ASTROCYTES-MICROGLIA CROSSTALK: HOMEOSTATIC MODULATION OF HIV EXPRESSION IN MICROGLIA

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BACKGROUND

The brain cells crosstalk involves a wide range of signaling pathways that can affect gene transcription. We have previously shown that neurons-microglia crosstalk regulates HIV expression in microglia cells. Since the astrocytes are essential homeostatic and immune cells in the brain, we expanded our work to explore the astrocyte-microglia crosstalk in the context of HIV infection. ***We hypothesize that astrocytes-released factors can regulate microglia activation and therefore silence HIV in infected microglia.***

METHODS

- We used Pluripotent Stem cells (iPSC) derived astrocytes (iA) and the HIV-latently infected microglia cell line HC69 to identify astrocytes soluble factors regulating HIV. This microglia cell line is an HIV-latently infected clone with a single round HIV-1 containing a short-lived GFP.
- Cell tracing marked HC69 cells were co-cultured with iA for 24-72 hours.
- GFP expression was determined by microscopy and cytometry analysis.
- HC69 were also treated with increasing concentration of possible iA-released factors (ATP, BDNF, GDNF, IL10).
- Coculture of iA and iPSC-microglia (iMG) was stablished to confirm the results obtained with HC69. iMG was also infected with the same HIV provirus expressing GFP.
- Triculture of iA, iPSC-neurons (iN) and iMG was used to confirm regulatory activity of brain cells over HIV-infected microglia.

RESULTS

- iA-microglia cocultures showed a significant reduction of HIV expression in microglia cells (Figure 2).
- We identified ***astrocytes-released*** ATP as responsible for such reduction. Since astrocytes-released ATP can be catabolized to adenosine by CD73, we blocked the CD73 activity with a specific inhibitor (AB-680). The ATP regulatory effect on HIV expression was then diminished, suggesting the effect was indeed due to ***adenosine*** and not by ATP itself (Figure 3).
- Same effect was observed when AB-680 was added to the iA-microglia cocultures. We also observed that low concentrations of ATP (0.5 to 5 mM) would activate the virus. However, with concentrations higher than 10 mM a clear reduction of HIV expression was observed.

Astrocytes-released Adenosine induces HIV silencing in microglia cells.

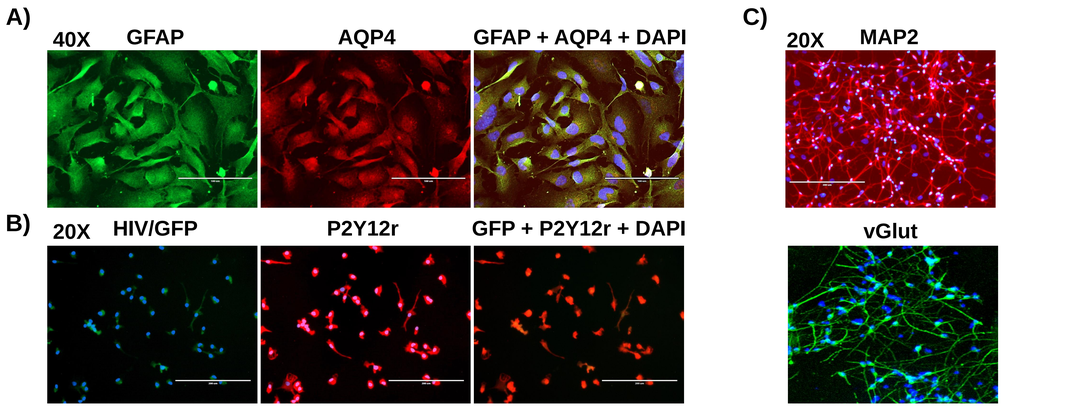

Figure 1. iPSC derived brain cells express specific markers. A) iPSC-Astrocytes (iA) express GFAP and Aquaporin 4 (AQP4); B) iPSC-microglia (iMG) HIV-infected (GFP) express P2Y12r; iPSC-neurons express MAP2 and vGlut.

