News Release

Title

Embryonic neocortical microglia express Toll-like receptor 9 and respond to plasmid DNA injected into the ventricle: technical considerations regarding microglial distribution in electroporated brain walls

Key Points

- **In the brains subjected to *in utero* electroporation (IUE), which is widely used for *in vivo* gene-transfer in neurodevelopmental studies, intra-ventricularly administered plasmid DNA caused microglial aberrant accumulation along the luminal surface of the cerebral wall and in the choroid plexus.**
- **Co-administration of Toll-like receptor 9 (TLR9) antagonist into the ventricle together with plasmid DNA restored the normal dispersed localization of microglia in the mid-embryonic cortex, indicating that microglial response is mediated by TLR9.**
- **Our results strongly suggest that the behavior of microglia in brain primordia subjected to IUE should be carefully interpreted.**

Summary

In this study, Professor Takaki Miyata and Postdoctoral Fellow Yuki Hattori in Nagoya University Graduate School of Medicine (dean: Kenji Kadomatsu, M.D., Ph. D.) demonstrated that microglia, the resident immune cells in the central nervous system, showed abnormal distribution pattern in the cerebral wall and choroid plexus of the brains subjected to the *in utero* electroporation (IUE), which is widely used for *in vivo* gene-transfer in neurodevelopmental studies.

Microglia, despite being only a minor population of the cells that constitute the cerebral wall, promote the differentiation of neural progenitor cells by extensively surveying the developing cortex. Although microglia are normally distributed homogeneously throughout the mid-embryonic cortical wall with only limited luminal entry, the intraventricular presence of exogenously derived plasmid DNAs induced microglia to accumulate along the apical surface of the cortex and aggregate in the choroid plexus. This effect was independent of capillary needle puncture of the brain wall or application of electrical pulses. The microglial response occurred at plasmid DNA concentrations lower than those routinely used for IUE, and was mediated by activation of Toll-like receptor 9 (TLR9), an innate immune sensor that recognizes unmethylated cytosine-phosphate guanosine (CpG) motifs abundant in microbial DNA. Administration of plasmid DNA together with oligonucleotide (ODN) 2088, the antagonist of TLR9, restored the normal (dispersed) intramural localization of microglia and decreased luminal accumulation of these cells. Thus, via TLR9, intraventricular plasmid DNA administration causes aberrant

distribution of embryonic microglia, suggesting that the behavior of microglia in brain primordia subjected to IUE should be carefully interpreted.

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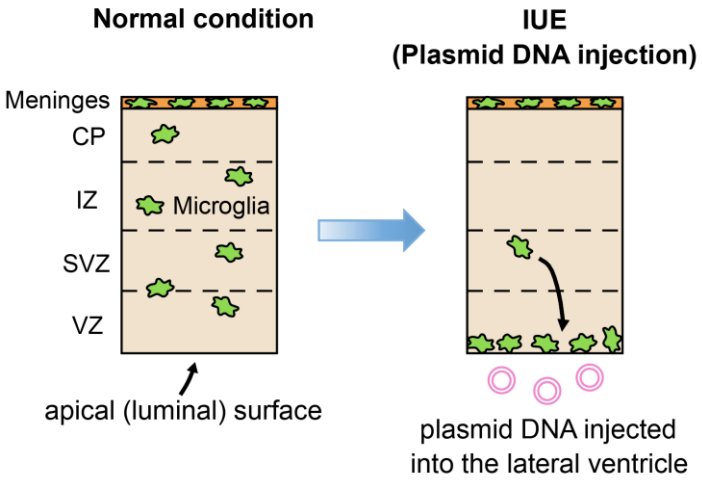
Research Background

The IUE technique established in 2001 is very useful for labeling and genetic modification of neural lineage cells of embryonic mammalian brains. In this method, plasmid DNAs or RNAs are injected into the lateral ventricle of the fetal brain *in utero* using a glass capillary needle, and then electrical pulses are externally applied across the embryo's head. This technique enabled us to easily manipulate gene expression *in vivo* such as overexpressing or suppressing the target genes. In trials of dual imaging approach of microglia and neural progenitor cells, however, the research group unexpectedly found that conventional IUE of the embryonic mouse cerebral wall markedly altered microglial distribution in the cortex. In this study, the biological mechanisms underlying abnormal microglial distribution were investigated.

