Periodontitis Exacerbates Cognitive Impairment via Endothelial Inflammation: Insights from Single-Nucleus Transcriptomics

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Objective: *To explore the underlying mechanisms of the association between periodontitis and cognitive impairment.*

Methods: *Single-nucleus transcriptomics of mice were used to investigate the impact of periodontitis on brain and hippocampal cells to gain insights into disease progression. After data processing, functional enrichment, pathway analysis and cell-cell communication analysis were used to identify the distinct pathways and genes upregulated in Alzheimer's disease (AD) linked to periodontitis. After cell culture, real-time quantitative polymerase chain reaction (RT-qPCR) and enzyme-linked immunosorbent assay (ELISA) were used to confirm the results.*

Results: *The present authors identified endothelial inflammation as a key factor in periodontitis-related AD. The findings revealed that the upregulation of Mgll expression in endothelial cells was linked to increased antigen presentation and exacerbated neuroinflammation, potentially worsening AD. Moreover, the present authors elucidated MGLL-2AG-CB2 signalling, through which MGLL activation suppresses CB2 expression and leads to heightened inflammation, while MGLL inhibition restores CB2 levels and mitigates inflammation.*

Conclusion: *The present study revealed the role of MGLL in linking periodontitis to hippocampal endothelial inflammation and antigen presentation in cognitive impairment. This research will enhance the understanding of how periodontitis impacts cognition and explore potential therapeutic strategies to alleviate periodontitis-associated cognitive impairment.*

Keywords: *cognitive impairment, endothelial inflammation, MGLL, periodontitis, single-nucleus transcriptomics*

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Periodontitis is an inflammatory disease caused by plaque microbiota and results in the degradation of tooth-supporting tissues, which in turn leads to tooth mobility and loss. It has also been reported to be closely associated with cognitive disorders such as Alzheimer's disease (AD) through pathogenic microorganisms and inflammation in both mice and humans.[1,2] Cognitive disorders mainly occur in individuals who are aged over 60 years, with an incidence rate of up to 10%.[3] As the population ages worldwide, the prevalence of these diseases is expected to increase. Currently, effective treatment strategies for both periodontitis and cognitive disorders are lacking. Thus, investigating the mechanisms through which periodontitis influences the development of cognitive disorders and identifying preventive measures could mitigate the risk they pose.

Recent studies have revealed a strong correlation between periodontitis and AD. Individuals with higher levels of periodontitis exhibit faster hippocampal atrophy and cognitive deterioration.[4] Periodontitis can facilitate the infiltration of pathogenic bacteria, virulence factors and inflammatory mediators into the

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brain via the blood-brain barrier (BBB).[5-7] These substances then change the brain microenvironment, triggering neuroinflammation and neurodegeneration, ultimately leading to cognitive decline. The present authors' previous research showed that periodontitis impairs macrophage function and promotes reactions of hippocampal cells to virulence factors and bacteria, inducing astrocyte abnormalities and neuroinflammation.[8] Nevertheless, the precise mechanisms by which factors damage the BBB and change the microenvironment in the hippocampus require further research.

The brain microvasculature has been proven to be a key player in the development of AD. Previous studies have shown vascular disruption in early AD development.[9,10] Endothelial cells (ECs) are crucial for cleaning beta-amyloid (Aβ) and other harmful substances from the central nervous system, whereas inflammation in them reduces Aβ clearance.[11] Furthermore, ECs can secrete cytokines and directly activate dysfunction of surrounding neurons, astrocytes and microglia, leading to neuroinflammation and cognitive impairment.[12-14] However, the precise mechanisms underlying damage to the brain endothelium from initial contact with periodontitis pathogens and toxins remain unclear.

Single-nucleus transcriptomics enables the examination of cellular changes, shedding light on the mechanisms by which periodontitis impacts cognitive function. In the present study, the authors generated single-nucleus maps of the brain and hippocampus, delineating key cell populations in these regions, and revealed that periodontitis upregulates genes in hippocampal ECs linked to AD and suppresses neurogenesis. Specifically, periodontitis induces the upregulation of monoacylglycerol lipase (MGLL) expression in ECs, leading to neuroinflammation. The authors demonstrated that increasing antigen presentation through MGLL-related pathways in ECs while inhibiting MGLL expression improved periodontitis-induced endothelial dysfunction. This research not only elucidates the pathogenesis of cognitive dysfunction associated with periodontitis but also lays the groundwork for targeted therapeutic interventions for cognitive impairment.

Materials and methods

Data acquisition

The methods used were described in a previous study by the present authors.[8] In brief, male C57BL/6 mice for sequencing were purchased from the National Resource Center of Model Mice (Nanjing, China) and housed in a specific pathogen-free facility at the Laboratory Animal Center of Sun Yat-sen University. The 12-month-old mice were separated into two groups (n = 6 in each group): the healthy group (Heal), which acted as a control, and the periodontitis group (PD). Ligature-induced periodontitis models were generated in PD group mice. After 6 weeks, they were euthanised by CO_2 inhalation for brain and hippocampus collection. All experiments were approved by the Animal Care and Use Committee of Sun Yat-sen University (SYSU-IACUC-2018-109 000135).

Single-nucleus RNA sequencing (snRNA-seq) was performed by the DNBelab C Series High-Throughput Single-Cell System from MGI (Shenzhen, China) and pre-processed by STAR (v2.5.3). The data were uploaded to the National Genomics Data Center (NGDC).

Data processing

The present authors used the Seurat (v5.1.0) package of R (v4.4.0) for subsequent processing.[15] We selected cells that contained between 500 and 4,000 genes and had less than 10% mitochondrial DNA and normalised the data with the *SCTransform* function (Fig S1, provided on request). After data integration and principal component analysis (PCA), we removed batch effects from the data with the *RunHarmony* function of the Harmony (v1.2.0) package[16] and used uniform manifold approximation and projection (UMAP) for dimensionality reduction in the PCA embeddings. Then, we annotated cell clusters based on marker genes identified by the *FindAllMarkers* function (Fig S2, provided on request) and mentioned in previous studies.[8,17-20]

Functional enrichment, pathway analysis and scoring

Differentially expressed genes (DEGs) were identified by the *FindMarkers* Seurat function with a Wilcoxon test ($P < 0.05$). DEGs in the brain and hippocampal ECs between the PD group and Heal group were selected with a minimum expression ratio of 0.2 and an average log fold change (avg logFC) of positive number. DEGs of hippocampal ECs with high *Mgll* expression (expression > 0) were selected with a minimum average log fold change (avg logFC) of 3 or a maximum average log fold change (avg logFC) of −3. Functional enrichment and pathway analyses were performed using Metascape (https://www.metascape.org) based on the selected DEGs to identify enriched functional terms.[21] Then, we integrated the results and visualised them in R with the Treemapify (v2.5.6) package. We chose visualised terms with the 10 highest counts. In addition, we calculated

module scores of significant functional terms with the *AddModuleScore* Seurat function.

