News Release

Title

Identification of the “new route” of microglial colonization into the brain

Key Points

- **The research group found a key gateway system of microglial colonization into the mouse embryonic brain.**
- **E12.5 brain allows intraventricular macrophages to efficiently enter the brain.**
- **Infiltrated macrophages into the brain thereafter acquire microglial properties.**
- **Establishment of *ex utero* intravital imaging system for E12.5 mouse embryos**

Summary

In this study, Lecturer Yuki Hattori and Professor Takaki Miyata in Department of Anatomy and Cell Biology, Nagoya University Graduate School of Medicine (dean: Hiroshi Kimura, M.D., Ph.D.), demonstrated the mechanism underlying how microglia, the resident immune cells in the brain, colonize the brain in the embryonic stage.

The brain, which controls the various functions in the whole body, is constituted not only by neural lineage cells but also other cell types, such as the immune cells and vascular-composing cells. These multiple cell types collaboratively work so as to maintain the brain function. Microglia are the immune cells in the central nervous system. These cells play multiple functions from embryonic to adult stage, such as patrolling the brain structure to maintain the environmental homeostasis, supporting the differentiation and positioning of neural lineage cells, and regulating neuronal network. However, when and how microglia colonize the brain remain to be elucidated.

Previous studies have revealed that microglia originate from erythromyeloid progenitors (EMPs) in the yolk sac. Yolk sac EMPs also generate macrophages, which are localized at the interface between the brain primordium and the surrounding vasculature-associated system. These two cell types have been suggested to segregate their fate decision early in development at yolk sac. However, the research group demonstrated that brain microglia are not only derived from yolk sac progenitors committed early but

also supplied later during brain development/maturation from macrophages through the newly established intravital imaging system for mouse embryos and cell tracking methods. They found that macrophages originally localized in the ventricle infiltrate the brain, and these infiltrated macrophages subsequently differentiate into microglia in response to the surrounding environmental factors in the brain. In other words, the group found that the brain is equipped with the “additional route” to supply some microglial populations.

Recent studies using single-cell analysis have elucidated the spatial and developmental heterogeneity of microglia in the developing mouse brain. Their findings shed light on the possibility that differences in microglial colonization routes or timing of entry into the pallium could be among the reasons for the diversity in microglial characteristics.

Recently, multiple studies reported that elevated maternal inflammation during pregnancy is associated with the emergence of separate psychological outcomes in their offspring later in development. Understanding for the behavior of microglia in the physiological stage is important to investigate the abnormality of microglia in the inflammatory state, and will contribute to the establishment of the novel preventive and therapeutic platform against fetal brain disfunction in the future.

This study was performed with the cooperation of Professor Hiroaki Wake and Lecturer Daisuke Kato in Department of Anatomy and Molecular Cell Biology, Nagoya University Graduate School of Medicine, Associate Professor Hiroyuki Konishi in Department of Functional Anatomy and Neuroscience, Nagoya University Graduate School of Medicine, Professor Ayano Kawaguchi in Okayama University, Professor Takahiro Masuda in Kyushu University, and Professor Marco Prinz in Freiburg University.

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1. Research Background

The brain, which controls the various functions in the whole body, is constituted not only by neural lineage cells but also other cell types, such as the immune cells and vascular-composing cells. These multiple cell types collaboratively work so as to maintain the brain function. Microglia are the immune cells in the central nervous system. These cells play multiple functions from embryonic to adult stage, such as patrolling the brain structure to maintain the environmental homeostasis, supporting the differentiation and positioning of neural lineage cells, and regulating neuronal network. However, when and how microglia colonize the brain remain to be elucidated.