Research Results

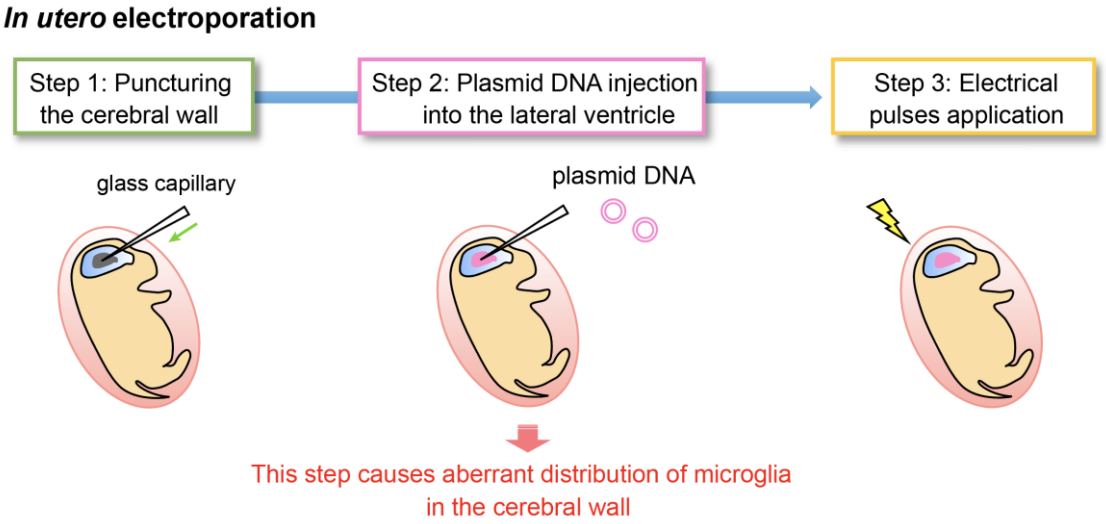
Normally at embryonic day (E) 14, microglia are distributed diffusely throughout the pallium along the radial (ventricle-to-pia) axis, and are found in the ventricular zone (VZ), subventricular zone (SVZ), and intermediate zone (IZ). In brains subjected to IUE, however, microglia were extremely scarce in both the SVZ and IZ and aberrantly accumulated along the apical surface (Figure 1). IUE-applied brains also exhibited densely accumulated microglia in the choroid plexus and the ventricle, whereas no such massive luminal infiltrations were observed in non-IUE controls.