Cell-cell communication analysis

We analysed cell-cell communication with the CellPhoneDB (v5.0.0) package in Python (v3.12.0),[22] then integrated the results and visualised them in R with Ggalluvial (v0.12.5). We chose visualised receptors with the five highest significant means and corresponding ligands.

Cell culture

The immortalised human cerebral microvascular endothelial cell line (HCMEC/D3) and human umbilical vein endothelial cell line (PUMC-HUVEC-T1) were purchased from Pricella (Wuhan, China) and cultured in complete culture medium for ECs from Pricella in a 37°C humidified incubator with 5% CO_2. The cells were expanded through three passages and seeded in 6-well culture plates with 1,000,000 cells per well.

Lipopolysaccharide (LPS) from *Porphyromonas gingivalis* was purchased from Sigma-Aldrich (St Louis, MO, USA) and stored as a 1 mg/ml stock solution in HBSS at −20°C. ABX-1431 was purchased from Selleck (Houston, TX, USA) and stored as a 20mM stock solution in DMSO at −80°C. The cells were cultured in serum-free medium with healthy, 200ng/ml LPS, or 200ng/ml LPS + 10μM ABX-1431 for 12 hours.

Real-time quantitative polymerase chain reaction (RT-qPCR)

Total RNA was collected using an RNA quick extraction kit from GOONIE (Guangzhou, China) according to the manufacturer's instructions, and stored at −80°C. The cDNA was obtained with a PrimeScript RT reagent kit (Takara, Kusatsu, Japan) according to the manufacturer's instructions. Real-time PCR was conducted with the Taq Pro Universal SYBR qPCR Master Mix (Vazyme, Nanjing, China) and a QuantStudio 7 Flex Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturers' instructions. Primers were synthesised by Tsingke Biotech (Beijing, China), and the sequences are listed in the supplemental information (Table S1, provided on request).

Enzyme-linked immunosorbent assay (ELISA)

Media were collected and stored at −20°C. ELISA was conducted with an ELISA Kit (MEIMIAN, Yancheng, China) according to the manufacturer's instructions. The optical density (OD) at 450 nm was detected using a microplate reader (BioTek, Winooski, VT, USA).

Statistical analysis

Statistical analyses of the snRNA-seq data were performed in R with default parameters. A Wilcoxon test was employed to compare differences of *Mgll* expression, and an unpaired Student *t* test was used to compare differences of module score in violin plots. Differences with $P < 0.05$ were considered to indicate statistical significance. Statistical analyses of the cellular experiments were performed in GraphPad Prism (v10.1.2; Dotmatics, Boston, MA, USA) and are presented as means ± standard errors of the means (SEMs). A one-way analysis of variance (ANOVA) followed by a Tukey multiple comparisons test was used for multiple comparisons. Differences with $P < 0.05$ were considered to indicate statistical significance.

Results

Construction of the single-nucleus transcriptomic atlases of mouse brains and hippocampi

To investigate disorders in the brain and hippocampus caused by periodontitis, the present authors obtained single-nucleus transcriptomic data from the brains and hippocampi of healthy mice and those with experimental periodontitis (Fig 1a). We successfully constructed the cell atlases of the brain (Fig 1b and c) and hippocampi (Fig 1d and e) of mice with periodontitis. The present study identified cells as eight clusters according to known markers, including inhibitory neurons (In) (*Gad1*$^+$, *Gad2*$^+$), excitatory neurons (Ex) (*Slc17a7*$^+$, *Camk2a*$^+$), astrocytes (Ast) (*Aqp4*$^+$, *Gfap*$^+$), oligodendrocytes (Oli) (*Mobp*$^+$, *Mog*$^+$), oligodendrocyte progenitor cells (OPCs) (*Olig1*$^+$, *Olig2*$^+$), microglia (Mic) (*Csf1r*$^+$, *P2ry12*$^+$) and endothelial cells (ECs) (*Flt1*$^+$, *Cdh5*$^+$). The present authors also revealed that the proportions of inhibitory neurons, microglia and ECs were decreased in both the brain and hippocampus, that of excitatory neurons and astrocytes was increased, and that of the entire neural population was slightly decreased (Fig 1f and g). This finding suggests that periodontitis can affect the brain and hippocampus in a similar pattern. The atlases formed the basis of the subsequent analyses.

