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Context matters: hPSC-derived microglia thrive in a humanized brain environment *in vivo*

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Abstract

It remains challenging to create a physiologically relevant human-brain-like environment that would support maturation of human pluripotent stem cell (hPSC)-derived microglia (hMGs). Schafer et al.[1] (*Cell*, 2023) now develop an *in vivo* neuroimmune organoid model with mature homeostatic hMGs for the study of brain development and disease.

Once considered passive bystanders of brain physiology, microglia have emerged as key players in brain homeostasis and disease.[2,3] As immune sentinels of the central nervous system, microglia continuously survey their local environment, mount an immune response to inflammatory stimulation, and clear cellular debris. Moreover, microglia refine neuronal circuitry in development by synaptic pruning, whereas microglial dysfunction is associated with various neurological disorders.[2,3] Although animal models have provided tremendous insights into microglial biology, human-cell-based microglia models have the potential to reveal human-specific features of microglial function.[4] Human pluripotent stem cells (hPSCs), including induced pluripotent stem cells (iPSCs) and embryonic stem cells (ESCs), are an excellent source of cellular models of human origin.[5] Indeed, hPSC-derived microglia (hMGs) have become a popular *in vitro* model of human microglia.[6] However, it remains difficult to obtain mature homeostatic hMGs in the context of a human-brain-like environment. In their recent study published in *Cell*, Schafer et al.[1] achieved remarkable hMG maturation in a physiologically relevant human cellular environment by transplanting hPSC-derived cortical organoids colonized with hMGs into the mouse cortex.

As tissue-resident macrophages, microglia develop in the context of their local microenvironment that is critical for establishing microglial identity.[2,7] However, *in vitro* models of human microglia lack the complexity of the *in vivo* environment that could support their maturation. This limitation may be partially overcome by transplantion of hMGs into the brain parenchyma of transgenic immunocompromised mice, which promotes hMG maturation.[6] Yet, the absence of other brain cell types of human origin prevents the study of physiologically relevant cell-cell interactions between hMGs and non-microglial cells in the chimeric mouse model. Alternatively, hMGs can be introduced into hPSC-

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DECLARATION OF INTERESTS

The authors declare no competing interests.

derived brain organoids that exhibit primitive human brain tissue architecture and cellular diversity.[6,8] Indeed, brain organoids have provided unprecedented access to a human-brain-like environment *in vitro*, and various hMG-containing neuroimmune organoids have been developed.[8] One critical limitation of brain organoids in supporting hMG maturation and long-term survival is a lack of organoid vascularization, leading to internal hypoxia, cellular stress, and necrosis of the organoid core.[8] Consequently, hMGs exhibit non-physiological activation and gradually die in neuroimmune organoids *in vitro*.[1] Although efforts to create vascularized brain organoids *in vitro* are ongoing,[8] it remains challenging to perfuse organoids in a physiologically relevant manner. Recently, transplantation of brain organoids into the rodent brain has emerged as an alternative approach to achieve organoid vascularization and advanced maturation.[9,10] Upon transplantation, brain organoids are robustly vascularized by host animal's vasculature, preventing extensive cell death in the organoid and promoting remarkable neuronal maturation.[9,10] Given the advanced brain organoid maturation *in vivo*, Schafer et al. hypothesized that transplantation of hMG-containing neuroimmune organoids would enhance hMG maturation as well as provide a physiologically relevant human-brain-like environment for hMGs.

To develop the *in vivo* neuroimmune organoid model, Schafer et al. co-cultured hPSC-derived hMG progenitors with cortical organoids to allow hMG progenitors to infiltrate into the organoids; the resulting neuroimmune organoids were subsequently transplanted into the retro-splenial cortex of immunocompromised mice. At 8 weeks post-transplantation (wpt), hMGs exhibited mature ramified morphology, which resembled that of primary human microglia. Moreover, transcriptomic profiling of hMGs at 11 wtp revealed expression of key microglial markers, including *P2RY12*, *TMEM119*, and *SALL1*, indicating homeostatic surveillant microglial state.[2] Gene expression profiling also revealed a cluster of proliferative microglia as well as the absence of non-physiological hMG activation that was apparent in neuroimmune organoids *in vitro*. Notably, the authors used a mouse model that was not humanized for colony-stimulating factor 1 (*CSF1*), required for hMG survival *in vivo*. None-theless, hMGs could survive in the organoid environment *in vivo* for months, which could be explained by high-level expression of the microglial CSF-1 receptor (CSF-1R) ligands *CSF1* and interleukin 34 (*IL-34*) in the brain organoid tissue.

Temporal profiling of hMG gene expression in transplanted neuroimmune organoids revealed gradual hMG maturation, reminiscent of human and mouse microglial development *in vivo*. hMGs progressively gained expression of microglial immune sensome genes as well as human-brain-environment-dependent genes.[4] The brain organoid tissue similarly exhibited transcriptional maturation from fetal to postnatal stages of brain development, indicating the potential application of the *in vivo* neuroimmune organoid model for studying human microglial biology across early human brain development. Interestingly, whereas hMGs transplanted within the organoid environment gained human-specific gene expression, hMGs transplanted directly into the mouse brain parenchyma gained both mouse- and human-specific gene expression. These findings underscore the importance of a physiologically relevant human-brain-like environment to attain human-specific microglial identity.

