Leptomeningeal Cells Induce Synaptic Failure via Cathepsin B-mediated IL-1 β Production after Porphyromonas Gingivalis Infection

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Keywords: Porphyromonas gingivalis, Leptomeningeal cells, Alzheimer's disease, Cathepsin B, IL-1 β , BDNF

Posted Date: November 13th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-104469/v1>

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Abstract

Background: Synaptic failure is the earliest sign before Alzheimer's disease (AD) onset and closely associated with cognitive decline. Clinical studies have shown that periodontitis is positively correlated with both the onset and pathological progression of AD, and preclinical studies have shown that *Porphyromonas gingivalis* (*P. gingivalis*), the key pathogen in periodontitis, and its virulence factors induced memory decline in mice. However, the mechanisms underlying the involvement of *P. gingivalis* in memory decline remain unclear.

Methods: We used primary leptomeningeal cells and primary cortical neurons to evaluate the effects of leptomeningeal cells on synaptic generation and plasticity after *P. gingivalis* infection in vitro. The expression of the related molecules was examined by mRNA in real time and by protein levels using Western blotting. Pharmacological and genetic approaches were used to explore the mechanism underlying the involvement of leptomeningeal cells in synaptic changes after *P. gingivalis* infection.

Results: NLRP3 inflammasome activation was involved in augmenting the IL-1 β secretion by primary leptomeningeal cells after *P. gingivalis* infection, as determined by the knockdown of NLRP3 with siRNA. Cathepsin B (CatB) mediated the activation of both NLRP3 inflammasomes and NF- κ B for the production of IL-1 β by *P. gingivalis*-infected primary leptomeningeal cells, as determined by the pharmacologically specific inhibition of CatB. In contrast, *P. gingivalis*-infected leptomeningeal cells induced an IL-1 β -dependent decrease in pre- and post-synaptic molecules in primary cortical neurons, as determined by the pharmacological blockage of the IL-1 receptor. *P. gingivalis*-infected leptomeningeal cells also induced the IL-1 β -dependent suppression of BDNF signaling in cultured N2a neurons, a stable mouse neural cell line. Furthermore, propolis, which is produced by honeybees, suppressed the expression of IL-1 β but increased that of BDNF in *P. gingivalis*-infected leptomeningeal cells.

Conclusion: The CatB-mediated IL-1 β production was augmented in leptomeningeal cells, resulting in synaptic failure and blockage of BDNF signaling in neurons during *P. gingivalis* infection. These findings highlight a new mechanism underlying the involvement of periodontitis in AD initiation and suggest that CatB may be an early intervention therapeutic target for delaying the onset of AD during *P. gingivalis* infection.

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Figures

Figure 1

NLRP3 inflammasome involved in IL-1 β secretion from primary leptomeningeal cells after *P. gingivalis* infection A, The relative cell viability in *P. gingivalis* infected leptomeningeal cells with different MOIs and time points. Each column and bar represent the mean $\pm$ SEM (n=3, each). B, The secretion of IL-1 β from leptomeningeal cells after infection with *P. gingivalis* for 1, 3, 6, and 12 hours. Each column and bar represent the mean $\pm$ SEM (n=3, each). Asterisks indicate a statistically significant difference from the control group (***P<0.001, **P<0.01, t-test). C, D, The relative mRNA expression of NLRP3(C) and Caspase-1(D) in leptomeningeal cells after *P. gingivalis* infection with MOI=10 for 1, 3, 6, and 12 hours. Each column and bar represent the mean $\pm$ SEM (n=3, each). Asterisks indicate a statistically significant difference from the control group (***P<0.001, t-test). E, Relative mRNA expression of NLRP3 in leptomeningeal cells with *P. gingivalis* infection for 3 hours in the presence or absence of NLRP3 siRNA. Each column and bar represent the mean $\pm$ SEM (n=3, each). Asterisks indicate a statistically significant difference between two groups (***P<0.001, **P<0.01, t-test). F, The immunoblots show the pro- and mature-type of caspase-1 in leptomeningeal cells after infection with *P. gingivalis* for 3 hours in the

