Neuritogenic function of microglia in maternal immune activation and autism spectrum disorders

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Autism spectrum disorder and maternal immune activation: Environmental factors during pregnancy, such as infections, maternal stress or autoimmunity, are closely associated with the prevalence of neurodevelopmental disorders including autism spectrum disorder (ASD), bipolar disorder and schizophrenia. It has been shown that severe infections during pregnancy can cause perturbations in maternal immune activation (MIA) and significantly increase the risk of ASD in the offspring although the mechanism is poorly understood. Many rodent MIA studies support this causal link by showing that offspring of dams administered with polyinosinic-polycytidylic acid (poly:C), a viral mimic Toll-like receptor 3 agonist, exhibit long-lasting ASD-like behavioral abnormalities such as increased repetitive behavior, impaired social interaction and communication. Interestingly, MIA also induces neuroinflammation and persistent immune dysregulation in the offspring. This is due to the generation of inflammatory cytokines persisting through development and adulthood in the brain of the offspring, suggesting that chronic immune dysfunction plays a role in mediating the deleterious effects of MIA on neurodevelopment (Garay et al., 2013).

Microglial role in synaptogenesis: Recent studies suggested that microglia, the primary innate immune cells in the brain, are potential key players in mediating the effects of MIA-induced neurodevelopmental changes and aberrant synaptogenesis (Mattel et al., 2017). Detailed microglial influence on the MIA phenotype in the offspring is yet to be elucidated. Deregulated microglial cells act as a warning system to the brain insult to ensure the clearance and regeneration of neuronal cells. They are also critical in brain development and maturation, such as clearing apoptotic cells, supporting neurogenesis and neuronal circuitry through the synaptic formation and pruning (Miyamoto et al., 2016). Microglia migrate from the yolk sac into the developing brain around E9.5 in mice. Their number in the brain dramatically increases in early postnatal life, reaching a maximum by postnatal day 18 (P18) (Pattison et al., 2016) and continue to mature and accelerate during the first postnatal week and reaches to a maximum level by postnatal day 30 (P30) (Sheng et al., 2008). Migration of microglia actively support the early synaptogenesis through their interaction with neurons. Deficiencies in microglial neurotrophic factors, results in deficits in learning-related synapse formation. Deletion of microglial phagocytic genes, such as fractalkine microglial receptor (Cx3cr1) or programlin, leads to altered synaptogenesis and neural circuitry, and enhancement of repetitive behavior. These studies suggest that the intricate positive and negative regulation of synaptic network by microglia is critical for brain development and functions.

Neuritogenic function of microglia in MIA: In our recent paper, “Inhibition of colony stimulating factor 1 receptor (CSF1R) corrects maternal inflammation-induced microglial and synaptic dysfunction and behavioral abnormalities” (Ikekuzi et al., 2020), we uncovered novel roles of microglia in the pathogenesis of MIA and identified microglia as a promising therapeutic target. In this study, we depleted and repopulated microglia in MIA offspring and showed that inhibitory microglia significantly lowered the functional deficits during the juvenile period following MIA induction at E9.5 to determine the effect of immune perturbed microglia on offspring development. Strikingly, depletion and repopulation of microgliaameliorated MIA-induced behavioral abnormalities, including social deficits and repetitive behavior, as determined by social interaction and self-grooming tests at P60. To understand the underlying molecular mechanisms, we performed RNA-sequencing of freshly isolated microglia from the brain of control (saline-injected pregnant dams) and MIA (poly:C-injected pregnant dams) offspring at E17, P7, P20 and P60, as well as from P60 control or MIA offspring fed with chow containing CSF1R inhibitor between P21–42, which were analyzed by Database for Annotation, Visualization and Integrated Discovery (DAVID) (Figure 1A). Interestingly, the gene expression pattern of MIA microglia isolated at P60 showed enhanced “synaptogenic” properties. DAVID Gene Ontology (GO) biological process analysis revealed that the most enriched GO terms upregulated by MIA and corrected by microglial repopulation at P60 were “synaptic vesicles” and “neurotransmission”, while the most enriched GO terms down-regulated by MIA and corrected by microglial repopulation were “antigen presentation”, “complement activation” and “innate immune response.” MIA microglia are thus synaptogenic, while repopulated microglia restored their neurosupportive functions. MIA may also have an impact on how microglia repopulate: repopulated control microglia possessed a robust immature microglia phenotype, characterized by upregulation of inflammatory cytokines, chemotaxis and proliferation, also reminiscent of disease-associated/neurodegenerative microglia. In contrast, repopulated MIA microglia showed an enhancement of homeostatic phenotype, characterized by upregulation of Tmem119 and Cx3cr1, suggesting maternal inflammation acceleration in MIA that persists through microglial repopulation. Acceleration of microglial maturation in MIA offspring was also reported previously (Matcovitch-Natan et al., 2016). Microglia isolated from MIA offspring replicated mainly from previously non-mitotic (bromodeoxyuridine negative) microglia, which may revert to a non-proliferative gene expression phenotype seen in MIA microglia (Figure 1B). These results suggest that MIA-induced filopodia formation, phagocytic genes, such as fractalkine and pleiotropin, a secretory growth factor (Ptn) and wingless-type MMTV integration site family member S (Wnt5a), an axon guidance and synaptic neurotrophic factor, and cytokine production by microglia, which can be reversed by therapeutic microglial repopulation.