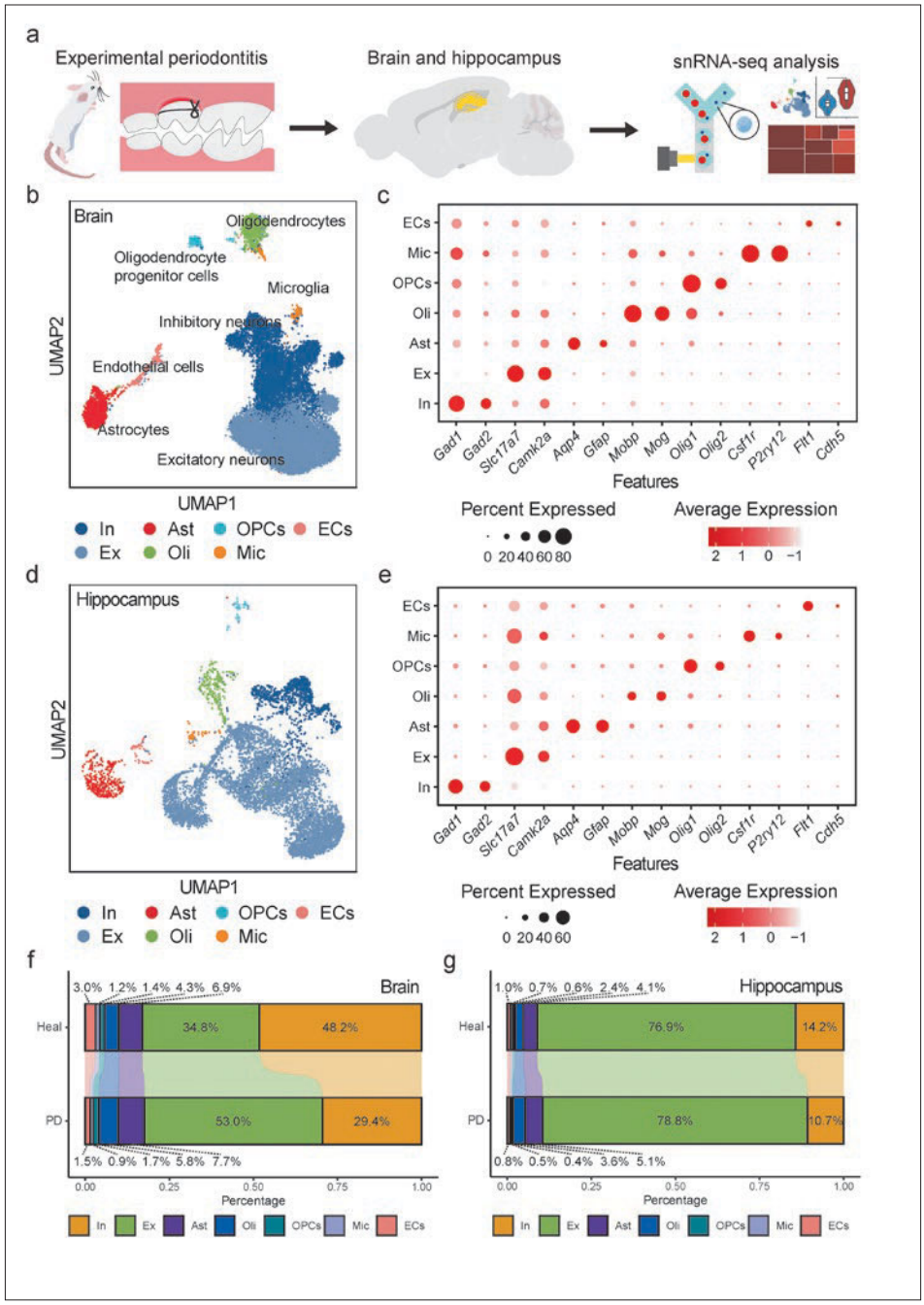

Fig 1a to g The single-nucleus transcriptomic atlases of the mouse brain and hippocampus. Overview of the processing of snRNA-seq data **(a)**; UMAP showing cell clusters in the brain **(b)**; dot plots showing marker genes of every cluster in the brain **(c)**; UMAP showing cell proportions in the hippocampus **(d)**; dot plots showing marker genes of every cluster in the hippocampus **(e)**; proportions of different cell types in the brain **(f)** and the hippocampus **(g)**.

Changes in the function of brain and hippocampal ECs and communication between the ECs and neighbouring cells in the hippocampal area

To explore the initiating mechanism of cognitive disorders in the hippocampus, the present authors compared the functions of activated ECs in both the brain and hippocampus of mice with periodontitis through functional enrichment and pathway analysis (Fig 2a and b). ECs form the BBB as the first line of defence with pericytes and fibroblasts in every area of the brain.[23] The present research revealed that ECs in the hippocampus have different gene expression patterns and functions from those in other areas of the brain when affected by periodontitis. The upregulated genes in ECs of the hippocampus were enriched mainly in the terms 'negative regulation of neurogenesis' and 'Alzheimer's disease (AD)' (Fig 2b). The present study suggests that ECs in the hippocampus may initiate the development of periodontitis-induced AD.