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Figure 2

CatB involved in NLRP3 inflammasome activation in primary leptomeningeal cells after *P. gingivalis* infection A, CLMS images of *P. gingivalis* infected leptomeningeal cells for 1 hour. *P. gingivalis* was labeled with FITC (Green), endosome/lysosome was labeled with LAMP2 (Red). Scale bar = 20µm. B, CLMS images of acridine orange in control and *P. gingivalis* infected leptomeningeal cells for 2 hours. Scale bar = 20µm. C, The immunoblots show the CatB in the cytosol of leptomeningeal cells after infection with *P. gingivalis* for 2 hours. D, The quantitative analyses of the immunoblot in (C). Each column and bar represent the mean $\pm$ SEM (n=3, each). Asterisks indicate a statistically significant difference from the control group (**P<0.01, t-test). E, The immunoblots show the pro- and mature-type of caspase-1 in leptomeningeal cells after infection with *P. gingivalis* for 3 hours in the presence or absence of 10 µM CA-074 Me. F, G, The quantitative analyses of the immunoblots in (E). Each column and bar represent the mean $\pm$ SEM (n=3, each). Asterisks indicate a statistically significant difference from the control group (***P<0.001, **P<0.01, t-test). Daggers indicate a statistically significant difference from the *P. gingivalis* infection group. (†††P<0.001, ††P<0.01, t-test). H, The IL-1β released from leptomeningeal cells after infection with *P. gingivalis* for 3 hours in the presence or absence of CA-074Me. Each column and bar represent the mean $\pm$ SEM (n=3, each). Asterisks indicate a statistically significant difference from the control group (***P<0.001, t-test). Daggers indicate a significant difference from the *P. gingivalis* infection group. (†††P<0.001, t-test). I, The immunoblots show the pro- and mature-type of IL-1β in leptomeningeal cells after infection with *P. gingivalis* for 3 hours with or without 1 µM CytoD. J, K, The quantitative analyses of the immunoblot of pro- (J) and mature-type (K) of IL-1β in (I). Each column and bar represent the mean $\pm$ SEM (n=3, each). Asterisks indicate a statistically significant difference from the control group (***P<0.001, t-test). Daggers indicate a statistically significant difference from the *P. gingivalis* infection group. (††P<0.01, t-test). L, The secretion of IL-1β from leptomeningeal cells after infection with *P. gingivalis* for 3 hours with or without Cyto D. Each column and bar represent the mean $\pm$ SEM (n=3, each). Asterisks indicate a statistically significant difference from the control group (***P<0.001, t-test). Daggers indicate a statistically significant difference from the *P. gingivalis* infection group. (†††P<0.001, t-test).

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CatB involved in NF-κB activation for IL-1β production in leptomeningeal cells after *P. gingivalis* infection

A, B, The relative mRNA expression of TLR2(A) and CatB(B) in leptomeningeal cells after *P. gingivalis* infection with MOI=10 for 1, 3, 6, and 12 hours. Each column and bar represent the mean ± SEM (n=3, each). Asterisks indicate a statistically significant difference from the control group (***P<0.001, **P<0.01, t-test). C, The immunoblots show the CatB in leptomeningeal cells after infection with *P. gingivalis* for 0.5, 1, 2, and 3 hours. D, The quantitative analyses of CatB in the immunoblot shown in (C). Each column and bar represent the mean ± SEM (n=3, each). Asterisks indicate a statistically significant difference from the control group (***P<0.001, **P<0.01, *P<0.05, t-test). E, The immunoblots show the IκBα in leptomeningeal cells after infection with *P. gingivalis* for 1 hour with or without pretreatment with 10 μM CA-074 Me. F, The quantitative analyses of total IκBα in the immunoblot shown in (E). Each column and bar represent the mean ± SEM (n=3, each). Asterisks indicate a statistically significant difference from the control group (**P<0.01, t-test). A dagger indicates a significant difference from the *P. gingivalis* infection group. (†P<0.05, t-test). G, H, I, Relative mRNA expression of NLRP3 (G), Caspase-1

