

Immune response in autism, revealed by in-vivo studies of xenotransplanted human brain organoids

Brain organoids facilitate the study of cell-cell interactions in a human-brain-like micro-environment but cannot form the primary immune cells of the central nervous system, microglia, on their own. Researchers have now transplanted forebrain organoids, colonized by stem-cell-derived microglial cells, into mice brains. And indeed, the stem-cell-derived microglial cells developed human-microglia-like transcriptomic and functional characteristics. This made it possible, for the first time, to study in-vivo immune responses in organoids derived from stem cell lines from patients with ASD and macrocephaly.

By Rohini Subrahmanyam

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New Ground article reviewed by: Simon T. Schafer and Fred H. Gage

Microglia, the primary immune cells of the central nervous system, play an important role in human brain development and disease. To study this role in detail, researchers use both two-dimensional cultured microglia systems and three-dimensional brain organoids, i.e., simplified stem-cell-derived in-vitro versions of brains, measuring a few millimeters in diameter. Nevertheless, our understanding of microglia's role in several brain disorders, autism spectrum disorder (ASD) being one of them, is still quite limited.

This is chiefly due to the lack of model systems that can effectively replicate the interactions between microglia and surrounding cells, like neurons and other glia, in the human brain. Two-dimensional systems fail to mimic human microglia in terms of their gene expression profile, presumably because of the absence of other human brain cell types around them. Researchers can also study the development of microglia derived from human pluripotent stem cells (HPSCs) that

are transplanted directly into the brains of immunocompromised mice. But again: The absence of a natural human environment greatly limits the insights that can be gained from doing so.

Three-dimensional brain organoids more closely resemble the actual brain, with a more comparable tissue architecture and a broader variety of cell types. But microglia still have to be added: They are of non-ectodermal origin, meaning they do not develop in the brain itself. Instead, they are known to originate from yolk-sac-derived erythromyeloid progenitor cells (EMPs) that migrate into the brain only at around the fifth gestational week. In the lab, this process is replicated by creating microglia from HPSCs and incorporating them into existing human brain organoids.

But crucial questions are still largely unanswered. We know little about whether and how HPSC-derived microglia mature *in vitro*, let alone *in vivo*. Do they actually resemble microglia in the human brain in terms of their genes and functions? Do they act and fulfil their roles like they do in actual human brains?

Xenotransplantation of brain organoids into brains of immunocompromised mice

To find answers, Simon Schafer, Abed AlFatah Mansour, and an international team led by Fred H. Gage took a new approach and published their findings in *Cell*: “An *in vivo* neuroimmune organoid model to study human microglia phenotypes.” First, they co-cultured stem-cell-derived microglia progenitor cells (EMPs) with human cerebral organoids and assessed the EMPs’ ability to colonize the organoids. Secondly, they used their previously developed xenotransplantation technique, published in *Nature* (see here: <https://doi.org/10.1038/nbt.4127>), to transplant these organoids into the brains of immunocompromised mice and study their development over time.

The international team found that the EMPs efficiently integrated into the organoids and eventually developed into cells that morphologically, functionally, and in terms of their transcriptomes closely resembled microglia in the actual human brain. The researchers also observed that the microglia in the organoids responded to environment-induced lesions and that they played a role in the brain-environment-induced immune response in a patient model with ASD and macrocephaly.

Microglial progenitor cell colonization of organoids

For their study, the researchers decided to grow forebrain organoids, hypothesizing that these would allow microglial differentiation. To facilitate visual distinction between microglia progenitor cells and the organoid tissue, they grew populations of CD43⁺ EMPs by using stem-cell lines that expressed a red fluorescent protein, tdTomato (tdT). The stem cells from which they grew the organoid tissue, in contrast, expressed the green fluorescent protein (GFP).

The team found that the EMPs efficiently migrated into and robustly colonized the cortical organoids, displaying rudimentary ramified-like morphologies – in higher numbers than in the case of 2D cultured microglia and more similar to those in an actual brain. Single-cell RNA sequencing (scRNA-seq) data on the EMPs revealed that the cells’ transcriptome resembled that of human fetal microglia. The researchers noticed, however, that even after a longer period of differentiation, the microglia didn’t express markers that would indicate their maturity or their having reached a homeostatic state. The appearance of necrotic cores within cerebral organoids, they assumed, was not favorable for long-term co-culture, hence preventing the microglia from reaching their fully mature state.

Xenotransplantation of human brain organoids into mice brains

To address these issues, the researchers transplanted the EMP-containing immunocompetent organoids into the brains of immunocompromised mice. By doing so, they obtained a

xenotransplanted immunocompetent human brain organoid (iHBO) system that would not be rejected by its host and allow *in-vivo* studies. After transplantation, the immature microglial cells migrated even further into the organoid tissue but did not migrate into the host brain. More importantly, around 8 weeks post transplantation, the microglia began expressing mature microglial markers and morphologies as well. scRNA-seq profiling of the microglia revealed that they expressed human-specific transcripts of mature microglial markers like TMEM119, P2RY12, and SALL1. This helped confirm that tdT⁺ human microglia cells (hMGs) with characteristics of mature homeostatic microglia were now present in the immunocompetent human brain organoids.