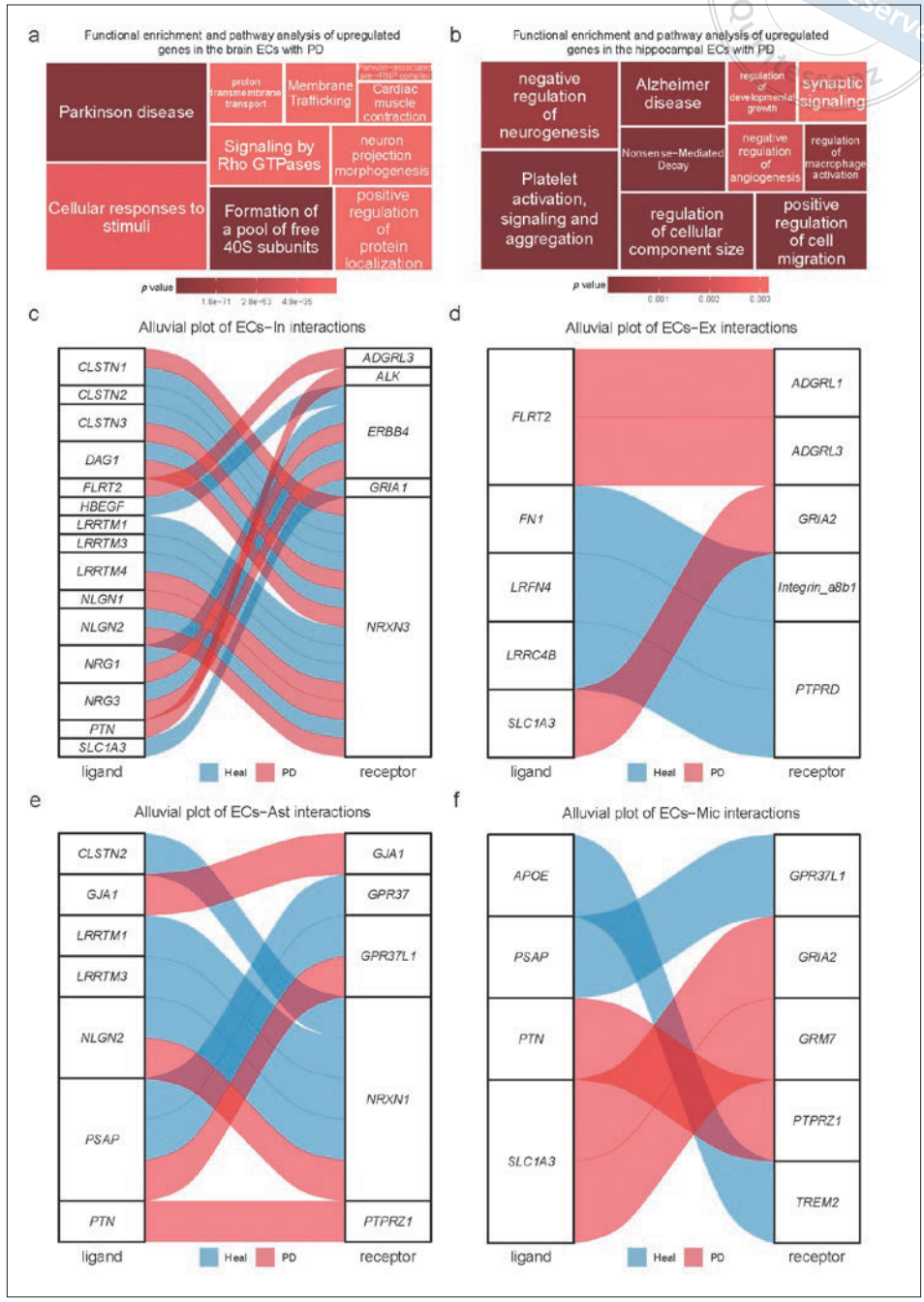

Fig 2a to f Changes in the function of brain and hippocampal ECs and communication between the ECs and neighbouring cells in the hippocampal area. Tree maps showing functional analyses of upregulated genes in the brain and hippocampus with periodontitis **(a and b)**; alluvial plots showing the main interactions between endothelial cells and surrounding cells, including inhibitory neurons **(c)**, excitatory neurons **(d)**, astrocytes **(e)** and microglia **(f)**, in the hippocampus of different groups.

The present authors also investigated the interactions between ECs and other significant clusters in the neurovascular unit, and observed that ECs in the hippocampus of mice with periodontitis had disrupted communication with neurons, astrocytes and microglia compared to those in healthy mice. Specifically, in ECs, *FLRT2* expression was upregulated, and expression of *FLRT2* receptor was correspondingly upregulated on neurons (Fig 2c and d). FLRT2 can regulate synapse formation, which may contribute to the imbalanced activation of neurons.[24,25] For ligands to astrocytes, ECs downregulated the expression of *CLSTN2*, *LRRTM1* and *LRRTM3*, whereas *GJA1* and *PTN* expression increased (Fig 2e). Moreover, ECs heightened the expression of *PTN* and *SLC1A3* ligands as well as matching receptors on microglia (Fig 2f). These findings suggest that periodontitis alters intercellular communication between hippocampal ECs and neighbouring cells, potentially contributing to cognitive impairment.

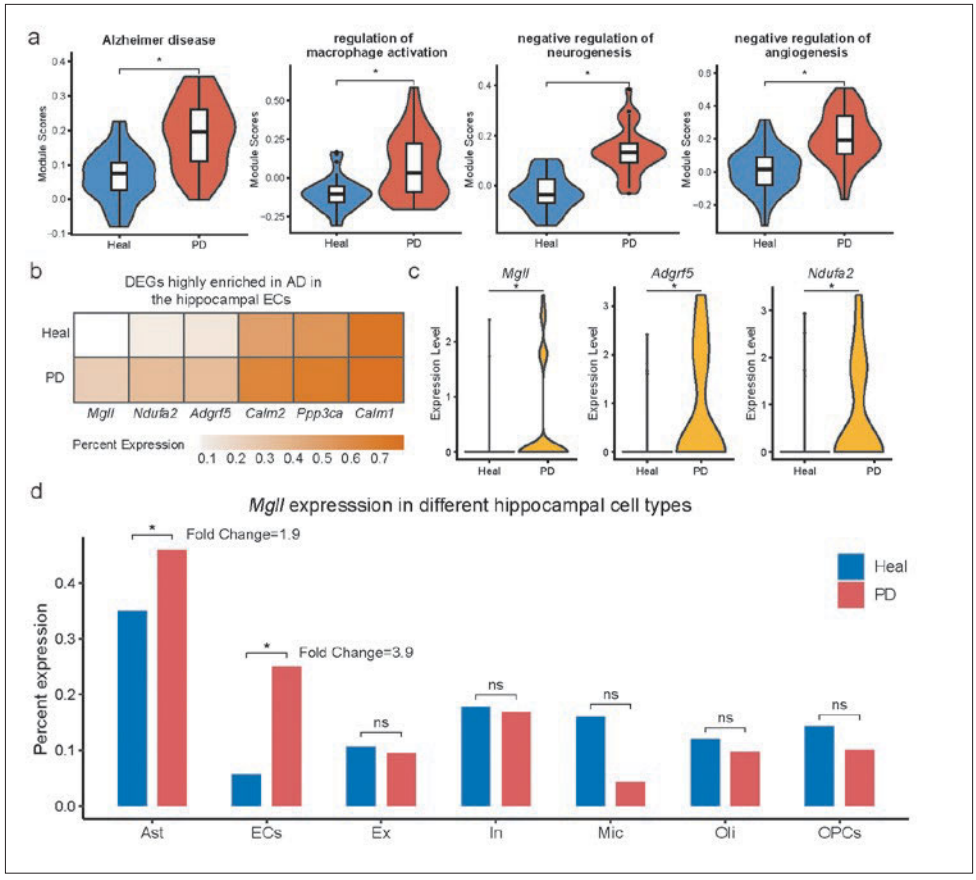

Fig 3a to d Activation of the *Mgll* in the hippocampal ECs with periodontitis. Module scores of significantly enriched pathway genes in the hippocampal endothelial cells with periodontitis **(a)**; heatmap showing DEGs highly enriched in AD in the hippocampal ECs with periodontitis **(b)**; violin plots showing the top three most highly expressed genes enriched in AD in hippocampal ECs in different groups **(c)**; bar plots of *Mgll* expression in different hippocampal cell types **(d)**. **P* < 0.05.