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Figure 4

P. gingivalis infected leptomeningeal cells induced an IL-1 β -dependent synaptic distribution in neurons Condition medium from leptomeningeal cells infected by *P. gingivalis* for 3 hours with MOI=10(Pg LCM) in the presence or absence of NLRP3 siRNA (Pg+NLRP3kd LCM) or CA-074 Me (Pg+CA LCM) was

collected and 30% of the neuron condition medium was replaced to culture primary neurons for 24 hours. A, The relative cell viability in control and Pg LCM neurons. Each column and bar represent the mean $\pm$ SEM (n=3, each). B, CLMS images of primary neurons (MAP2, red) with or without Pg LCM treatment. C, The neurite length analyses of the neurons in(B). Each column and bar represent the mean $\pm$ SEM (n=10, each). Asterisks indicate a statistically significant difference from the control group (**P<0.01, t-test). D, The relative mRNA expression of SYP, VGLUT1, SYN1, and PSD95 in control and Pg LCM neurons. Each column and bar represent the mean $\pm$ SEM (n=3, each). Asterisks indicate a statistically significant difference from the control group (***P<0.001, t-test). E, Pearson's correlation between the VGLUT1 and SYP in(D). E, Pearson's correlation between the secreted IL-1 β in Pg LCM and synaptic components in(D). G, The immunoblots show SYP in control, Pg LCM, Pg+NLRP3kd LCM, and Pg+CA CLM incubated primary neurons. H, Quantitative analyses of the immunoblots in (G). Each column and bar represent the mean $\pm$ SEM (n=3, each). Asterisks indicate a statistically significant difference from the control group (***P<0.001, t-test). Daggers indicate a statistically significant difference from the Pg LCM group. (†††P<0.001, †P<0.05, t-test). I, The immunoblots show SYP in primary neurons with or without 1 μ g/ml IL-1Ra pretreatment followed by incubation with Pg LCM. J, Quantitative analyses of the immunoblots in (I). Each column and bar represent the mean $\pm$ SEM (n=3, each). Asterisks indicate a statistically significant difference from the control group (***P<0.001, t-test). Daggers indicate a statistically significant difference from the Pg LCM group. (†††P<0.001, t-test).

Figure 4

P. gingivalis infected leptomenigeal cells induced an IL-1 β -dependent synaptic distribution in neurons. Condition medium from leptomenigeal cells infected by *P. gingivalis* for 3 hours with MOI=10 (Pg LCM) in the presence or absence of NLRP3 siRNA (Pg+NLRP3kd LCM) or CA-074 Me (Pg+CA LCM) was collected and 30% of the neuron condition medium was replaced to culture primary neurons for 24 hours. A, The relative cell viability in control and Pg LCM neurons. Each column and bar represent the mean $\pm$ SEM (n=3, each). B, CLMS images of primary neurons (MAP2, red) with or without Pg LCM treatment. C, The neurite length analyses of the neurons in (B). Each column and bar represent the mean $\pm$ SEM (n=10, each). Asterisks indicate a statistically significant difference from the control group (**P<0.01, t-test). D, The relative mRNA expression of SYP, VGLUT1, SYN1, and PSD95 in control and Pg LCM neurons. Each column and bar represent the mean $\pm$ SEM (n=3, each). Asterisks indicate a statistically significant difference from the control group (***P<0.001, t-test). E, Pearson's correlation between the VGLUT1 and SYP in (D). F, Pearson's correlation between the secreted IL-1 β in Pg LCM and synaptic components in (D). G, Western blot analysis of SYP (38kDa) and Actin (42kDa) in control (con), Pg LCM, Pg+NLRP3kd LCM, and Pg+CA LCM neurons. The Pg LCM group shows a significant decrease in SYP expression compared to the control group. H, Bar graph showing Relative immunoreactivity (Y-axis, 0.4 to 1.2) for SYP in control (con), Pg LCM, Pg+NLRP3kd LCM, and Pg+CA LCM neurons. The Pg LCM group shows a significant decrease in SYP immunoreactivity compared to the control group (***P<0.001, t-test). I, Western blot analysis of SYP (38kDa) and Actin (42kDa) in control (con), Pg LCM, and L-1Ra+Pg LCM neurons. The L-1Ra+Pg LCM group shows a significant increase in SYP expression compared to the Pg LCM group. J, Bar graph showing Relative immunoreactivity (Y-axis, 0.0 to 1.5) for SYP in control (con), Pg LCM, and L-1Ra+Pg LCM neurons. The L-1Ra+Pg LCM group shows a significant increase in SYP immunoreactivity compared to the Pg LCM group (***P<0.001, t-test).