Aberrant microglia-synapse interactions in MIA: To understand how these transcriptomic and proteomic changes in MIA microglia are reflected in MIA offspring, we investigated the effect of MIA on synaptic density and microglial interactions with pre- and post-synaptic neuronal structures in the layer V of the mPFC at P60. For this purpose, we isolated the dendritic spines of biocytin-filled intrinsically bursting neurons in the layer V of the mPFC, whose electrophysiological properties were significantly altered after MIA by showing slower action potential kinetics and increased excitatory postsynaptic currents. Notably, total and filopodia (filar, immature dendritic spines that may develop into mature synapses) filopodia were significantly increased in MIA compared to the control adult offspring. Confocal imaging and three-dimensional reconstruction analysis of MIA microglia revealed a significant difference in microglial branching, which were interacted with spines in closer proximity in MIA compared to control adult offspring. Most importantly, the changes caused by MIA microglia aberrant depletion and repopulation either normalized or negated the effect on spine and microglial morphology and synaptic density. Taken together, these data indicate the pathological function of microglial neurotrophic factors and consequent spine dysgenesis and aberrant microglia-synapse interaction. Further investigation is required to understand causal effect of these changes on MIA abnormal behavior.

Relevance to other MIA studies: Finally, we cross-examined our results with other studies related to MIA or microglia biology. There is evidence to support our finding for the increased spine density in MIA offspring at P60. Recently, Ragan et al. (2011) also found that prenatal poly:C injection decreased synaptophysin- and glutamic acid decarboxylase-67- positive synapses in the neuronal cell bodies in the upper-layer frontal cortical neurons and increased the dendritic spine density in postnatal 8-weeks-old offspring. Additionally, evidence from postmortem ASD human brain tissue showed an increase in spine density on apical dendrites of pyramidal neurons from cortical layer II in frontal, temporal and parietal lobes and layer V in the temporal lobe, which was inversely correlated with cognitive function (Hutsler and Zhang, 2010). Recent studies support the notion that microglial interaction with spines may facilitate spine formation. Microglial contacts to dendritic shafts induced filopodia formation (Weinhard et al., 2018). Miyamoto et al. observed higher filopodia formation rates by microglial contact compared to control sites, and the presence of both MIA-induced filopodia to functional excitatory synapses (Miyamoto et al., 2016). These previous studies may explain our findings on microglia morphology and their increased interaction of spines led to aberrantly increased spine densities in MIA offspring. Finally, aberrant neurotrophic factors produced in MIA microglia in our study have been reported to affect spine formation. For instance, Ncam2 is expressed in both neurons and microglia, and could trigger intracellular Ca2+ elevation that may subsequently recruit actin and elicit filopodia formation (Sheng et al., 2018).
et al., 2015). Wnt5a has been found to stimulate dendritic spine morphogenesis and induce de novo formation of spines (Varela-Nallar et al., 2010).

Another study, however, revealed reduced synaptic densities in MIA offspring. In vivo multiphoton imaging in the cortex of young MIA offspring showed a reduction in number and turnover rates of dendritic spines, which persisted into adulthood (Coiro et al., 2015). The discrepancy in results could be explained by the difference in the time frame of MIA induction between their model (E12.5 poly IC injection) and our model (E9.5 poly IC injection), the critical time period when microglia migrate from the yolk sac). Another plausible explanation for this discrepancy can be the reduced expression levels of microglial neurotrophic factors at P20, around the time frame when Coiro’s study found a reduction in microglial function due to maternal immune activation and autism spectrum disorders. Neuritogenic function of microglia in maternal immune activation causes age- and region-specific changes in brain cytokines in offspring throughout development. Brain Behav Immun 31:54-68.

In summary, our findings of aberrantly increased neuritogenic factors from MIA microglia during neurodevelopment may shed light on the molecular mechanism of microglia-mediated MIA phenotype in the offspring, such as increased synapticogenesis found concurrently with behavioral deficits in the children of both groups. These results raise the possibility that increased neurotrophic factors from maternal immune activation could lead to increased spine and synaptic pruning in MIA-mediated ASD, which can be detected in cerebrospinal fluid or blood as future biomarkers for ASD.

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