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Current Perspective

Connection Between Periodontitis and Alzheimer's Disease: Possible Roles of Microglia and Leptomeningeal Cells

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Abstract. Neuroinflammation, inflammation of the brain, is strongly implicated in Alzheimer's disease (AD), which can be enhanced by systemic inflammation. Therefore, the initiation and progression of AD are affected by systemic diseases such as cardiovascular disease and diabetes. This concept suggests a possible link between periodontitis and AD because periodontitis is a peripheral, chronic infection that elicits a significant systemic inflammatory response. There is now growing clinical evidence that chronic periodontitis is closely linked to the initiation and progression of AD. Recent studies have suggested that leptomeningeal cells play an important role in transducing systemic inflammatory signals to the brain-resident microglia, which in turn initiate neuroinflammation. Furthermore, it is apparent that senescent-type microglia respond in an exaggerated manner to systemic inflammation. It is estimated that a high percentage of adults are suffering from periodontitis, and the prevalence of periodontitis increases with age. Therefore, chronic periodontitis can be a significant source of covert systemic inflammation within the general population. The present review article highlights our current understanding of the link between periodontitis and AD.

Keywords: Alzheimer's disease, leptomenongeal cell, microglia, periodontitis, systemic inflammation

1. Introduction

Dementia is an acquired and persistent compromise in multiple cognitive domains that is severe enough to interfere with everyday functioning. With increased longevity, dementia has emerged as a major health concern in elderly people worldwide. Alzheimer's disease (AD) is the most common cause of primary degenerative dementia among elderly people. As the population ages and the lifespan increases, the prevalence of AD will likely continue to increase. Although the molecular mechanisms involved in the etiology and pathogenesis of AD have not been completely characterized, the accumulation of β-amyloid (Aβ) and the hyperphosphorylation of tau in the brain are pathological hallmarks of AD. Furthermore, neuroinflammation is considered to be a factor involved in accelerating the onset and progression of AD. Because effective approaches that can delay the onset, slow the progression, or reverse the disease process have not yet been established, the identification of treatable factors involved in neuroinflammation is important to find ways to prevent AD.

Neuroinflammation has generally been recognized as a prominent feature of AD and probably plays a substantial role in the cognitive dysfunction of AD (1). Interleukin-1β (IL-1β) is the key molecule involved in the neuroinflammation in AD, because IL-1β drives the release of multiple inflammatory mediators by activated microglia, leading to self-propagating neuroinflammation. Activated microglia promote the progression of AD by secreting proinflammatory molecules (2). Clinical studies have given evidence of the extent of microglial activation in early AD and mild cognitive impairment (MCI) (3). IL-1β expression has been detected in the plaque-surrounding microglia in the AD brain (4), suggesting that the IL-1β released from the plaque-surrounding microglia may contribute to neuronal dysfunction by promoting the formation of dystrophic

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neurites and via direct neurotoxicity.

It is well recognized that systemic infection/inflammation can alter the neuroinflammation in the brain (5). Periodontitis is a common chronic multi-bacterial infection affecting the supporting structures of the teeth, which results in a significant bacterial and inflammatory load in the body. As a form of chronic systemic inflammation, periodontitis not only causes other systemic inflammatory diseases, such as atherosclerosis and diabetes, but also initiates or hastens the rate of progression of AD directly (6). An increasing number of clinical studies have demonstrated the impact of periodontitis on AD, and recent experimental studies clarified the route of transduction of inflammatory signals from periodontitis to the brain. We herein review the current understanding of the link between periodontitis and AD.

2. Clinical evidence for periodontitis as a potential risk factor for AD

Kamer et al. have proposed that periodontal disease induces systemic inflammation, which stimulates the production of Aβ and tau protein in the brain leading to Alzheimer's neuropathology (7). In addition to the low-grade chronic inflammation itself, periodontitis causes or promotes other chronic systemic inflammatory diseases, including atherosclerosis, cardiovascular disease, and diabetes, indicating that periodontitis is a significant source of systemic inflammatory molecules (6). On the basis of the contribution of periodontitis to systemic inflammation and the potential role of systemic inflammation in neuroinflammation, it is reasonable to consider that chronic periodontitis is a risk factor for the incidence and progression of AD.

