HIV-infected microglia drive transcriptional and metabolic changes in complex human brain organoids

631

Leanne C. Helgers[1,2*] Pamela C. Capendale[3,4*], Anoop T. Ambikan[5], Renata Vieira de Sá[4,6], Teunis B.H. Geijtenbeek[1,2], Adithya Sridhar[3,4,7], Ujjwal Neogi[5], Dasja Pajkrt[3,4,7]

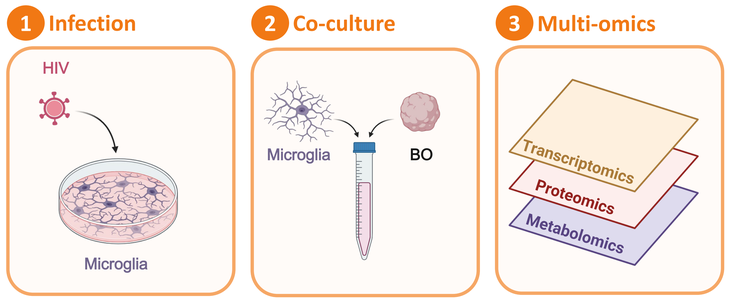
[1] Department of Experimental Immunology, Amsterdam UMC Location University of Amsterdam, Amsterdam The Netherlands
[2] Amsterdam institute for Infection and Immunity, Amsterdam The Netherlands
[3] OrganoVIR Labs, Emma Children's Hospital, Department of Pediatric Infectious Diseases, Amsterdam UMC, location Academic Medical Center, Amsterdam Institute for Infection and Immunity, Amsterdam Institute for Reproduction and Development, University of Amsterdam, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands
[4] OrganoVIR Labs, Department of Medical Microbiology, Amsterdam UMC, location Academic Medical Center, Amsterdam Institute for Infection and Immunity, University of Amsterdam, Meibergdreef 9, 1100 AZ Amsterdam, The Netherlands
[5] The Systems Virology Lab, Division of Clinical Microbiology, Department of Laboratory Medicine, Karolinska Institutet, ANA Futura, Campus Flemingsberg, Stockholm, Sweden
[6] UniQure Biopharma B.V., Department of Research & Development, Paasheuvelweg 25A, 1105 BE Amsterdam, The Netherlands
[7] Emma Center for Personalized Medicine, Amsterdam UMC, Amsterdam, the Netherlands.
* These authors contributed equally to this work

BACKGROUND

- HIV enters the central nervous system early after infection which can result in HIV-associated neurocognitive disorder (HAND).
- Microglia are resident immune cells in the brain targeted by HIV.
- Residual HIV production causes microglia, and subsequently, astrocytes activation to initiate an inflammatory immune response.
- Activation of microglia and astrocytes increases their energy demand, which causes the neurons to perform less optimal.

METHODS

Here, we investigated how HIV-infected microglia impact neuro-inflammation and metabolism using an advanced 3D human brain organoid model. First, microglia are exposed to HIV for 3 days before being co-cultured with brain organoids. After a co-culture of 3 days, we employed transcriptomics, coupled with genome-scale metabolic modeling (GEM) to delineate neuro-inflammatory and metabolic responses elicited by HIV-infected microglia in the brain.



To control for Microglia induced changes in brain organoids, different conditions were included. We use distinct terminology to distinguish between brain organoids without microglia (BOs), and BOs embedded with microglia (MBOs). Marker expression of microglia was validated by flow cytometry for CD11b, CD45+ and CCR5+ (data not shown).

BO conditions
- BO - Mock
- BO - HIV (inactive)
- BO - HIV

MBO conditions
- MBO - Mock
- MBO - HIV

1 Microglia are infected with HIV

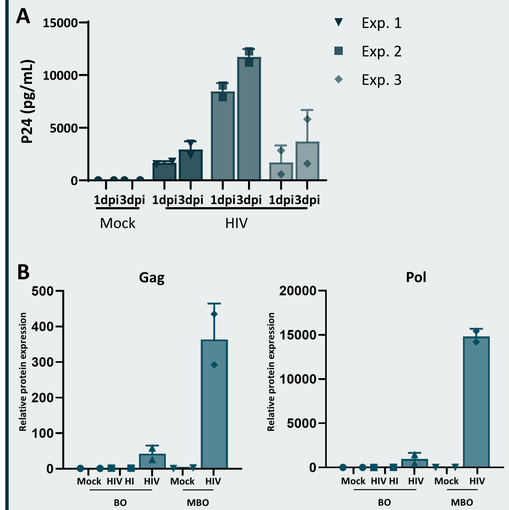


Fig 1. Microliga are exposed to mock or HIV (MOI 0.5) for 3 days where after cells were lysed. HIV infection is determined by means of p24 detection using ELISA (**A**). Experimental set-up for BOs and MBOs as described in methods. RT-qPCT was used for the detection of *Gag* and *Pol* in BOs and MBOs (**B**).