2. Research Results

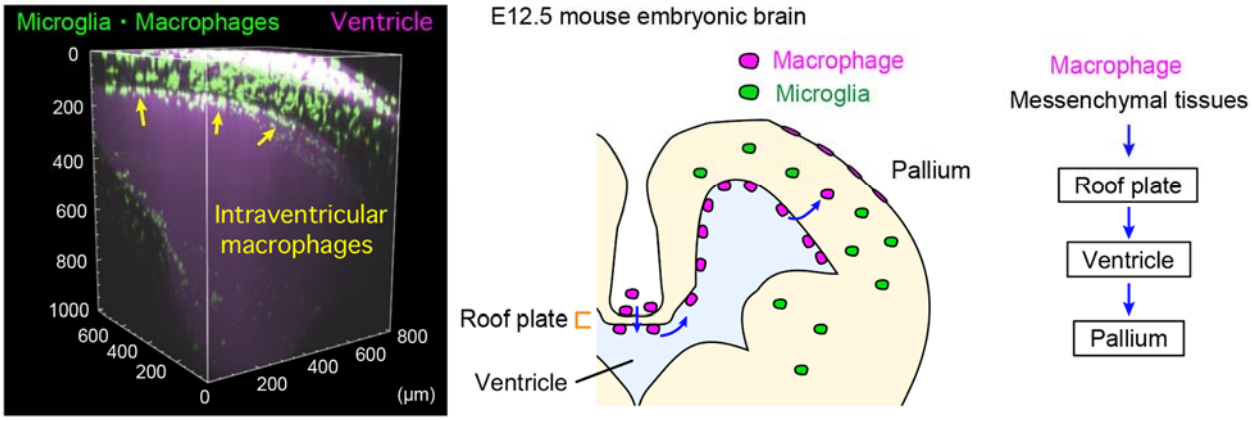
Previous studies have revealed that microglia originate from erythromyeloid progenitors (EMPs) in the yolk sac. Yolk sac EMPs also generate macrophages, which are localized at the interface between the brain primordium and the surrounding vasculature-associated system. These two types of cells have been suggested to segregate their fate decision early in development at yolk sac. However, the research group demonstrated brain microglia are not only derived from yolk sac progenitors committed early but also supplied later during brain development/maturation from macrophages through the newly established intravital imaging system for mouse embryos and cell tracking methods. They found that macrophages originally localized in the ventricle infiltrate the brain, and these infiltrated macrophages subsequently differentiate into microglia in response to the surrounding environmental factors in the brain. In other words, the group found that the brain is equipped with the “additional route” to supply some microglial populations.

(1) The infiltration of intraventricular macrophages into the pallium at E12.5

First, the research group found that microglia and macrophages are intermingled in the pallium in the early embryonic mouse brain, such as at E12.5 and E13.5, whereas these cells show distinct distribution pattern at E14.5. To test whether macrophage in the pallium are supplied from the surrounding tissue, the research group performed live imaging of macrophages/microglia in cultured brain slices. They found that macrophages attached to the ventricular surface frequently enter the pallium at E12.5. The infiltration frequency was the highest at E12.5, but it was dramatically reduced at E13.5 and E14.5, suggesting that E12.5 is the most prominent stage of the infiltration of intraventricular macrophages during the period tested.

Furthermore, the group also found that macrophages transmigrate the midline roof plate from the mesenchymal side toward the ventricle. These results demonstrated that the “roof plate→ventricle→pallium” route is an essential path for microglial colonization into the embryonic mouse brain (**Figure 1**).