There is growing clinical evidence that chronic periodontitis is closely linked to the initiation and progression of AD. Noble et al. identified a cross-sectional association between a serologic marker of a common periodontitis pathogen, *Porphyromonas gingivalis* (*P. gingivalis*), and poor cognitive test performance among patients older than 60 years in the Third National Health and Nutrition Examination Survey (NHANES-III) (8). This preliminary study suggested that periodontitis is associated with cognitive impairment, especially in the elderly. Furthermore, Stein et al. examined the serum antibody levels to periodontal bacteria in participants who were eventually diagnosed with AD in comparison with those in non-AD controls (9). They found that there were elevated levels of antibodies to periodontal bacteria in subjects years before cognitive impairment, suggesting that periodontitis could potentially contribute to the risk of AD onset and/or progression.

More recently, Poole et al. have assessed the presence of the major three periodontal bacteria, the so called "Red complex", including *Treponema denticola*, *Tannerella forsythia*, and *P. gingivalis*, and/or bacterial components in the brain tissue of individuals with and without dementia (10). They obtained statistically significant evidence of the presence of lipopolysaccharide (LPS) from *P. gingivalis* in AD cases, with a 12-h maximum postmortem delay using the immunolabeling and the immunoblotting of brain tissues from individuals with and without dementia. No evidence of *P. gingivalis* LPS was detected in the non-AD control tissue, even with a longer postmortem delay of up to 43 h. This study confirms that LPS from periodontal bacteria can access the AD brain during life. Furthermore, the fact that a known chronic oral pathogen–related virulence factor reaches the human brains suggests that inflammation has a role in the existing AD pathology. Riviere et al. found the presence of oral *Treponema* in the trigeminal ganglia, the brain stem, the hippocampus and the cortex of AD patients using molecular and immunological techniques (11). Taken together, the clinical evidence suggests that periodontitis could potentially contribute to the risk of AD onset/progression; however, the exact route by which periodontitis transduces the systerm inflammatory signal into the brain remains unclear.

3. Possible routes of communication between the systemic immune system and the brain

Several routes by which systemic immune signals can be transmitted to the brain have been intensively studied (5, 12). First, the direct pathway involves the circumventricular organs, which are specialized regions lacking a contiguous blood–brain barrier (BBB). In the circumventricular organs, pathogen-associated molecular patterns induce the production and release of proinflammatory cytokines by macrophage-like cells expressing Toll-like receptors (TLRs). These cytokines can enter the brain by volume diffusion. A second route involves the IL-1 receptors located on the perivascular macrophages and endothelial cells of brain capillaries. The activation of the IL-1 receptors by circulating cytokines initiates the release of cytokines into the brain, without the physical entry of the BBB constituents. A third route comprises cytokine transporters overflowing into the systemic circulation, which can then gain access to the brain through these saturable transport systems. A fourth route involves the transmission of systemic immune signals via the autonomic nervous system. Systemic cytokines directly stimulate primary afferent nerves, such as the vagal nerves, which, in turn, activate central pathways involved in sickness behavior.

In addition to these four "classical" routes, we have

further found that the leptomeningeal pathway may be involved. The leptomeninges covering the surface of the brain parenchyma play a role by providing a physical boundary at the cerebrospinal fluid–blood barrier. The activation of leptomeningeal cells by circulating cytokines induces the production and release of proinflammatory cytokines into the brain (13, 14). Therefore, leptomeningeal cells can transmit signals from systemic immune cells into the brain-resident microglia.