Activation of Mgll in the hippocampal ECs in mice with periodontitis

The present authors then conducted a detailed analysis of the key genes involved in pathways associated with cognitive dysfunction upregulated in hippocampal ECs in response to periodontitis. The examination of these pathways revealed a significant upregulation in the AD pathway (Fig 3a). Pathways related to the negative regulation of angiogenesis, neurogenesis and macrophage activation were also upregulated in ECs affected by periodontitis. Heatmap visualisation highlighted specific genes linked to the AD pathway, such as *Mgll*, *Calm1*, *Ppp3ca*, *Calm2*, *Adgrf5* and *Ndufa2*, whereas *Mgll* exhibited the greatest increase in expression (Fig 3b and c). The analysis also indicated that *Mgll* is predominantly expressed in hippocampal astrocytes and ECs under the influence of periodontitis, whereas its upregulation in ECs was most notable (Fig 3d). Consequently, the present authors focused on the expression of *Mgll*, as its upregulation could potentially disrupt endothelial function and impact cellular communication. These findings suggest that *Mgll* is activated in the hippocampal ECs in mice with periodontitis.

Activation of Mgll exacerbates inflammation and antigen presentation in the hippocampal ECs

The present authors further investigated the impact of *Mgll* activation on the functions of ECs by comparing the gene expression profiles of ECs with high and low *Mgll* levels. Enrichment analysis of differentially expressed genes revealed that genes associated with the immune response, apoptotic signalling regulation and antigen presentation via MHC class II were upregulated in ECs with high Mgll expression, whereas genes associated with developmental growth pathways were downregulated in those cells (Fig 4a and b). Statistical analysis confirmed the significant upregulation of immune response and apoptotic signalling regulation and the downregulation of developmental growth in high *Mgll*-expressing cells (Fig 4c). Examination of immune-related genes in high *Mgll*-expressing cells identified H2-Eb1, Cd74 and H2-Aa as the top three genes with significantly increased expression compared to *Mgll*-negative cells, indicating enhanced MHCII complex regulation and inflammatory functions in ECs with high *Mgll* expression (Fig 4d and e). The activation of proinflammatory ECs was found to exacerbate neuroinflammation and cognitive dysfunc-

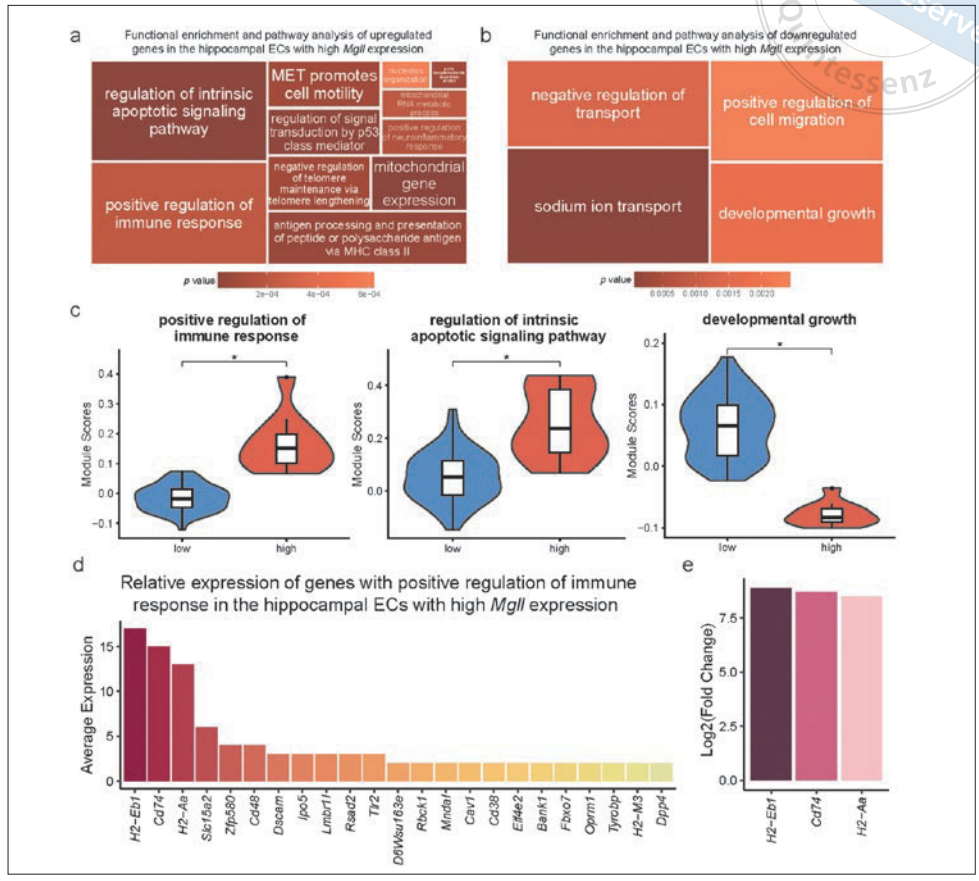

Fig 4a to e Activation of *Mgll* exacerbates inflammation and antigen presentation in the hippocampal ECs. Tree maps showing functional analyses of upregulated **(a)** and downregulated genes **(b)** in hippocampal ECs with high *Mgll* expression; module scores of significant pathway genes in functions enriched in the hippocampal ECs with high *Mgll* expression **(c)**; bar plot showing the relative expression of genes that positively regulate the immune response in hippocampal ECs with high *Mgll* expression **(d)**; bar plot showing the top three genes with the greatest changes in the expression of genes associated with positive regulation of the immune response in hippocampal ECs with high *Mgll* expression **(e)**. **P* < 0.05.

tion by stimulating T cells and microglia. These findings suggest that *Mgll* activation amplifies inflammation and antigen presentation in hippocampal ECs.

