Towards the generation of NeuroImmune Assembloids for the study of neurodevelopmental disorders

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Abstract: Microglia are the tissue-resident macrophages colonizing the brain in early stages of development and whose role is emerging to be crucial in neuropsychiatric disorders, such as autism spectrum disorders (ASD). We aim at exploring how alterations in microglia maturation during neurodevelopment contribute to the onset of ASD, by characterizing foetal microglia maturation in human brain tissue and by establishing a 3D *in vitro* platform leveraging human Pluripotent Stem Cells (hPSCs)-derived cortical organoids (hCO), where hPSCs-derived microglia-progenitors are incorporated.

Exploring, by immunofluorescence, microglia abundance and morphological maturation during development in early and mid-fetal human brain sections, we observed that few amoeboid IBA1+ cells colonize the human frontal cortex during the early fetal life and that they increase over time, by acquiring a ramified morphology. To investigate which ASD risk genes (gene.sfari.org) are enriched in fetal microglia, cell clustering analyses were performed on a published single-cell dataset of human fetal microglia, suggesting that early or late microglia express two distinct sets of ASD-risk genes, implicating different stages of microglia development in ASD. To model the early stages of microglia development, we incorporated tdTomato+ hPSCs-derived erythromyeloid progenitors (EMPs) in early stage hCOs, finding that EMPs-like cells can colonize and expand in the hCO. Moreover, we found that the microenvironment of hCO drives EMPs maturation towards a more mature microglia-like phenotype, whose cellular expansion is promoted by MCSF and IL34. To investigate how the hCO microenvironment and the addition of external growth factors affect the molecular signature of microglia-like cells, bulk RNA-seq has been performed.

Our data suggest that, during fetal development, microglia undergo a morphological and molecular maturation mirroring different functional states, whose impairments at different developmental stages leading to altered functionality may result in ASD features. Hence, the NIA model can be leveraged as a reliable *in vitro* tool to mimic the brain microenvironment driving the final microglia specification and to study the neuroimmune interplay.