Adenosine, Guardian of the Brain: Possible Role in Protecting from HIV Neuropathogenesis

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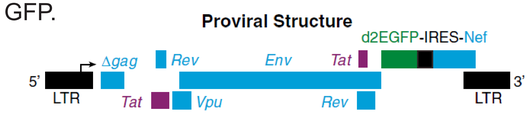
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BACKGROUND

- Due to its regulatory properties, adenosine has been recognized as a guardian molecule with many protective functions in and out of the brain. For example, HIV silencing in T-cells has been associated with upregulating the adenosine-producing ecto-5'-nucleotidase Nt5e (known as CD73). We hypothesized that ***under HIV-induced inflammatory conditions in the brain, extracellular ATP/adenosine would increase, and it will lead to reduce microglial activation and silence HIV in infected microglia cells***.

METHODS

- Our studies were performed using iPSC-derived astrocytes (iA), neurons (iN), microglia iMG, and the immortalized microglia cell line HC69, a clone latently infected with a single round HIV-1 containing a short-lived GFP.

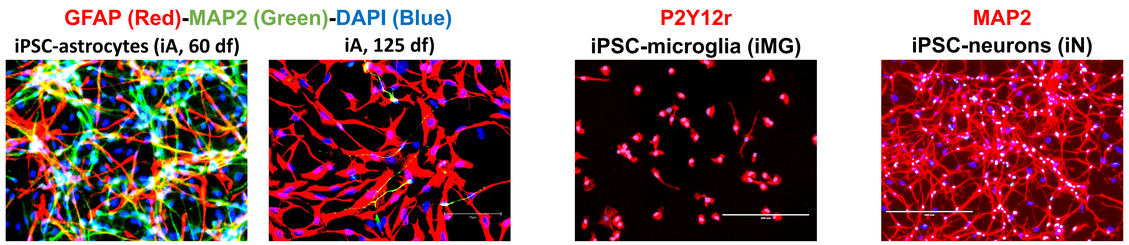


- Commercially available iPSC-microglia from Tempo (iMGT), which lack expression of the adenosine receptor 3 (A3), were infected with the single round reporter and used as control.
- HC69 monoculture and iA+HC69 coculture were used in the initial evaluation of A3 agonists and antagonists.
- iMG were added to the co-/tri-cultures on day 12 of differentiation, allowing them to differentiate fully.
- The cultures were then infected with the macrophage-tropic HIV-1 AD8 virus on day 26.
- The cultures were treated 24 h post-infection with 2 μM NECA (A3 agonist) or adenosine receptor antagonists (1 μM) for four days. Untreated cells were used as control.
- RNA and DNA were extracted and analyzed by qPCR. A decrease in the RNA-to-DNA ratio was used to measure the extent of viral suppression.
- Flow cytometry and microscopy were also used to evaluate HIV expression and latency.

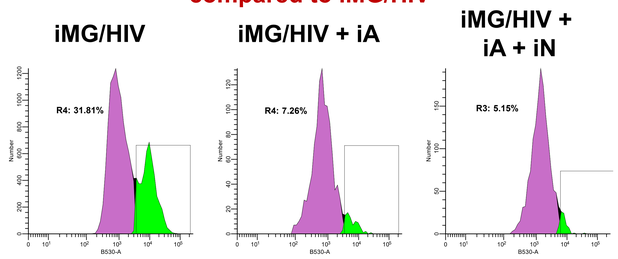
Adenosine receptor 3 agonist NECA potently reduces HIV-1 replication in microglia cells.