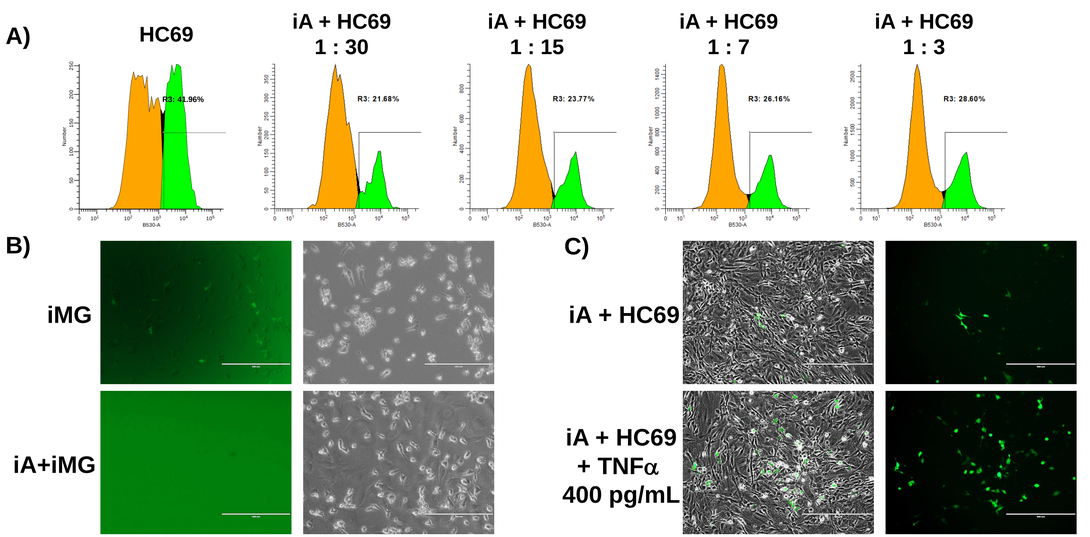

Figure 2. iPSC-Astrocyte (iA) induce HIV silencing in microglia cells. A) iA cocultured with HC69 for 24 h induced HIV silencing in a rate dependent manner; B) The silencing effect was reproduced using iMG; C) Silenced HIV can be reactivated by cytokine treatment (TNFα 400 pg/mL).

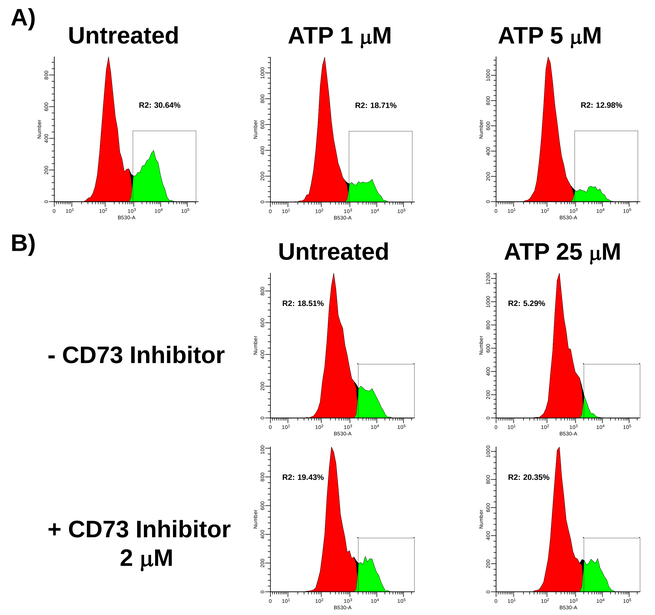

Figure 3. Adenosine induces HIV silencing in HC69 microglia cells. A) HC69 cells were treated with ATP for 48h, then detached and analyzed by flowcytometry. B) HC69 were pretreated with CD73 inhibitor (AB-680) 2h previous ATP treatment (48h) then analyzed by flow.

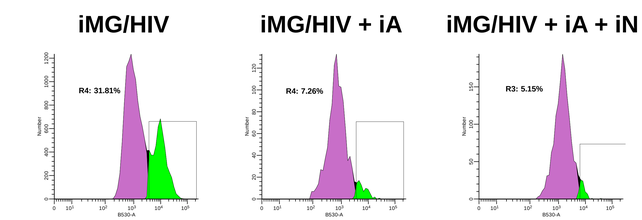

Figure 4. iPSC-Astrocytes and Neurons modulate HIV expression in HIV-infected iPSC-microglia. Three days post infection iMG were marked with CellTrace violet and cultured with iA or iA + iN for 48 h. Analyzed then by flow.

CONCLUSIONS

- Data from iA-iMG and iA-iMG-iN cultures suggest regulatory-homeostatic mechanisms that could be explored to reduce inflammation and HIV expression in the brain. One of those mechanisms could be related to Adenosine.
- Our results support the importance of studying the crosstalk among different brain cell types to identify possible therapeutic targets.