4. Chronic systemic inflammation and neuroinflammation

It is well known that chronic systemic inflammation has causal links to neuroinflammation via systemically released proinflammatory mediators including IL-1β, and microglia are the primary brain cells that respond to systemic inflammation (5, 12). Repeated LPS-induced chronic systemic inflammation in mice resulted in prolonged IL-1β production, as well as microglial activation in the brain (15). The mice that received a systemic inflammatory challenge in late gestation were predisposed to develop AD-like neuropathology during the course of aging. These mice displayed chronic elevation of IL-1β, increased expression of hippocampal amyloid precursor protein (APP) and its proteolytic fragments, and increased tau phosphorylation, resulting in significant impairments of working memory in old age. More importantly, the phenotype was strongly exacerbated when this prenatal infection was followed by a second systemic inflammatory challenge in adulthood, further suggesting that systemic inflammation represents a major risk factor for the development of AD (16).

More recent research showed that obesity and high fat feeding led to an increase in circulating pro-inflammatory mediators, which initiated microglia-related neuroinflammation, resulting in a disruption of the cognitive function (17). This indicates that obesity-induced systemic inflammation leads to neuroinflammation and negative outcomes on cognition. Moreover, systemic challenge with LPS elevated the levels of TLR2 mRNA in the frontal cortex and cerebellum (18).

5. Chronic systemic inflammation and AD pathologies, including Aβ accumulation and tau hyperphosphorylation

There is increasing evidence that neuroinflammation induces the production of Aβ. Sheng et al. reported that chronic systemic treatment with LPS increases APP expression and processing, with the accumulation of Aβ in the lateral parietal cortex of APPswe transgenic mice (19). Although the mechanism underlying LPS-induced amyloidogenesis remains unclear, it is possible that the changes in secretase activities are involved. Lee et al. found that a single systemic injection of LPS incresed the activity of β-site APP–cleaving enzyme 1 (BACE1), a rate-limiting enzyme that initiates Aβ formaion, and also led to changes in the concentration of brain Aβ_{1-42} in adult, but not young, mice (20). Furthermore, chronic treatment with LPS induced the intracellular acumulation of Aβ_{1-42} in hippocampal pyramidal neurons in adult mice. It is likely that proinflammatory cytokines, including IL-1β and tumor necrosis factor-α (TNF-α), play significant roles in LPS-induced Aβ formation because these proinflammatory cytokines induce Aβ formation (21), and the process may be related to the increased expression and activity of BACE1 though the loss of peroxisome proliferator-activated receptor-γ (PPAR-γ)-mediated repression of BACE1 transcription in neuronal cells (22). Therefore, LPS-induced neuroinflammation could influence APP processing through the enhancement of BACE1 activity, thereby inducing increased accumulation of Aβ.

In addition to Aβ, we have recently found that chromogranin A (CGA), a neurosecretory acidic glycoprotein, which is also present in the senile plaques of AD patients, can activate microglia to induce the IL-1β production (23 – 25). Our preliminary experiments showed that chronic systemic treatment with *P. gingivalis* LPS induced the intracellular accumulation of Aβ_{1-42} and CGA in the hippocamal pyramidal neurons and led to memory deficits in middle-aged mice (Wu et al., unpublished observations).

It is noteworthy that the IL-1β secreted from activated microglia further accelerates tangle formation in cortical neurons through tau hyerphosphorylation, indicating that activated microglia also play important roles in the tau pathology in AD (26).