RESULTS

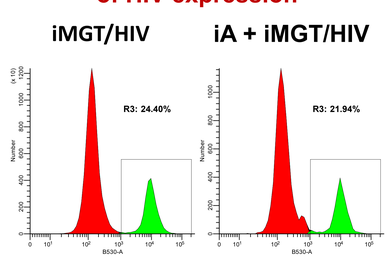
iPSC-brain cells differentiation. Staining for cell type markers



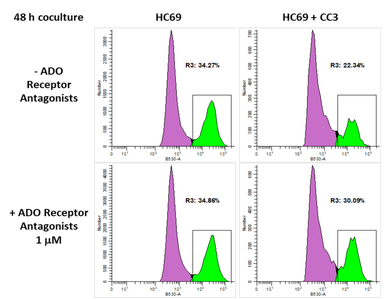
Co- and tri-cultures with HIV-infected iMG showed a significant reduction of HIV expression compared to iMG/HIV



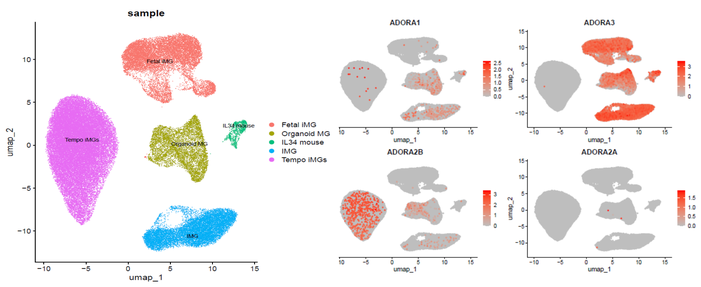
Cocultures of iA + control iMGT/HIV did not show reduction of HIV expression



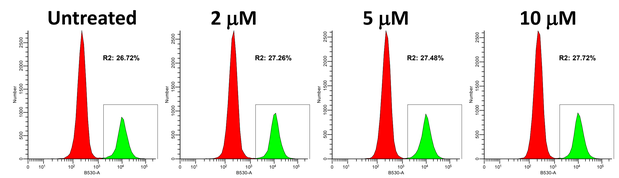
iA-induced HIV silencing in HC69 is related to Adenosine



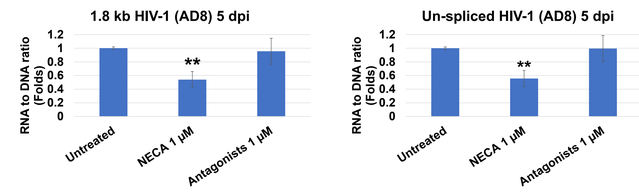
iPSC-microglia (iMGT) from Tempo lack expression of adenosine receptor 3 (ADORA3)



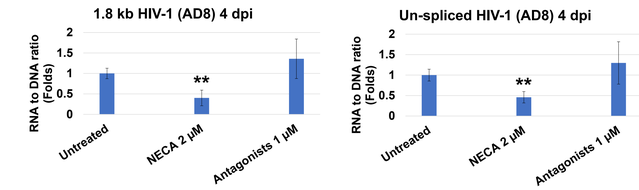
Adenosine receptor 3 (A3) agonist NECA does not induce HIV silencing iMGT/HIV



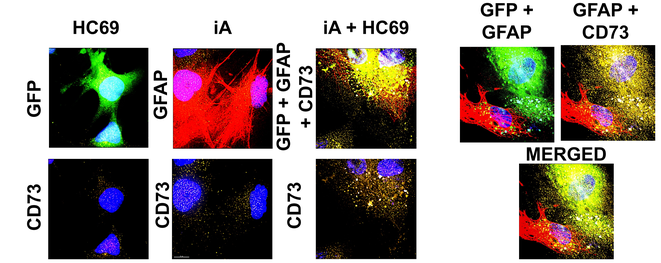
HIV replication is reduced in the presence of A3 agonist (NECA) in iMG/HIV (AD8) monocultures



HIV replication is reduced in the presence of A3 agonist (NECA) in iA+ iMG/HIV (AD8) cocultures



Higher CD73 expression in the iA-HC69 coculture



CONCLUSIONS

- The potent suppression of HIV expression by NECA strongly suggests that the anti-inflammatory properties of adenosine can be exploited to reduce HIV expression in the brain. This approach, which mimics the natural regulatory/homeostatic mechanisms that play a role in reducing inflammation and HIV expression in microglia cells, suggests new avenues for HIV treatment in the CNS.