To also study the hMGs' transcriptomic development, the researchers performed scRNA-seq at different points in time – 6, 11 and 24 weeks post transplantation – and found that the cells continuously upregulated homeostatic and microglia-specific genes like TMEM119, TLR4, and CX3CR1, to name a few.

Upon comparing the scRNA-seq data on the hMGs with recently published scRNA-seq data on actual microglia in the developing human brain, obtained during the 9th and 18th gestational week, they also found that the former showed steadily increasing expression of human-brain-environment-specific genes. To test the extent to which the human-brain-like environment affects the shaping of microglial identity, the team compared the transcriptomic signatures of hMGs in the transplant with those of stem-cell-derived hMGs that were directly transplanted into a mouse brain. And indeed, the former responded to their human-brain-like micro-environment with a higher expression of many more human-specific microglia genes.

Microglia sensing and reacting to perturbations

In addition to identifying transcriptomic similarities, the researchers then proceeded to check for functional similarities between hMGs and actual microglia. Upon using 2-photon fluorescence imaging to study the hMGs *in vivo*, the team found that, similar to resting-state human microglia, the hMGs were highly ramified and displayed multiple remarkably motile processes, or elongated extensions, as would be expected in an actual brain. Through continuous extension and retraction of the processes, they appeared to be constantly engaged in surveying the surrounding environment. Additionally, the density of hMGs increased from 12 to 24 weeks post translation, which is consistent with what has been observed in naturally developing brains.

Subsequently, the researchers locally applied 2-photon-induced focused laser lesions, or perturbations, to the organoids. They observed a rapid response, with the cells almost immediately extending their processes toward the injury site in a directed manner. The distance and speed with which the processes travelled to reach the injury site gradually decreased over the course of their development, while the density of the cells increased, hence indicating that at different points in the brain's development, the microglia will respond differently to any disruptions in their environment.

Would the cells also respond to systemic inflammatory cues? To assess this aspect, the researchers administered LPS, the bacterial cell-wall endotoxin lipopolysaccharide. It is known that, when binding to the microglia's pattern-recognition receptor TLR4, LPS triggers a cascade of downstream immune responses in them. When subjected to higher doses of LPS, the hMGs exhibited profound morphological changes, with rounded morphologies, a lack of characteristic primary processes, and an increase in the number of filopodia – all of which would be expected in an actual brain as well. Hence, in the model, the hMGs appeared to be not just transcriptionally, but also functionally similar to true microglia in the human brain, constantly monitoring their environment and capable of responding to particular pathological and physiological cues.

Studying microglia response to an autistic brain environment

Finally, and for the first time, the researchers used their organoid model with hMGs to study *in vivo* how a specific neurodevelopmental condition affects the crosstalk between microglia and their brain environment. Their choice fell on ASD, a complex disorder with a very heterogeneous set of phenotypes. Specifically, they were looking at ASD in combination with the phenotype macrocephaly, which was well characterized in a previous study by Schafer, Mansour, Gage, and others (see here: <https://www.nature.com/articles/s41593-018-0295-x>). Using isogenic induced pluripotent stem cell lines from patients with ASD and macrocephaly and from neurotypical control subjects, they created GFP+ organoids and tdT+ EMPs and integrated the latter into the former, for the patients and controls alike. The ASD and control organoids were then transplanted into the brains of mice to study the development of hMGs. True to their immune-sensing nature, the microglia derived from ASD patients appeared to be better suited to mounting an immune response, with larger soma and more filopodia.

To confirm that the changes in the hMGs were due to their brain environment rather than their innate genetic disposition, the researchers also generated neurotypical control and ASD organoids, both harboring neurotypical hMGs. Their observations that the hMGs reacted to the ASD brain environment but not to the control brain environment confirmed that this behavior was actually driven by the ASD brain environment and not the microglia themselves.

Summary and outlook

Schafer, Mansour, and Gage's team carefully characterized human microglia in an immunocompetent brain organoid model over the course of their development, finding that the hMGs not only displayed transcriptional similarities to their counterparts in the human brain but were also capable of sensing and responding to perturbations in the environment via their morphological processes. The researchers also described the first experimental evidence of disease-associated immune responses in hMGs triggered by specific changes in the brain environment of patients with autism spectrum disorder and macrocephaly.

Some limitations are duly noted. Larger groups of ASD patients and controls are needed to confirm the observed effects, as the researchers concede in their publication. Furthermore, they cannot yet rule out the possibility that the organoids' host mice brains influence the physiology of the cell types being investigated.

Most importantly, however: The team explored a new way to study *in vivo* – at least in brain organoids transplanted into mice brains – the crosstalk between microglia, neurons, and other types of glia in development and disease. For potential therapies, a better understanding of cell-cell interactions in the brain and how they lead to various brain diseases is crucial. Looking ahead, experimentation with even more advanced human brain organoids that also mimic the natural brain environment by developing their own vasculature could further advance this field of research. ●

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