6. Age-dependent differences in the microglial responses through leptomeninges during chronic systemic inflammation

It is interesting to note that chronic systemic inflammation induces age-dependent differential responses in microglia. Using a model of stable chronic inflammation, rat adjuvant arthritis (AA), we found that activated microglia in the proximity of the leptomeninges produce anti-inflammatory cytokines, such as IL-10 and transforming growth factor-β1 (TGF-β1), in young adult AA rats (13, 14, 27). In contrast, activated microglia in close proximity to the leptomeninges in middle-aged AA rats produced IL-1β and exhibited increased expression of prostaglandin E_2 (PGE_2) synthesizing enzymes, cyclooxygenase-2 and microsomal prostaglandin synthase-1

(27). In cultured leptomeningeal cells, IL-1β and PGE_2, respectively, caused a marked loss of occludin and ZO-1, two major tight junction proteins. Pretreatment with IL-10 and TGF-β1 significantly antagonized their effects. Therefore, chronic systemic inflammation induces age-dependent differential phenotypic changes in microglia, yielding an anti-inflammatory cell phenotype in young rats and a proinflammatory cell phenotype in middle-aged rats. Furthermore, age strongly influences the barrier functions of the leptomeninges through the age-dependent differential microglial responses during chronic systemic inflammation (27).

These observations prompted further investigation of the functional outcomes of the resultant differential microglial phenotypic changes during chronic systemic inflammation. We therefore examined the effects of chronic systemic inflammation on the formation of long-term potentiation (LTP) in the hippocampus in young adult and middle-aged rats because LTP is the cellular substrate for learning and memory (28). The formation of LTP in the Schaffer collateral-CA1 synapses was not affected in young adult AA rats. In contrast, the formation of hippocampal LTP was significantly impaired in middle-aged AA rats. Systemic administration of minocycline, a known inhibitor of microglial activation, significantly restored the magnitude of LTP in middle-aged AA rats. These observations suggest that chronic systemic inflammation induces deficits in the hippocampal LTP in middle-aged rats through neuroinflammation, which is mainly induced by brain-resident microglia.

Therefore, it is considered that microglia might be primed during aging even by middle-age. Primed microglia are hyperresponsive to secondary stimuli and thus can produce an exaggerated inflammatory response in the brain. It is considered that age-dependent autophagic and lysosomal dysfunctions allow the dominance of reactive oxygen species (ROS)-hypergenerating older mitochondria in microglia. Increased intracellular ROS, in turn, activate the redox-sensitive transcription factors, such as nuclear factor–κB (NF-κB), to provoke an exaggerated inflammatory response (29). Therefore, increased oxidative stress and the resultant activation of redox-sensitive transcription factors during aging may drive the emergence of senescent-type microglia. This may explain why Aβ, which cannot sufficiently activate NF-κB, is able to induce IL-1β secretion by activated microglia isolated from the aged mouse brain, but not from the young adult mouse brain (25). These observations may partly explain the reason why senescence is an important causative factor for AD.

7. Leptomeninges as a possible communication route between periodontal bacteria and the brain-resident microglia

As mention above, the leptomeninges can transmit systemic inflammatory signals to the brain (13, 14, 27). Furthermore, leptomeningeal cells express both TLR2 and TLR4, the receptors of *P. gingivalis* LPS. These facts prompted us to further examine the possibility that *P. gingivalis* LPS may activate leptomeningeal cells to elicit the release of inflammatory molecules directly into the brain, resulting in the activation of the brain-resident microglia. Using an in vitro system, we found that leptomeningeal cells can transmit the inflammatory signals from peripheral macrophages to brain-resident microglia in response to *P. gingivalis* LPS stimulation (30). The mean expression levels of IL-1β and TNF-α secreted by leptomeningeal cells after treatment with the conditioned medium from *P. gingivalis* LPS–stimulated macrophages were significantly higher than those after treatment with *P. gingivalis* LPS alone. Furthermore, the mean expression levels of IL-1β and TNF-α secreted by activated microglia after treatment with the conditioned medium from *P. gingivalis* LPS–treated leptomeningeal cells were significantly higher than those after treatment with *P. gingivalis* LPS alone. These observations suggest that the leptomeningeal cells transmit systemic inflammatory signals from macrophages to brain-resident microglia by secreting inflammatory mediators during periodontitis. However, in vivo evidence is needed to confirm these findings.