The MGLL inhibitor ABX-1431 mitigated inflammation and antigen presentation in ECs

ECs exhibiting high *Mgll* expression demonstrate heightened proinflammatory and enhanced antigen presenting properties; however, the precise mechanisms through which *Mgll* governs these EC functions remain ambiguous. Therefore, the present authors investigated the impact of LPS on simulating bacteria on HCMEC/D3 cells (Fig 5) and PUMC-HUVEC-T1 cells (Fig S3, provided on request). After LPS stimulation, elevated expression levels of type 2 cannabinoid receptors (*CB2*) were observed, as well as the proinflammatory and antigen presenting genes *HLA-DRB1* and *CIITA* (Fig 5a). *CB1* expression had no significant change, which corresponded to a previous study.[26] 2-arachidonoylglycerol (2-AG) is the ligand of cannabinoid receptors, the activation of which is known to significantly mitigate neuroinflammation and aid disease-related damage repair.[27] MGLL predominantly hydrolyses 2-AG to arachidonic acid (AA), which induces cannabinoid receptors to exert inhibitory effects.[26] AA can also attribute to neuroinflammation.[28] The present authors observed consistent results, which affirmed that MGLL played an important role in the endothelial inflammation (Fig 5b). ABX-1431 is a common MGLL inhibitor.[29] Notably, treatment with ABX-1431 suppressed 2-AG hydrolysation, decreasing the expression of *HLA-DRB1* and *CIITA* and restoring the expression of CB2 (Fig 5b). The present findings clarified that MGLL decreased the levels of 2-AG and CB2, attributing to increased CIITA and MHCII expression, leading to neuroinflammation in surrounding cells (Fig 5c). These results suggest that MGLL inhibitors can diminish the inflammatory and antigen-presenting capacities of ECs by augmenting 2-AG levels and suppressing CIITA and MHCII expression, which could be potential therapies for periodontitis-associated AD.

Discussion

By utilising single-nucleus transcriptomics of brain and hippocampal cells, the present study revealed that periodontitis exacerbates cognitive impairment by inducing endothelial inflammation and dysfunction of antigen

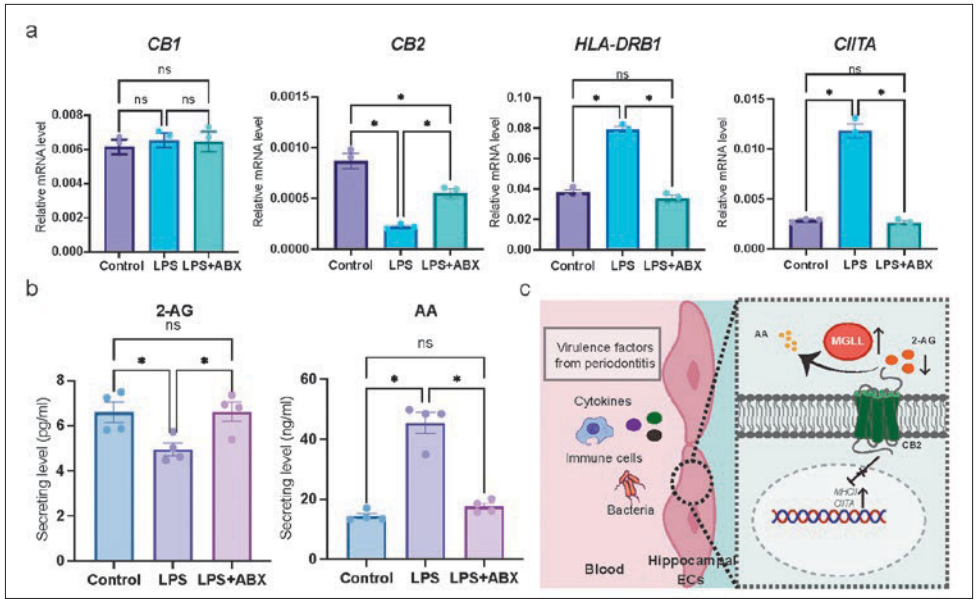

Fig 5a to c The MGLL inhibitor mitigated inflammation and antigen presentation in HCMEC/D3. RT-qPCR analyses of MGLL associated genes in different groups **(a)**; ELISA analysis of 2-AG and AA in different groups **(b)**; overview of the mechanisms by which MGLL induces neuroinflammation during the process by which periodontitis exacerbates AD **(c)**. ABX: ABX-1431. Error bars indicate the standard error of the mean (SEM). *Adjust $P < 0.05$.

presentation through upregulation of *Mgll* expression. This study offers valuable perspectives for the advancement of precise therapeutic interventions for cognitive impairment.

The present study revealed the key role of endothelial inflammation as a significant contributor to periodontitis-associated cognitive impairment. Previous research has emphasised the importance of endothelial dysfunction during the development of AD, but has not precisely explored the connection between endothelial dysfunction and periodontitis-associated AD. Abnormalities of brain ECs contributing to cognitive impairment have been observed in morphology, metabolism and immunity.[30,31] Periodontitis involves sophisticated mechanisms that can damage systemic health,[32-34] and may play a pivotal role in brain endothelial abnormalities that exacerbate neuroinflammation and cognitive decline; however, changes in brain ECs underlying periodontitis are unclear. Through the snRNA-seq of brain and hippocampal cells, the present authors identified the distinct pathways upregulated in the hippocampal ECs of mice with periodontitis that are linked to AD. Subsequent analysis of cell communication revealed aberrant interactions between ECs and surrounding cells involving neurons, astrocytes and microglia in the hippocampus, which may have intensified neuroinflammation. In summary, the present study provides a comprehensive understanding of the impact of periodontitis on hippocampal ECs and elucidates potential mechanisms that contribute to the exacerbation of neuroinflammation.

The present authors also identified the potential mechanism through which periodontitis exacerbates neuroinflammation via endothelial cell dysfunction. MGLL was identified as one of the key molecules leading to endothelial cell dysfunction. It can participate in endocannabinoid signalling, cause dysfunction of astrocytes and microglia and augment inflammatory mediators such as prostaglandin.[28] Previous studies have shown an imbalanced endocannabinoid system in gingival crevicular fluid in individuals with periodontitis.[35,36] In the central nervous system, a recent study observed elevated MGLL expression in protein extracts from the frontal cortex in cases of depression combined with periodontitis;[37] however, its contribution and underlying mechanisms in the hippocampus with periodontitis-associated AD is unclear. Thus, the present authors focused on its function by comparing cells that exhibited high and low *Mgll* expression in the hippocampal data of periodontitis-afflicted mice. Analysis of upregulated genes involved in AD-related pathways revealed the predominant expression of *Mgll* in ECs and astrocytes, and the most significant upregulation was observed in the ECs. Interestingly, the present authors observed an accompanying increase in the expression of genes associated with MHCII molecules, including a notable increase of more than sevenfold in the expression of *H2-Eb1*, *Cd74* and *H2-Aa*. MHCII molecules play a crucial role in the antigen presentation function of ECs, contributing to the activation of T cells and macrophages and the exacerbation of tissue inflammation.[38,39] The present authors infer that peri-

odontitis may aggravate neuroinflammation and cognitive impairment by activating the antigen presentation of ECs, leading to the activation of microglia, astrocytes and other cell types. Periodontitis may aggravate MGLL expression through inflammatory signalling, such as PI3K/AKT and NF-κB, as described in previous studies,[40-42] which should be studied further. Further experimental validation is imperative to substantiate the direct connection between periodontitis, MGLL activation in hippocampal ECs and upregulated antigen presentation.