HIV-infected microglia integrate into Brain organoids

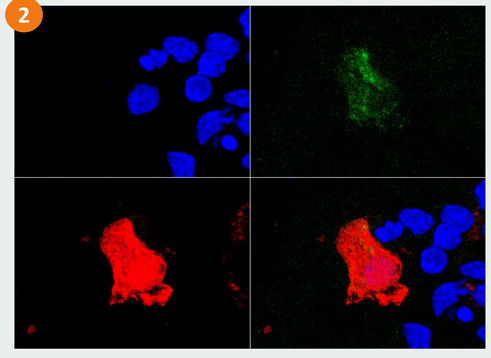


Fig 2. Microliga are exposed to mock or HIV (MOI 0.5) for 3 days, where after cells are co-cultured with brain organoids for an additional 3 days. MBOs were sliced and confocal microscopy was utilized to visualize co-localization of microglia and HIV in MBOs. **Cell nuclei** in blue (Hoechst), **Microglia** in red (Iba1), and **HIV** in green (p24). In addition, co-localization of HIV (p24) and astrocytes (GFAP), but not neurons (bTub), was also observed (data not shown).

3 Transcriptomics: Microglia are essential for HIV-induced transcriptional changes

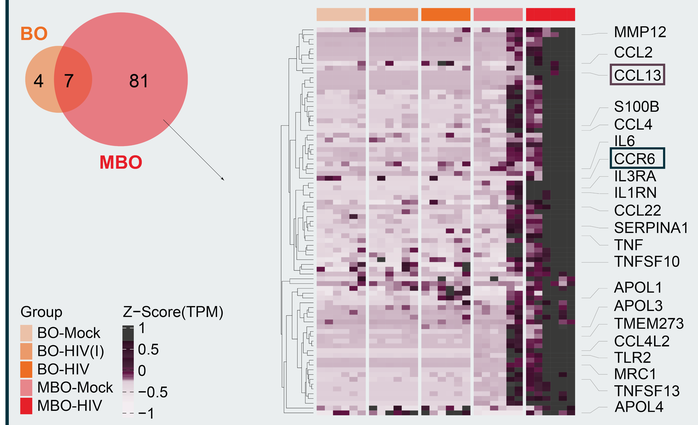


Fig 3. Experimental set-up for BOs and MBOs as described in methods. Differentially expressed genes (DEGs) comparing mock infection vs. HIV-1 infection in BOs and MBOs. In total, 81 DEGs were uniquely expressed in HIV-1 infected MBOs (differentiation plots not shown). The Heatmap represents these DEGs showing all conditions for BOs and and MBOs representing the 2 samples from 3 donors in the columns. A significant increase in gene expression is observed for several DEGs unique for HIV-1 infected MBOs, mainly including inflammatory genes. We identified **CCR6**, important for HIV-1 permissiveness, and **CCL13**, to be severely upregulated which was validated by RT-qPCR (data not shown).

3 Metabolomics : HIV-infected microglia induce changes in metabolic flux

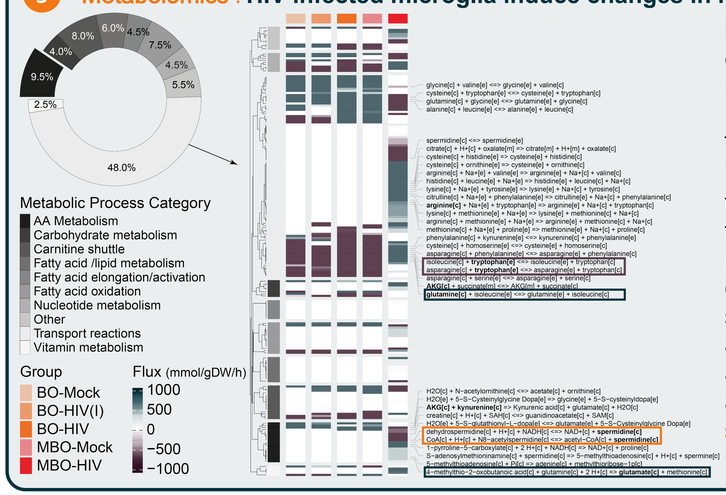


Fig 4. Experimental set-up for BOs and MBOs as described in methods. This GEM model is built using genomic data to identify shift in enzymatic reactions and metabolites in specific conditions. The results show percentage of changes observed in various metabolic process categories based on the number of metabolic reactions with altered flux in MBO-HIV compared to other groups. The heatmap displays the predicted flux distributions demonstrating shifts in pathway utilization in specific conditions. In the MBO-HIV condition analysis revealed pronounced disturbances in amino acid metabolism, particularly **polyamine** and **tryptophan** fluxes, but also include an altered **spermidine** metabolism.

CONCLUSION

- We observe successful integration of HIV-infected microglia in human brain organoids
  - *transfer of virus proteins or virus particles from microglia to astrocytes*
- Out data suggest that HIV-infected microglia induce transcriptional changes in BOs
  - *upregulation of genes involved in inflammatory pathways*
- Genome scale metabolic modeling shows an impaired metabolism mediated by HIV-infected microglia
  - *Impaired polyamine and tryptophan metabolism*
  - *Elevated spermidine levels*

ADDITIONAL KEY INFORMATION

Amsterdam UMC University Medical Centers
organovir LABS ORGANOIDS FOR VIROLOGY
Karolinska Institutet

l.c.Helgers@amsterdamumc.nl