8. Conclusions

During chronic periodontitis, proinflammatory macrophages and periodontal bacterial components, including LPS and flagellin, activate the receptors localized on the surface of leptomeninges, which in turn activate brain-resident microglia to evoke neuroinflammation. The intense neuroinflammation evoked by senescent-type microglia may contribute to the initiation and progression of AD, resulting in the cognitive deficits (Fig. 1). Therefore, early treatment of periodontitis may limit the severity and progression of AD. During normal aging, microglia undergo several morphological and functional changes, which involve the induction of exaggerated neuroinflammation in response to systemic inflammation. Therefore, pharmacological approaches aimed to rejuvenate senescent-type microglia may also constitute a promising avenue for future research to reduce the risk of AD. The notion that periodontitis is a risk factor for AD is still hypothetical at present. Additional cohort studies profiling the oral clinical pre-

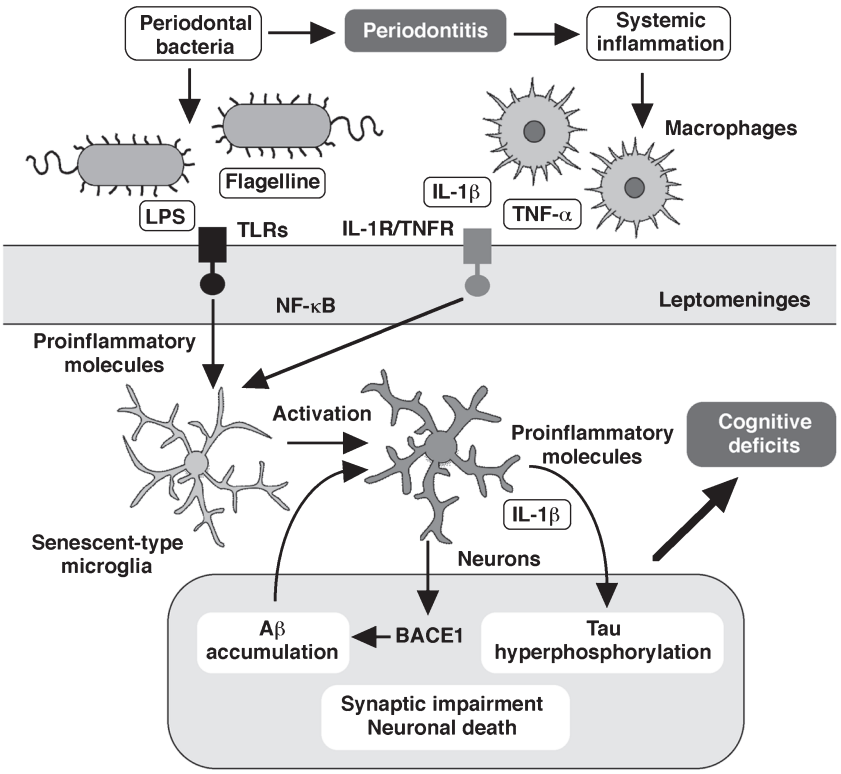

Fig. 1. A schematic illustration of the transduction of periodontal bacterial inflammatory signals to brain-resident microglia through the leptomeninges. During chronic periodontitis, IL-1β and TNF-α secreted by macrophages and periodontal bacterial components, including LPS and flagellin, activate IL-1R/TNFR and TLRs, which are localized on the surface of leptomeninges. These factors in turn activate senescent-type microglia in the brain. After cellular activation, senescent-type microglia secrete proinflammatory molecules, such as IL-1β, to increase the expression and activity of BACE1, resulting in the increased accumulation of Aβ. Furthermore, IL-1β secreted from activated senescent-type microglia also accelerates the tangle formation through tau hyperphosphorylation. These pathological features of AD may impair neuronal functions and promote cognitive deficits.

sentation of patients with different cognitive functions and during the progression of AD are warranted to clarify this hypothesis.

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