Specifically, the present authors elucidated that the MGLL-2AG-CB2 pathway activates antigen presentation and inflammatory phenotypes in ECs. MGLL can hydrolyse 2-AG to AA, while the expression of the 2-AG receptor CB1 and CB2 decreases in surrounding environment.[28] CB2 has been shown to play a crucial role in regulating inflammation and neuronal modulation in the brain, including the transition of microglia and astrocytes to the M2 phenotype, and the release of excitatory and inhibitory neurotransmitters.[43] CB2 is also widely expressed on immune cells such as macrophages and dendritic cells, which suggests that it may participate in antigen presentation.[44,45] In the present authors' hypothesis based on the analyses, CB2 suppresses CIITA expression and reduces MHCII levels, while decreasing CB2 expression can augment anti-inflammatory antigen presentation in ECs, trigger tissue inflammation and worsen tissue damage. Therefore, the present authors investigated whether inhibiting MGLL could enhance CB2 expression and decrease CIITA and MHCII levels in ECs. The findings revealed that MGLL inhibition increases 2-AG levels, activates endothelial CB2 and subsequently downregulates CIITA and MHCII. Targeting MGLL to reduce the anti-inflammatory antigen presentation and inflammatory profiles of ECs could represent a potential strategy for mitigating periodontitis-induced neuroinflammation and cognitive impairment; however, the use of LPS as a model of inflammation may not fully recapitulate the complex microbial and host interactions in vivo. Further experimental validation is also necessary to confirm the efficacy and feasibility of this approach, which will be the focus of follow-up studies.

Conclusion

In summary, with analyses of snRNA-seq data from brain and hippocampal cells, this study reveals the role of the MGLL-2AG-CB2 pathway in linking periodontitis to inflammation and antigen presentation of ECs, leading to neuroinflammation and cognitive impairment. These insights offer a foundation for targeted therapeutic approaches for cognitive impairment.

Conflicts of interest

The authors declare no conflicts of interest related to this study.

Author contribution

Drs Zong Shan SHEN, Ji Chen YANG and Bin CHENG designed and directed the study; Drs Zong Shan SHEN and Ji Chen YANG contributed to the collection and analysis of the data and the manuscript draft; Drs Chuan Jiang ZHAO and Bin CHENG critically revised the manuscript. All the authors approved the final submission.

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References

1. Dominy SS, Lynch C, Ermini F, et al. Porphyromonas gingivalis in Alzheimer's disease brains: Evidence for disease causation and treatment with small-molecule inhibitors. Sci Adv 2019;5:eaau3333.
2. Wang RP, Huang J, Chan KWY, et al. IL-1β and TNF-α play an important role in modulating the risk of periodontitis and Alzheimer's disease. J Neuroinflammation 2023;20:71.
3. Jia L, Du Y, Chu L, et al. Prevalence, risk factors, and management of dementia and mild cognitive impairment in adults aged 60 years or older in China: A cross-sectional study. Lancet Public Health 2020;5:e661–e671.
4. Yamaguchi S, Murakami T, Satoh M, et al. Associations of dental health with the progression of hippocampal atrophy in community-dwelling individuals: The ohasama study. Neurology 2023;101:e1056–e1068.
5. Lei S, Li J, Yu J, et al. Porphyromonas gingivalis bacteremia increases the permeability of the blood-brain barrier via the Mfsd2a/caveolin-1 mediated transcytosis pathway. Int J Oral Sci 2023;15:3.
6. Li F, Ma C, Lei S, et al. Gingipains may be one of the key virulence factors of porphyromonas gingivalis to impair cognition and enhance blood-brain barrier permeability: An animal study. J Clin Periodontol 2024;51:818–839.
7. Kerkis I, da Silva ÁP, Araldi RP. The impact of interleukin-6 (IL-6) and mesenchymal stem cell-derived IL-6 on neurological conditions. Front Immunol 2024;15:1400533.
8. Shen Z, Kuang S, Zhang Y, et al. Restoring periodontal tissue homoeostasis prevents cognitive decline by reducing the number of Serpina3nhigh astrocytes in the hippocampus. Innovation (Camb) 2023;5:100547.
9. Bryant A, Li Z, Jayakumar R, et al. Endothelial cells are heterogeneous in different brain regions and are dramatically altered in Alzheimer's disease. J Neurosci 2023;43:4541–4557.
10. Custodia A, Ouro A, Romaus-Sanjurjo D, et al. Endothelial progenitor cells and vascular alterations in Alzheimer's disease. Front Aging Neurosci 2022;13:811210.

