Cell Stem Cell

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Previews

Microglia modulate neurodevelopment in human neuroimmune organoids

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https://doi.org/10.1016/j.stem.2021.11.005

Dissecting contributions of microglia to human brain development and disease pathogenesis requires modeling interactions between these microglia and their local environment. In this issue of *Cell Stem Cell*, Popova et al. (2021) propose a transcriptomic "microglia report card" and create a neuroimmune organoid to model complex interactions involving human microglia.

As migrants from the earliest hematopoietic progenitor pools, microglia colonize the developing human nervous system during the first trimester (Bennett and Bennett, 2020). It is in this early milieu that radial glia neural stem cells and microglia first meet. Over the decades-long relationship afterward, microglia function as brain resident macrophages and clear dead cells, sculpt neuronal circuits, constantly survey local synapses, and respond quickly to brain injury and inflammation. Human genetics studies reveal that this bond might not always be so productive: microglia uniquely express some neuropsychiatric and neurodegenerative disease risk genes such as *Trem2*, *CD33*, *C4A*, and *CSF1R* (Prinz et al., 2019). While there is tremendous enthusiasm to understand how microglia might contribute to brain development and disease pathogenesis in humans, current experimental models are limited by the exquisite sensitivity of microglia to *ex vivo* conditions and the challenges of recapitulating complex human cell-cell interactions (Bennett and Bennett, 2020). In this issue of *Cell Stem Cell*, Popova et al. (2021) seek to adjudicate the degree to which existing models accurately preserve microglial identity and propose a new brain organoid-based model with engrafted microglia.

The transcriptomic identity of microglia across species is well known. Microglia-specific genes such as *Tmem119*, *P2ry12, Hexb,* and *Sall1* can distinguish microglia from other macrophages (Bennett et al., 2016). Microglial isolation and subsequent culture rapidly downregulate expression of this specific gene module, which can be fully re-induced upon engraftment *in vivo* (Bohlen et al., 2017). In addition to isolated primary cells, several protocols exist for creating human induced pluripotent stem cell (iPSC)-derived microglia (iMG). These approaches recapitulate many features of microglial transcriptomic identity, even more faithfully by xenotransplantation of human iMG into rodent brains (Abud et al., 2017). Like primary mouse cells, engrafted human iMG express microglia-specific genes typically lost *in vitro*. To enable cross-model comparisons, Popova et al. (2021) prepared a "report card" of sorts to compare transcriptomic signatures of human microglia between models, including acutely isolated microglia, serum-free cultured microglia, iMG in culture, and xenografted iMG in mouse brains. The authors showed that the transcriptome profiles are largely conserved, although the degree of microglial signature gene expression, as well as isolation artifacts, vary. Using acutely isolated fetal human microglia as the gold standard for comparison, they demonstrate the extent to which transcriptomically defined microglial states are represented by single-cell RNA sequencing, such as "homeostatic," axon-tract associated, and cytokine-associated microglia (Figure 1A). This axon-associated subset expresses genes, such as *Spp1* and *Lgals3,* appears in the developing nervous system, and in rodents is linked to oligodendrocyte development and myelination. In contrast, the cytokine-associated microglia subset expressing high levels of *TNFA* and *IL1B* is unique to human microglia and exists in the absence of immune stimulation. While further studies are required to see if these transcriptional states are truly functionally distinct, such an unbiased approach to understanding which pathways and genes are differentially regulated is a powerful resource for seasoned microgliologists and newcomers alike.

Brain organoids provide incredible insight into the complex development process of the human nervous system (Qian et al., 2019). By allowing for differentiation and self-organization of developing neural tissue from pluripotent stem cells in 3D, brain organoid models enable access to human disease models and phenotypes in a way not possible with traditional approaches (Qian et al., 2018). Microglia are not normally represented in forebrain organoid differentiation protocols, owing to their origins as mesodermal lineage cells. To model complex neural-microglial interactions, Popova et al. (2021) engrafted primary isolated human microglia into forebrain organoids at 5 weeks of differentiation, a time point that is both technically convenient and mirrors the arrival of microglia to the developing human nervous system. In the absence of exogenous growth factors, microglia engrafted and survived within organoids, likely from the respective expression of *CSF1*, *TGFβ*, and *IL34* by