G, The immunoblots show SYP in control, Pg LCM, Pg+NLRP3kd LCM, and Pg+CA CLM incubated primary neurons. H, Quantitative analyses of the immunoblots in (G). Each column and bar represent the mean $\pm$ SEM (n=3, each). Asterisks indicate a statistically significant difference from the control group (** P <0.001, t-test). Daggers indicate a statistically significant difference from the Pg LCM group. (††† P <0.001, † P <0.05, t-test). I, The immunoblots show SYP in primary neurons with or without 1 μ g/ml IL-1Ra pretreatment followed by incubation with Pg LCM. J, Quantitative analyses of the immunoblots in (I). Each column and bar represent the mean $\pm$ SEM (n=3, each). Asterisks indicate a statistically significant difference from the control group (** P <0.001, t-test). Daggers indicate a statistically significant difference from the Pg LCM group. (††† P <0.001, t-test).

Figure 4

P. gingivalis infected leptomeningeal cells induced an IL-1 β -dependent synaptic distribution in neurons Condition medium from leptomeningeal cells infected by *P. gingivalis* for 3 hours with MOI=10(Pg LCM) in the presence or absence of NLRP3 siRNA (Pg+NLRP3kd LCM) or CA-074 Me (Pg+CA LCM) was

collected and 30% of the neuron condition medium was replaced to culture primary neurons for 24 hours.

A, The relative cell viability in control and Pg LCM neurons. Each column and bar represent the mean $\pm$ SEM (n=3, each).

B, CLMS images of primary neurons (MAP2, red) with or without Pg LCM treatment.

C, The neurite length analyses of the neurons in(B). Each column and bar represent the mean $\pm$ SEM (n=10, each). Asterisks indicate a statistically significant difference from the control group (**P<0.01, t-test).

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E, Pearson's correlation between the VGLUT1 and SYP in(D).

F, Pearson's correlation between the secreted IL-1 β in Pg LCM and synaptic components in(D).

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H, Quantitative analyses of the immunoblots in (G). Each column and bar represent the mean $\pm$ SEM (n=3, each). Asterisks indicate a statistically significant difference from the control group (***P<0.001, t-test). Daggers indicate a statistically significant difference from the Pg LCM group. (†††P<0.001, †P<0.05, t-test).

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Figure 5

P. gingivalis infected leptomenigeal cells induced an IL-1 β -dependent suppression of BDNF signaling in neurons A, The relative mRNA expression of Arc in N2a cells after BDNF treatment for 10,30,60 and 120 minutes with or without 50% Pg LCM incubation. Each column and bar represent the mean $\pm$ SEM (n=3, each). Asterisks indicate a statistically significant difference from the control group (***P<0.001, t-test). Daggers indicate a statistically significant difference from the Pg LCM incubated 30 minutes neurons. (†††P<0.001, t-test). B, The expression of p-Akt and total Akt protein after treatment with Pg LCM, 10 ng/ml BDNF, and 1 μ g/ml IL-1Ra. C, The quantitative analyses of p-Akt/Akt in the immunoblot shown in (B). Each column and bar represent the mean $\pm$ SEM (n=3, each). Asterisks indicate a statistically significant difference (***P<0.001, *P<0.05, t-test). D, The expression of p- CREB and total CREB protein after treatment with Pg LCM, BDNF, and IL-1Ra. E, The quantitative analyses of p-CREB/CREB in the immunoblot shown in (D). Each column and bar represent the mean $\pm$ SEM (n=3, each). Asterisks indicate a statistically significant difference (**P<0.01, *P<0.05, t-test). F, CLMS images of p-CREB after treatment