11. Waigi EW, Pernomian L, Crockett AM, et al. Vascular dysfunction occurs prior to the onset of amyloid pathology and aβ plaque deposits colocalize with endothelial cells in the hippocampus of female APPswe/PSEN1dE9 mice. Geroscience 2024;46:5517–5536.
12. Zhang P, Fu G, Xu W, et al. Up-regulation of miR-126 via DNA methylation in hypoxia-preconditioned endothelial cells may contribute to hypoxic tolerance of neuronal cells. Mol Biol Rep 2024;51:808.
13. Linnerbauer M, Wheeler MA, Quintana FJ. Astrocyte crosstalk in CNS inflammation. Neuron 2020;108:608–622.
14. Huo A, Wang J, Li Q, et al. Molecular mechanisms underlying microglial sensing and phagocytosis in synaptic pruning. Neural Regen Res 2024;19:1284–1290.
15. Hao Y, Stuart T, Kowalski MH, et al. Dictionary learning for integrative, multimodal and scalable single-cell analysis. Nat Biotechnol 2024;42:293–304.
16. Korsunsky I, Millard N, Fan J, et al. Fast, sensitive and accurate integration of single-cell data with harmony. Nat Methods 2019;16:1289–1296.
17. Xing J, Ren L, Xu H, et al. Single-cell RNA sequencing reveals cellular and transcriptional changes associated with traumatic brain injury. Front Genet 2022;13:861428.
18. Li Y, Li Z, Wang C, et al. Spatiotemporal transcriptome atlas reveals the regional specification of the developing human brain. Cell 2023;186:5892–5909.e22.
19. Khrameeva E, Kurochkin I, Han D, et al. Single-cell-resolution transcriptome map of human, chimpanzee, bonobo, and macaque brains. Genome Res 2020;30:776–789.
20. Lau SF, Cao H, Fu AKY, Ip NY. Single-nucleus transcriptome analysis reveals dysregulation of angiogenic endothelial cells and neuroprotective glia in Alzheimer's disease. Proc Natl Acad Sci U S A 2020;117:25800–25809.
21. Zhou Y, Zhou B, Pache L, et al. Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. Nat Commun 2019;10:1523.
22. Troulé K, Petryszak R, Prete M, et al. CellPhoneDB v5: Inferring cell-cell communication from single-cell multiomics data. http://arxiv.org/abs/2311.04567. Accessed 23 July 2024.
23. Yang Q, Wei X, Deng B, et al. Cerebral small vessel disease alters neurovascular unit regulation of microcirculation integrity involved in vascular cognitive impairment. Neurobiol Dis 2022;170:105750.
24. Pederick DT, Lui JH, Gingrich EC, et al. Reciprocal repulsions instruct the precise assembly of parallel hippocampal networks. Science 2021;372:1068–1073.
25. Maestú F, de Haan W, Busche MA, DeFelipe J. Neuronal excitation/inhibition imbalance: Core element of a translational perspective on Alzheimer pathophysiology. Ageing Res Rev 2021;69:101372.
26. Zhou S, Ling X, Zhu J, et al. MAGL protects against renal fibrosis through inhibiting tubular cell lipotoxicity. Theranostics 2024;14:1583–1601.
27. Moe A, Rayasam A, Sauber G, et al. Type 2 cannabinoid receptor expression on microglial cells regulates neuroinflammation during graft-versus-host disease. J Clin Invest 2024;134:e175205.
28. Bajaj S, Jain S, Vyas P, Bawa S, Vohora D. The role of endocannabinoid pathway in the neuropathology of Alzheimer's disease: Can the inhibitors of MAGL and FAAH prove to be potential therapeutic targets against the cognitive impairment associated with Alzheimer's disease? Brain Res Bull 2021;174:305–322.
29. Müller-Vahl KR, Fremer C, Beals C, Ivkovic J, Loft H, Schindler C. Endocannabinoid modulation using monoacylglycerol lipase inhibition in tourette syndrome: A phase 1 randomized, placebo-controlled study. Pharmacopsychiatry 2022;55:148–156.
30. Leira Y, Vivancos J, Diz P, Martín Á, Carasol M, Frank A. The association between periodontitis and cerebrovascular disease, and dementia. Scientific report of the working group of the Spanish society of periodontology and the Spanish society of neurology. Neurologia (Engl Ed) 2024;39:302–311.
31. İş Ö, Wang X, Reddy JS, et al. Gliovascular transcriptional perturbations in Alzheimer's disease reveal molecular mechanisms of blood brain barrier dysfunction. Nat Commun 2024;15:4758.
32. Shen Z, Zhang R, Huang Y, et al. The spatial transcriptomic landscape of human gingiva in health and periodontitis. Sci China Life Sci 2024;67:720–732.
33. Lu J, Zhang S, Huang Y, et al. Periodontitis-related salivary microbiota aggravates Alzheimer's disease via gut-brain axis crosstalk. Gut Microbes 2022;14:2126272.
34. Herrera D, Molina A, Buhlin K, Klinge B. Periodontal diseases and association with atherosclerotic disease. Periodontol 2000 2020;83:66–89.
35. Nakajima Y, Furuichi Y, Biswas KK, et al. Endocannabinoid, anandamide in gingival tissue regulates the periodontal inflammation through NF-kappaB pathway inhibition. FEBS Lett 2006;580:613–619.
36. Özdemir B, Shi B, Bantleon HP, Moritz A, Rausch-Fan X, Andrukhov O. Endocannabinoids and inflammatory response in periodontal ligament cells. PloS One 2014;9:e107407.
37. Robledo-Montaña J, Díaz-García C, Martínez M, et al. Microglial morphological/inflammatory phenotypes and endocannabinoid signaling in a preclinical model of periodontitis and depression. J Neuroinflammation 2024;21:219.
38. Rodor J, Chen SH, Scanlon JP, et al. Single-cell RNA sequencing profiling of mouse endothelial cells in response to pulmonary arterial hypertension. Cardiovasc Res 2022;118:2519–2534.
39. Rustenhoven J, Drieu A, Mamuladze T, et al. Functional characterization of the dural sinuses as a neuroimmune interface. Cell 2021;184:1000–1016.e27.
40. Zhu W, Zhao Y, Zhou J, et al. Monoacylglycerol lipase promotes progression of hepatocellular carcinoma via NF-κB-mediated epithelial-mesenchymal transition. J Hematol Oncol 2016;9:127.
41. Gandhi AY, Yu J, Gupta A, Guo T, Iyengar P, Infante RE. Cytokine-mediated STAT3 transcription supports ATGL/CGI-58-dependent adipocyte lipolysis in cancer cachexia. Front Oncol 2022;12:841758.
42. Shen S, Fu B, Deng L, et al. Paeoniflorin protects chicken against APEC-induced acute lung injury by affecting the endocannabinoid system and inhibiting the PI3K/AKT and NF-κB signaling pathways. Poult Sci 2024;103:103866.
43. Grabon W, Rheims S, Smith J, Bodennec J, Belmeguenai A, Bezin L. CB2 receptor in the CNS: From immune and neuronal modulation to behavior. Neurosci Biobehav Rev 2023;150:105226.
44. Xiang W, Shi R, Kang X, et al. Monoacylglycerol lipase regulates cannabinoid receptor 2-dependent macrophage activation and cancer progression. Nat Commun 2018;9:2574.
45. Cabral GA, Ferreira GA, Jamerson MJ. Endocannabinoids and the immune system in health and disease. Handb Exp Pharmacol 2015;231:185–211.