How does the brain environment modulate microglial function in the context of neurological disorders? To address this question, Schafer et al. transplanted neuroimmune organoids derived from hPSCs of subjects with autism spectrum disorder (ASD), revealing a substantial increase in microglial reactivity as compared to that in neuroimmune organoids derived from neurotypical controls. Notably, the reactive microglial phenotype was evident even if hMGs were derived from hPSCs of neurotypical subjects, suggesting that the altered ASD-specific brain organoid environment was sufficient to elicit the microglial response. Future experiments should also assess how hMG-organoid interactions influence hMG-mediated synaptic pruning as well as neuronal activity, both of which may be altered in ASD.[3]

Finally, the *in vivo* neuroimmune organoid model can be used to study how systemic perturbations to animal physiology impact microglial phenotypes. As a proof of principle, Schafer et al. showed that intraperitoneal administration of bacterial lipopolysaccharide (LPS) triggered organoid-resident hMG activation within 24 h, indicating a rapid hMG response to a pro-inflammatory stimulus. Therefore, the effects of viral infection, blood-derived peripheral factors, and systemically administered drugs on microglial function may be assessed using the *in vivo* model.

In summary, Schafer et al. developed an advanced neuroimmune organoid model with hMGs that function within a physiologically relevant human-brain-like environment, exhibit remarkable maturation, and readily respond to environmental cues. Rapidly evolving brain organoid technology, which now includes the transplantation paradigm, opens new avenues for modeling cell-cell interactions in human brain development and disease. In addition to cortical neuroimmune organoids, diverse specialized organoids may be used to study distinct aspects of human brain physiology[8]; for example, neuroimmune myelin organoids may reveal the roles of microglia in developmental myelination programs. The *in vivo* neuroimmune organoid model will also pave the way for studying microglial dysfunction in neurodegenerative diseases, such as Alzheimer's disease, where neuroinflammation is a key hallmark of disease progression.[2] Overall, this[1] and other[9,10] pioneering studies of brain organoid transplantation mark a new era of modeling brain development and diseases, bringing the best of both worlds—the human origin of hPSC-derived cells and the physiological complexity of animal models—together.

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REFERENCES

1. Schafer ST, Mansour AA, Schlachetzki JCM, Pena M, Ghassemzadeh S, Mitchell L, Mar A, Quang D, Stumpf S, Ortiz IS, et al. (2023). An in vivo neuroimmune organoid model to study human microglia phenotypes. Cell 186, 2111–2126.e20. 10.1016/j.cell.2023.04.022. [PubMed: 37172564]
2. Butovsky O, and Weiner HL (2018). Microglial signatures and their role in health and disease. Nat. Rev. Neurosci 19, 622–635. 10.1038/s41583-018-0057-5. [PubMed: 30206328]

3. Neniskyte U, and Gross CT (2017). Errant gardeners: glial-cell-dependent synaptic pruning and neurodevelopmental disorders. Nat. Rev. Neurosci 18, 658–670. 10.1038/nrn.2017.110. [PubMed: 28931944]

4. Gosselin D, Skola D, Coufal NG, Holtman IR, Schlachetzki JCM, Sajti E, Jaeger BN, O'Connor C, Fitzpatrick C, Pasillas MP, et al. (2017). An environment-dependent transcriptional network specifies human microglia identity. Science 356, eaal3222. 10.1126/science.aal3222. [PubMed: 28546318]

5. Shi Y, Inoue H, Wu JC, and Yamanaka S (2017). Induced pluripotent stem cell technology: a decade of progress. Nat. Rev. Drug Discov 16, 115–130. 10.1038/nrd.2016.245. [PubMed: 27980341]

6. Abud EM, Ramirez RN, Martinez ES, Healy LM, Nguyen CHH, Newman SA, Yeromin AV, Scarfone VM, Marsh SE, Fimbres C, et al. (2017). iPSC-Derived Human Microglia-like Cells to Study Neurological Diseases. Neuron 94, 278–293.e9. 10.1016/j.neuron.2017.03.042. [PubMed: 28426964]

7. Bennett FC, Bennett ML, Yaqoob F, Mulinyawe SB, Grant GA, Hayden Gephart M, Plowey ED, and Barres BA (2018). A Combination of Ontogeny and CNS Environment Establishes Microglial Identity. Neuron 98, 1170–1183.e8. 10.1016/j.neuron.2018.05.014. [PubMed: 29861285]

8. Del Dosso A, Urenda JP, Nguyen T, and Quadrato G (2020). Upgrading the Physiological Relevance of Human Brain Organoids. Neuron 107, 1014–1028. 10.1016/j.neuron.2020.08.029. [PubMed: 32970996]

9. Mansour AA, Gonçalves JT, Bloyd CW, Li H, Fernandes S, Quang D, Johnston S, Parylak SL, Jin X, and Gage FH (2018). An in vivo model of functional and vascularized human brain organoids. Nat. Biotechnol 36, 432–441. 10.1038/nbt.4127. [PubMed: 29658944]

10. Revah O, Gore F, Kelley KW, Andersen J, Sakai N, Chen X, Li MY, Birey F, Yang X, Saw NL, et al. (2022). Maturation and circuit integration of transplanted human cortical organoids. Nature 610, 319–326. 10.1038/s41586-022-05277-w(2022). [PubMed: 36224417]