, 2018; Ghidoni et al., 2018; McKhann et al., 2021) and numerous trials of therapies that target the hallmarks of the disease – amyloid plaques and neurofibrillary tangles. The etiology of Alzheimer’s disease remains elusive. There is a growing body of evidence for an infectious trigger of Alzheimer’s disease, and, in particular, the focus has been on the oral pathogen Porphyromonas gingivalis (P. gingivalis). Reports of the expression of a misfolded form of p53 in non-neuronal cells (fibroblasts, peripheral blood mononuclear cells, and B cells) and serum, which appears several years before clinical symptoms manifest, may provide further support for the role of bacteria in general, and P. gingivalis in particular, in the initiation of the disease. This review presents a model of the pathway from initial oral infection with P. gingivalis to amyloid plaque formation and neuronal degeneration, via the steps of chronic periodontitis; secretion of the inflammasomes lipopolysaccharide and gingipains into the bloodstream; induction of an inflammatory response in both peripheral cells and tissues; disruption of the blood-brain barrier, and entry into the central nervous system of the inflammasomes and the P. gingivalis bacteria themselves. In this model, the misfolded p53 (or “unfolded p53”; up53) is induced in non-neuronal cells and upregulated in serum as a result of oxidative stress due to lipopolysaccharide from P. gingivalis. up53 is therefore a potential biomarker for early diagnosis of the presence of a causative agent of Alzheimer’s disease. Fastidious dental hygiene and aggressive antibiotic treatment may prevent the patient progressing to clinical Alzheimer’s disease if serum up53 is detected at this pre-symptomatic stage.

Search Strategy and Selection Criteria
The following keywords and search terms were searched on PubMed (https://pubmed.ncbi.nlm.nih.gov) using Safari between September 8, 2021 and October 26, 2021:

• “unfolded p53 and Alzheimer’s disease”
• “unfolded p53 and oxidative stress”
• “P. gingivalis and Alzheimer’s disease”
• “P. gingivalis and LPS”
• “gingipains and oxidative stress”

How to cite this article: French PW (2022) Unfolded p53 in non-neuronal cells supports bacterial etiology of Alzheimer’s disease. Neural Regen Res 17(12):2619-2622.

Innland Limited, Sydney, NSW, Australia
*Correspondence to: Peter W. French, PhD, pwf261@iinet.net.au. https://orcid.org/0000-0002-9290-1687 (Peter W. French)
Non-Neuronal Cells and Serum from Alzheimer’s Disease Patients Contain Conformationally Altered p53

Let us start in a possibly unexpected way. In 2002, it was reported that p53 is conformationally altered in non-neuronal cells from analysis of skin fibroblasts derived from sporadic AD patients (Uberti et al., 2002). It should be noted that those findings were not observed in neurons. Uberti et al. (2002) showed a change in the tertiary structure of p53 led to the lack of p53 activation in fibroblasts. However, the conformational change was not related to mutations, as commonly is the case in tumor cells. Interestingly, the mutation-independent formation of the conformational variant of the p53 protein was specific to AD as compared to other neurodegenerative diseases. This is a somewhat surprising observation in the context of AD, as it was seen in cells (fibroblasts) outside the central nervous system. The researchers did not propose an explanation, other than to suggest that oxidative stress could be a cause of the conformational change. The cause of the oxidative stress was not identified. In a follow-up study, the conformationally altered p53, or unfolded p53 (up53), was detected in immortalized lymphocytes from sporadic AD patients and patients with AD-related mutations. Confirmation that this conformational variant is expressed in peripheral blood cells was reported by Lanni et al. (2008), who stated that peripheral blood mononuclear cells from AD patients express a significantly higher amount of up53 compared to non-AD subjects, using a conformation-specific p53 antibody, which discriminates up53 from wild type p53 based on its tertiary structure. This finding was confirmed in peripheral blood mononuclear cells and extended to the serum by Arce-Varas et al. (2017). Interestingly, up53 has still not been observed in neuronal cells from AD patients. This indicates that AD may be caused by an agent that induces a systemic response (oxidative stress) in peripheral cells but acts differently in the CNS. In other words, up53 could be a marker of the presence of a causative agent of AD in the patient’s body.