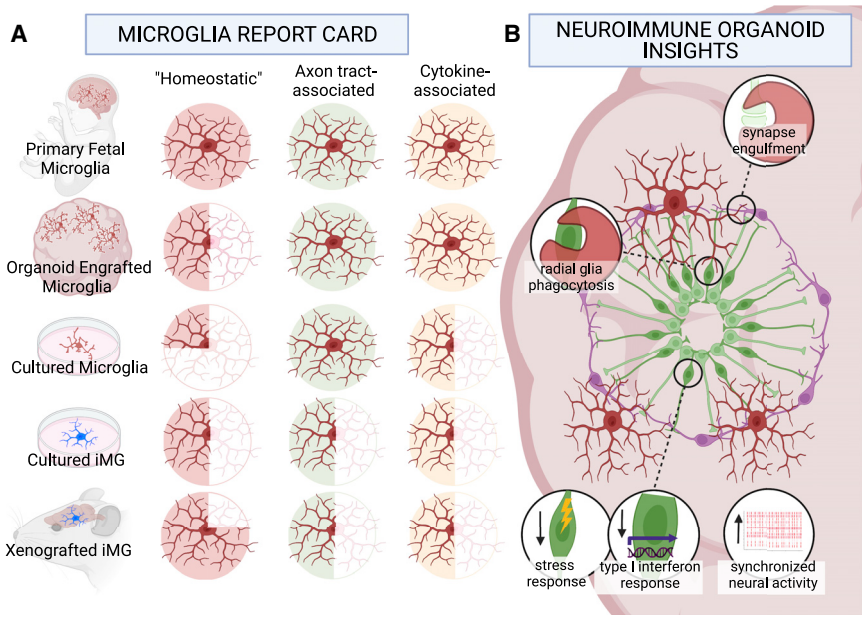

Figure 1. A microglia report card and insights from the neuroimmune forebrain organoid.
(A) Comparison by single-cell RNA-seq of fetal human microglia in culture or engrafted in 3D human forebrain organoids, with iPSC-derived microglia (iMG) in culture and xenografted into rodent brains. The "Microglia Report Card" depicts the relative score of these microglia compared to acutely isolated fetal human microglia ("Primary Fetal Microglia") based on a composite score calculated by the authors. It is depicted here as partially filled microglia, with stronger colors showing more faithful representation of microglia subsets expressing "homeostatic," "axon-tract associated," and "cytokine-associated," transcriptomic signatures in each condition.
(B) Neuroimmune organoid insights provided by Popova et al., (2021) including the function of forebrain organoid engrafted microglia in synapse and radial glia engulfment, decreased DNA damage and interferon signaling in radial glia and proliferating cells, and increased synchronized neuronal activity. Schematic created with Biorender.com.

radial glia, dividing progenitor cells, and excitatory neurons. These microglia are highly motile and engulf progenitor cells and synaptic components, much like their *in vivo* counterparts (Figure 1B). Microglia in brain organoids score high marks on the transcriptomic identity "report card" compared with those in 2D cultures, including representation of the unique cytokine-associated microglia subset. This neuroimmune organoid model revealed that the presence of microglia decreases interferon-response-related gene expression in radial glia. Normal human cortical organoids exhibit cellular stress, which is abated by xenotransplantation of organoids into rodent brains, a scenario that results in engraftment of rodent microglia (Bhaduri et al., 2020). The neuroimmune organoid model demonstrates that the presence of microglia attenuates previously reported markers of cellular stress in radial glia and proliferating cells. Finally, the authors show that microglia promote maturation of neuronal networks in organoids as measured by synchronized activity on multi-electrode arrays.

The molecular mechanisms underlying the influence of microglia on different cell types, the stress state, and neuronal maturation in brain organoids remain to be determined, but these initial phenotypic observations provide a glimpse into the types of discoveries possible with a human neuroimmune organoid. For example, manipulation of candidate cues arising from neural progenitor or immature neuron could aid the identification of the key factors that dictate the unique homeostatic signature of microglia that is downregulated in diseases. The extent to which microglia contribute to early human cortical patterning is also a critical unanswered question in neurodevelopment. Engraftment of microglia into assembled brain organoids could test hypotheses generated from rodent studies which suggest that human microglia regulate interneuron positioning and dopaminergic axon pathfinding and enable mechanistic studies. The model may also provide powerful insights on the impact of early viral infection, as with Zika virus and HIV (Fan et al., 2021), on brain development that cannot be discovered with traditional culture methods. Finally, future work using engineered and patient-derived cells promise critical insights into neurodevelopmental disorders and pathogenesis of neurodegeneration. Overall, the idea of a microglial report card will serve a rapidly growing field well as a metric against which others can compare existing or new models of human microglia. The human neuroimmune organoid is a framework upon which many cell autonomous and cell-cell interactions can be further investigated.

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