with Pg LCM, BDNF and IL-1Ra. Scale bar = 10 μ m. G, The quantitative analysis of relative p-CREB fluorescent in(F). Each column and bar represent the mean $\pm$ SEM (n=10, each). Asterisks indicate a statistically significant difference between indicated groups (***P<0.001, **P<0.01, *P<0.1, t-test).

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after treatment with Pg LCM, BDNF, and IL-1Ra. E, The quantitative analyses of p-CREB/CREB in the immunoblot shown in (D). Each column and bar represent the mean $\pm$ SEM (n=3, each). Asterisks indicate a statistically significant difference (**P<0.01, *P<0.05, t-test). F, CLMS images of p-CREB after treatment with Pg LCM, BDNF and IL-1Ra. Scale bar = 10 μ m. G, The quantitative analysis of relative p-CREB fluorescent in(F). Each column and bar represent the mean $\pm$ SEM (n=10, each). Asterisks indicate a statistically significant difference between indicated groups (***P<0.001, **P<0.01, *P<0.1, t-test).

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Figure 6

Propolis modulated the IL-1 β -related BDNF production in primary leptomenigeal cells after *P. gingivalis* infection A, B, The relative mRNA expression of BDNF(A) and IL-1 β (B) in leptomenigeal cells after *P. gingivalis* infection with MOI=10 for 1 and 3hours. Each column and bar represent the mean $\pm$ SEM (n=3,

each). Asterisks indicate a statistically significant difference from the control group (** $P < 0.001$, t-test). C, Cell viability of leptomeningeal cells after propolis treatment with different concentrations. Each column and bar represent the mean $\pm$ SEM ($n=3$, each). Asterisks indicate a statistically significant difference from the control group (** $P < 0.001$, t-test). D, E, The relative mRNA expression of BDNF(D) and IL-1 β (E) in leptomeningeal cells after *P. gingivalis* infection with MOI=10 for 3hours with or without 10 $\mu\text{g/ml}$ propolis 24 hours pretreatment. Each column and bar represent the mean $\pm$ SEM ($n=3$, each). Asterisks indicate a statistically significant difference from the control group (** $P < 0.001$, t-test). Daggers indicate a statistically significant difference from the *P. gingivalis* infection group. (††† $P < 0.001$, †† $P < 0.01$, t-test). F, Pearson's correlation between the IL-1 β and BDNF in(E). G, The relative mRNA expression of CatB in leptomeningeal cells after *P. gingivalis* infection with MOI=10 for 3hours with or without propolis 24 hours pretreatment. Each column and bar represent the mean $\pm$ SEM ($n=3$, each). Asterisks indicate a statistically significant difference from the control group (* $P < 0.05$, t-test). Daggers indicate a statistically significant difference from the *P. gingivalis* infection group. (†† $P < 0.01$, t-test). H, I, Pearson's correlation between the CatB in (G) and IL-1 β in(E)/BDNF in(D).

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Figure 7

leptomeningeal cells might induce synaptic failure via the CatB-mediated IL-1 β production after *P. gingivalis* infection. In the *P. gingivalis*-infected leptomeningeal cells, CatB is involved in positive feedback for NF- κ B activation by degrading I κ B α , and CatB leakage into the cytosol induces NLRP3 inflammasome activation, which subsequently induces mature IL-1 β production and release. In neurons, the IL-1 β secreted by *P. gingivalis*-infected leptomeningeal cells induces the reduction of pre- and post-synaptic molecules and blockage of BDNF signaling. Propolis decreases the IL-1 β production but increases that of BDNF in *P. gingivalis*-infected leptomeningeal cells.

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