Conformationally Altered p53 Is Produced in Response to Oxidative Stress

Oxidative stress is characterized by an accumulation of reactive oxygen species (ROS) and plays a key role in the progression of inflammatory diseases. Buizza et al. (2012) found that the expression of up53 was associated with markers of oxidative stress, leading the researchers to suggest that oxidative stress is responsible for the induced immunoinflammatory response in immortalized B cells from AD patients. They measured the expression of oxidative markers 4-hydroxy-2-nonenal (a product of lipid peroxidation) and 3-nitrotyrosine (a product of protein nitration) and the activity and levels of antioxidant enzymes. These biomarkers were significantly increased in the familial AD cells compared with the healthy control lymphocytes and the lymphocytes derived from spontaneous early-onset AD patients. However, further analysis using immunoprecipitation of the ant-up53 and anti wild-type p53 antibodies indicated that the number of nitrated tyrosine residues on the up53 molecule was greater in both the familial and early-onset AD-derived cells compared to the control cells. Furthermore, lymphocytes derived from healthy subjects expressed an intense band related to wild-type p53, while immunoreactivity to the up53 antibody was very low and extended to the serum by AD patients on the other hand demonstrated a higher immunoreactivity to up53, which correlated with a compromised p53 functionality.

Bacterial-Derived Lipopolysaccharide Can Induce Oxidative Stress

One inducer of oxidative stress in cells is bacterial-derived lipopolysaccharide (LPS). LPS is the main component of the membrane of Gram-negative bacteria. Lipopolysaccharide (10 kDa) monomer consists of a distal polysaccharide (or O-antigen), a non-repeating “core” oligosaccharide, and a hydrophobic domain known as lipid A (or endotoxin). Lipid A, the inner-most component, is the biologically active region of LPS. It can bind with pattern recognition receptors (PRRs) and trigger heterogeneous cell responses, which change according to microenvironmental conditions and affect the host immune signaling, thereby facilitating bacterial survival in the host (Al-Qutub et al., 2006). LPS has been shown to induce elevated ROS levels in endothelial cells (Sampath et al., 2009) and somatomedinogenesis (Rexer et al., 2006). This is pertinent, because LPS can be found in large amounts in the brain of AD patients compared to healthy controls. LPS co-localizes with amyloid plaques and is located around blood vessels in the brains of AD patients (Zhan et al., 2016). Furthermore, peripheral injection of LPS in mice can activate microglia, inducing the release of pro-inflammatory cytokines, such as interleukins and tumor necrosis factor-alpha (Godbout et al., 2005). In line with these observations, Lanni et al. (2003) demonstrated that injection of LPS presented an increased neuroinflammation associated with the enhanced expression and processing of APP and Ab<sub>40</sub> levels inside neurons. Additionally, Lee et al. (2010) showed that in mice expressing a mutated tau protein, LPS infusion increased microglial activation and neurofibrillary tangle formation.

Source of Bacterial Lipopolysaccharide in Alzheimer’s Disease

LPS is a major surface component of Gram-negative bacteria. This implicates an infectious (bacterial) etiology for AD. Moreover, it has been widely reported that periodontitis and gingivitis are linked to a higher risk of AD (Singhrao et al., 2015). Periodontitis and gingivitis are chronic inflammatory diseases that affect not only the tissues of the oral cavity but also the entire body. They have been associated with type 2 diabetes, cardiovascular disease, and rheumatoid arthritis (Carter et al., 2017), which are additional risk factors for the development of AD.