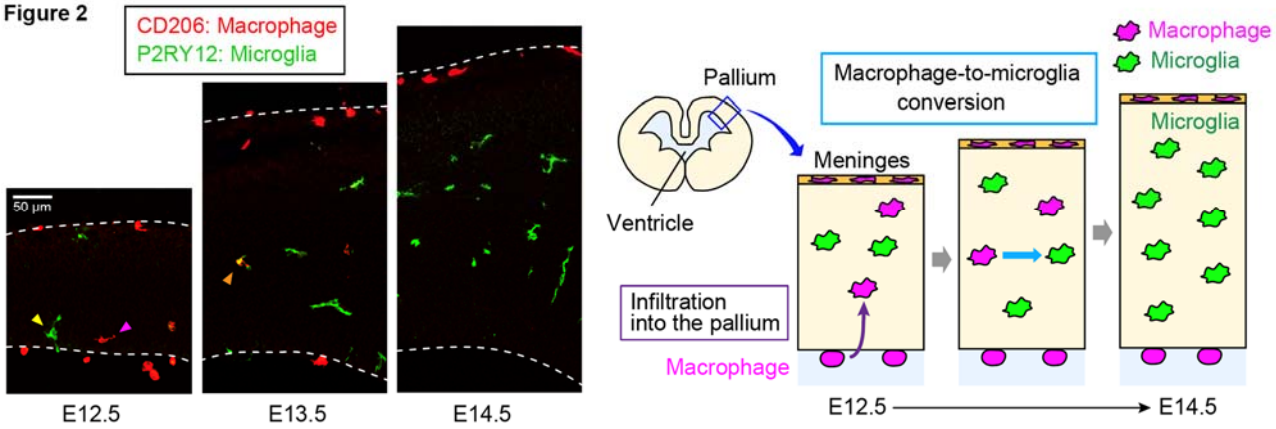
Figure 1



(2) Infiltrated macrophages in the pallium differentiate into microglia

To next investigate whether intraventricular macrophages that have infiltrated the pallium differentiate into microglia, isolated GFP-labeled macrophages derived from genetically modified mice were transplanted into the ventricle of wild-type mice. Two days after transplantation, the GFP^+ cells that stayed in the ventricle still maintained their macrophage properties, whereas those that had entered the pallium acquired microglial properties. These data suggest that once macrophages enter the pallium, they can transform into microglia. In addition, the group also performed *in vivo* cell tracing method for intraventricular macrophages using a Flash tag system. The data demonstrated a clear contribution of the labeled intraventricular macrophages to the microglial population. Taken together, these results strongly support the model of the conversion of postinfiltrated macrophages into microglia in the pallium, which is probably induced by environmental signals in the pallium (**Figure 2**).