Fig. 1



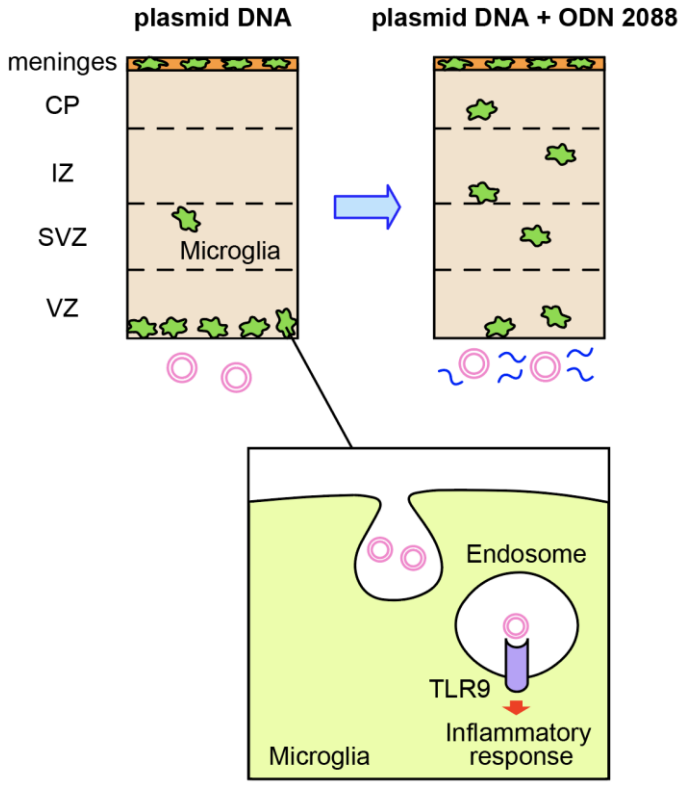
To determine which of the steps involved in IUE (1, puncturing the cerebral wall with a glass capillary needle; 2, injection of plasmid vector DNA into the lateral ventricle; 3, electrical pulses) causes microglial aberrant accumulation, the distribution of microglia between embryos subjected to each of these procedures were separately examined. The results strongly suggest that the presence of exogenously sourced plasmid DNAs in embryonic mouse ventricle caused abnormal microglial distribution (Figure 2).

Fig. 2



It is known that Toll-like receptor 9 (TLR9) recognizes unmethylated cytosine-phosphate guanosine (CpG) motifs, which are abundant in bacterial and viral DNAs. When mammalian cells intracellularly expressing TLR9, such as macrophages and dendritic cells, sense microbial DNAs, they elicit immune response with various inflammatory cytokine production. Based on the gene expression analysis revealing that embryonic microglia highly expressed TLR9, oligonucleotide (ODN) 2088, the antagonist of TLR9, was co-injected with plasmid DNA into the ventricle. The research group found that administration of plasmid DNA together with ODN 2088 partially restored the normal intramural localization of microglia (i.e., the number of microglia accumulated near the apical surface in the VZ was significantly decreased, whereas the number of those positioned in the SVZ/IZ increased), and also decreased luminal accumulation of these cells (Figure 3). These results strongly suggest that microglia expressing TLR9 may sense intraventricularly injected plasmid DNA and subsequently accumulate near the apical surface in the VZ and in the choroid plexus. Furthermore, we confirmed that performing IUE in the presence of ODN 2088 enabled us to monitor microglia by live-imaging in slice cultures with their almost normal behaviors.

Fig. 3



Further, the research group proposed that the presence of bacterial endotoxin, lipopolysaccharide (LPS), in plasmid preparations might influence the distribution of embryonic microglia as these cells highly expressed TLR4, a receptor for LPS. Since plasmid DNA is purified from *E. coli.*, endotoxin, the component of the bacterial cell wall, might be contaminated in plasmid DNA solution and the level of its contamination is dependent on the purification method used. LPS itself disturbed microglial localization in the brain in a separate manner from TLR9 signaling, indicating that LPS contamination in plasmid DNA solution should be also carefully interpreted.

Research Summary and Future Perspective

The research group showed that injection of plasmid DNA into the lateral ventricle for IUE induced microglia to accumulate near the luminal surface and aggregate in the choroid plexus through TLR9 recognition. In addition, LPS contaminated in plasmid DNA solution has been shown to have a potential to elicit microglial response. These facts indicate that the experimental results might be influenced depending on the amount of plasmid DNA used for IUE and that of LPS contained in the plasmid DNA solution. Although microglia are a minor population of the cells composing the cerebral wall, these cells contribute to the differentiation of neural progenitors by extensively surveying the developing cortex. Therefore, the microglial abnormal behavior in the IUE-applied brains should be interpreted with caution. Further, co-injection of a TLR9 antagonist (ODN 2088) into the ventricle along with plasmid DNA restored the normal, dispersed localization pattern of microglia, suggesting that it might be helpful for avoiding microglial aberrant localization in IUE-applied brains. These findings may contribute to better understand for the mechanisms of brain development and neurogenesis.

Publication

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