There is increasing evidence for the role of P. gingivalis as the “master bacteria” inducing gingivitis and periodontitis. They are obligate anaerobic bacteria that are known to live in the community of microorganisms in the oral cavity (Hajishengallis and Lamont, 2014). P. gingivalis is a Gram-negative anaerobe that produces many virulence factors, including LPS and gingipains, that can destroy portalional tissues either directly, or indirectly via the host’s inflammatory response (How et al., 2016). Of relevance to this discussion, LPS derived from P. gingivalis can induce oxidative stress in cells (fibroblasts) (Golz et al., 2014). It is pertinent to note that the effects of LPS from P. gingivalis on the cells of the gingiva were reported by Liu et al. (2018) to be mediated by p53. These researchers showed that LPS-induced up53 activity and localization in mitochondria led to cellular redox imbalance and mitochondrial dysfunction, thus triggering the cellular inflammatory response as indicated by increased secretion of interleukin-1β, interleukin-6, and tumor necrosis factor-alpha. Furthermore, the cellular redox imbalance and inflammation induced by LPS could be reversed by inhibiting p53 activity. p53 expression followed by LPS-induced inflammation could also be restricted by restricting ROS generation. This provides evidence that LPS from P. gingivalis could lead to the unfolding of p53 via the production of ROS in cells not only locally, but also throughout the body. P. gingivalis-secreted virulence factors can enter the bloodstream (Hira et al., 2020).

Supporting the proposition that LPS from bacteria, including P. gingivalis, is involved in the progression of AD, Zhang et al. (2018) demonstrated that chronic systemic exposure to LPS from bacteria can lead to AD-like symptoms in rodents. When administered intraperitoneally, LPS from P. gingivalis caused a significant increase in apoptosis in peripheral lymphocytes as well as the gut microflora of infected mice. In another study, young (2 months old) and middle-aged (12 months old) mice were systemically exposed to LPS from P. gingivalis daily for five weeks. Chronic systemic exposure to LPS induced learning and memory deficits and other epidemiological phenotypes observed in AD patients. Furthermore, P. gingivalis-secreted virulence factors can enter the bloodstream (Hira et al., 2020).

Gingipains and Alzheimer’s Disease

In addition to LPS, P. gingivalis also produces gingipains. Gingipains are trypsin-like cysteine proteases that are broadly classified into two main categories – the arginine gingipains A and B, and the lysine gingipain, which can exist in both soluble and membrane-bound forms (Guo et al., 2010). Gingipains express cathespin B proteolytic enzymatic activity that enables cleavage of the amyloid-B precursor protein resulting in the formation of amyloid-beta plaques. Tau is also an established substrate for gingipains, which can cleave tau into subunits 3R and 4R. These proteolytic activities could lead to the accumulation of misfolded tau protein, and intracellular Aβ accumulation in the middle-aged mice in a cathespin B-dependent manner (Wu et al., 2017).

Gingipains have also been shown to contribute to Alzheimer’s disease by activating the innate immune system and contributing to neuroinflammation (Dominy et al., 2019). The western blots from all three AD brains revealed protein, LPS infusion increased microglial activation and neurofibrillary tangle formation.

Evidence for the Presence of Bacteria in Alzheimer’s Disease Brains

To induce neurological disease such as AD, Panza et al. (2019) proposed that pathogens could perhaps directly cross a weakened blood-brain barrier (BBB), reach the CNS, and cause neurological damage by eliciting neuroinflammation. Alternatively, they suggested that the pathogens may cross a weakened intestinal barrier, reach the vascular circulation and then cross the BBB (Panza et al., 2019). BBB dysfunction is a hallmark of AD. van de Haar et al. (2016) demonstrated global BBB leakage in patients with early AD and that leakage is associated with cognitive decline. They hypothesized that the compromised BBB may be a part of a cascading sequence of events that eventually lead to cognitive decline and dementia. There are several mechanisms by which P. gingivalis can disrupt the BBB (Olson, 2021).

In support of this direct effect of bacteria on causing AD, there is evidence for the presence of both C. pneumoniae and P. gingivalis in the brains of AD patients, as well as other bacteria and fungi (Pisa et al., 2017). The presence of P. gingivalis was determined by Western blotting of AD brain lysates that were immunoprecipitated with gingipain-specific antibodies (Dominy et al., 2019). The Western blots from all three AD brains revealed
It is known that deposits of Aβ in the brain can be detected 10–20 years before the cognitive decline and a diagnosis of AD. At least 10 years is required for periodontitis to become systemic. Whilst association is not causation, there is now sufficient evidence from a wide variety of experimental and observational approaches to support the view that P. gingivalis plays a key initiating role in AD. The detection of up53 in non-neuronal cells and tissues may be the first indication that a chronic infection that can initiate AD is present. Thus, a diagnostic based on this, coupled with stringent dental hygiene and an effective, targeted antibacterial strategy, may result in a significant decrease in the incidence of disease.