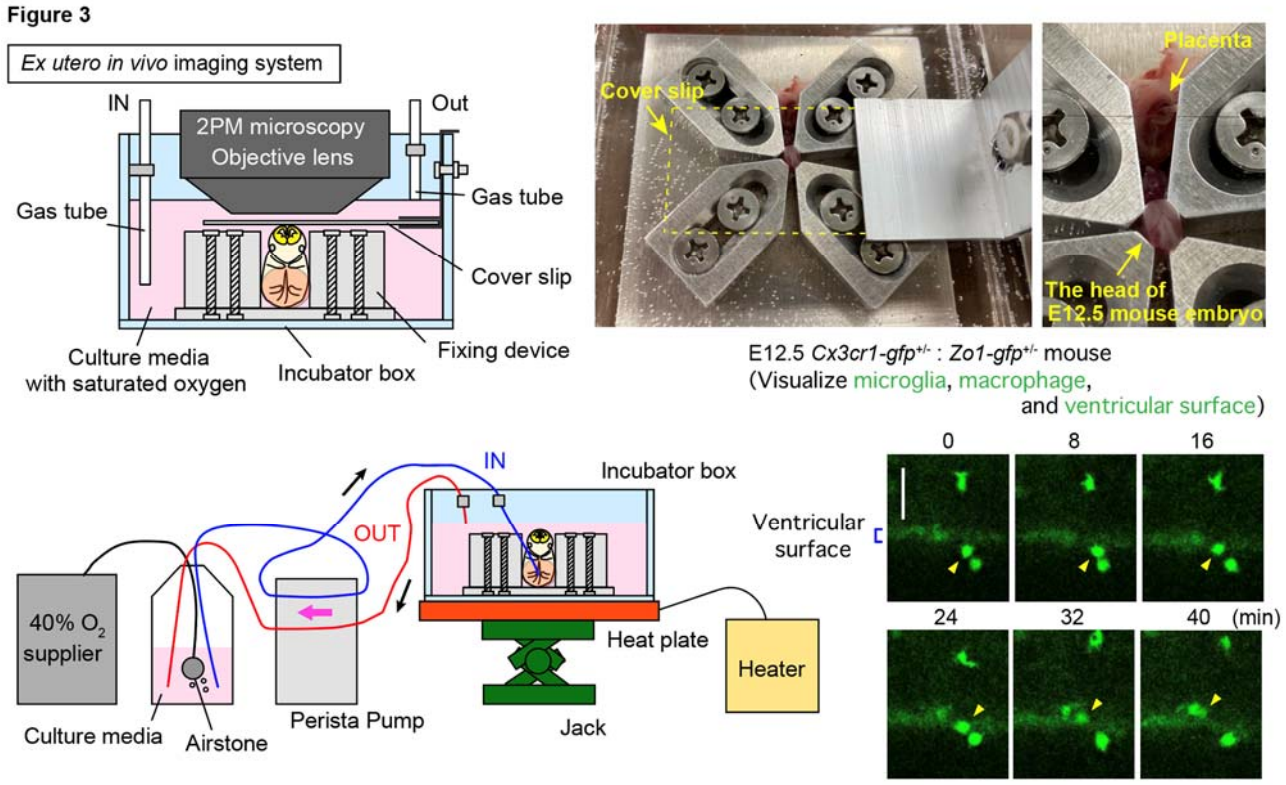
Moreover, to separately evaluate the proportion of microglia derived from extraparenchymal macrophages, the research group performed a fate mapping analysis. The result revealed that about one sixth amount of microglia at E14.5 are supplied by macrophages.



(3) Intravital imaging of mouse embryos to detect macrophage infiltration

Finally, to further and directly confirm the infiltration of the intraventricular macrophages into the embryonic mouse brain wall *in vivo*, the research group performed intravital imaging of E12.5 mouse embryos. They previously established an *in utero in vivo* imaging system for the E14.5 mouse embryos through preparative surgical treatments to mobilize the uterine horn (Hattori et al., *Nat Commun.*, 2020). However, this system could not be applied for E12.5 embryos because the E12.5 embryos were extremely small and therefore not suitable for fixation inside the amniotic membrane. Thus, the research group separately established a new *ex utero* intravital imaging system

for E12.5 embryos using two-photon microscopy. Notably, intravital imaging demonstrated that macrophages originally positioned in the ventricle infiltrated the pallium at E12.5. Thus, this *in vivo* observation confirms that intraventricular macrophages enter the pallium in the early embryonic stage and contribute to the microglial population (**Figure 3**).



3. Future Perspective

They found that macrophages originally localized in the ventricle infiltrate the brain, and these infiltrated cells subsequently differentiate into microglia in response to the surrounding environmental factors in the brain. Recent studies using single-cell RNA-sequence analysis have elucidated the spatial and developmental heterogeneity of microglia in the developing mouse brain. Their findings shed light on the possibility that differences in microglial colonization routes or timing of entry into the pallium could be among the reasons for the diversity in microglial characteristics. The investigation which clarifies whether differences in the origin of microglia affect their functions should be conducted in the future.

Recently, multiple studies reported that maternal excessive immune activations (in the state of infection, nutritional status, and environmental factors) are associated with the emergence of separate psychological outcomes in their offspring later in development. Understanding for the behavior of microglia in the physiological stage is important to investigate the abnormality of microglia in the inflammatory state, and will contribute to the

establishment of the novel preventive and therapeutic platform against fetal brain disfunction in the future.

4. Acknowledgements

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5. Publication

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Title: CD206^{+} macrophages transventricularly infiltrate the early embryonic cerebral wall to differentiate into microglia

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