iPSC-DERIVED MICROGLIA-INTEGRATED FOREBRAIN ORGANOIDS: A NOVEL MODEL FOR HIV INFECTION IN THE CNS.

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Microglial integration into brain organoids provides a model to study neuro-immune interactions during neurodevelopment and pathologic processes such as CNS viral infections. Here, we describe a novel model of integration of human iPSC-derived microglia (iMg) to human iPSC-derived forebrain organoids and demonstrate the capacity of integrated iMg to harbor productive HIV replication. We also present data suggesting that the iMg integration enhances neuronal maturation. Human forebrain organoids were generated from two independent iPSC lines and were matured for 45–55 days before they were used in a series of integration experiments. Briefly, common myeloid progenitors were differentiated from iPSCs of adult human fibroblasts and further differentiated into iMg. Organoid-iMg integration was performed by coculturing organoids on a layer of iMg for 10 days in organoid media containing M-CSF and IL-34. For viral transduction, 1 ng/mL of the microphage-tropic HIV-ADA strain was added to either ongoing organoid-iMg cocultures or iMg-integrated organoids transferred to new plates. Robust HIV infection was achieved within 5–7 days, with nearly all IBA-1 positive cells displaying HIV p24 marker. Productive HIV infection was confirmed by qPCR and p24 alphalisa. We also demonstrated that the productive HIV infection could be transferred by coculturing infected organoids over a layer of uninfected iMg or with uninfected iMg-integrated organoids. Overall, we achieved robust iMg integration of total cells in the iMg-integrated organoids. The microglia in iMg-integrated organoids, without HIV infection, displayed ramified morphology. qPCR analysis of iMg-integrated organoids revealed a strong correlation between markers of neuronal differentiation (Nestin and Ctip2) and the myeloid marker CD14 ($r > 0.7$, $p < 0.001$), and this correlation did not change in the presence of HIV. These results demonstrate the suitability of our model to study neuro-immune interactions in the presence of HIV infection and developmental studies of the CNS.