Once chronic gingivitis has taken hold, treatment options are much more limited. This is due to the characteristic biofilms that the bacterium can form. Biofilms can be up to 500 times less sensitive to some antibiotics, although azithromycin can still be effective (Mazeo et al., 2011). Over 74% of patients with periodontitis harbor pathogens resistant to at least one standard antibiotic (Rams et al., 2014). Periodontitis isolates of P. gingivalis have been demonstrated to be resistant to penicillin, amoxicillin, erythromycin, azithromycin, clindamycin, and tetracycline (Gerits et al., 2017).

This emphasizes the need to promote meticulous dental hygiene as an effective prophylactic approach for AD, rather than relying on antibiotic treatment. However, following a diagnosis of early-onset Alzheimer’s dementia, an aggressive antibiotic therapy could be considered (Panza et al., 2019). This could be tested in a clinical study.

These approaches could prevent the manifestation of clinical symptoms, and has the potential to significantly reduce the burden of this highly morbid disease. Further work is needed to explore the utility of a screening test based on the detection of unfolded p53 in the circulation.

Acknowledgments: I thank my colleagues Carl Stubbings, Dr. Emily Stein, and Dr. Emile Mistry for commenting on general aspects of this topic over the years.

Author contributions: PWF designed and wrote the manuscript, collected the data, and approved the final version of the manuscript.

Conflicts of interest: The author declares no conflicts of interest. No conflicts of interest exist between Innlimaz Limited and Publication of this paper.

Open access statement: This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

References

Al-Qutub MN, Brahman PH, Karimi-Naser LM, Liu X, Genco CA, Darveau RP (2006)
Hemin-dependent modulation of the lipid A structure of Porphyromonas gingivalis lipopolysaccharide. Infect Immun 74:4474-4485.

Arce-Varas N, Abate G, Prandelli C, Martínez C, Cueto F, Menéndez M, Marziano M, Cabrera-García D, Fernández-Sánchez MT, Novelli A, Memo M, Uberti D (2017)
Comparison of extracellular and intracellular blood compartments highlights Redox alterations in Alzheimer’s and mild cognitive impairment patients. Curr Alzheimer Res 14:112-122.

Blennow K, Zetterberg H (2018) Biomarkers for Alzheimer’s disease: current status and prospects for the future. J Intern Med 284:643-663.

Buizza L, Cenini G, Lanni C, Ferrari-Toninelli G, Prandelli C, Govoni S, Buoso E, Racchi M, Barcikowska M, Styczynska M, Szybinska A, Butterfield DA, Memo M, Uberti D (2012) Conformational altered p53 as an early marker of oxidative stress in Alzheimer’s disease. PLoS One 7:e29789.

Carter CJ, France J, Crean S, Singhrao SK (2017) The Porphyromonas gingivalis/host interaction shows enrichment in GWASdb genes related to Alzheimer’s disease, diabetes and cardiovascular diseases. Front Aging Neurosci 9:408.

Castellani RJ, Rolston RK, SMA MA (2010) Alzheimer disease. Dis Mon 56:484-546.

Deshpande RG, Khan MB, Genco CA (1998) Invasion of aortic and heart endothelial cells by Porphyromonas gingivalis. Infect Immun 66:5337-5343.

Dominy SS, Lynch C, Ermin F, Benedek M, Marczuk A, Konradi A, Nguyen M, Hadtsch 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. J Neuroinflammation 16:230.

Fulop T, Witkowski JM, Bourgade K, Khalil A, Zerif E, Larbi A, Hirokawa K, Pawelec G, Bocti C, Lacombe G, Dupuis G, Frost EH (2018) Can an infection hypothesis explain the beta amyloid hypothesis of Alzheimer’s disease? Front Aging Neurosci 10:224.

Gerits E, Verstraeten N, Michiels J (2017) New approaches to combat Porphyromonas gingivalis biofilms. J Oral Microbiol 9:1300366.
