Developing a Microglial HIV-1 Latency Reporter Cells for Investigating Viral Latency and Treatment Strategies

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Abstract

Despite the success of combination antiretroviral therapy (cART) in controlling HIV replication, a definitive cure remains unattainable due to persistent viral reservoirs[1]. A major barrier is the establishment of latent HIV within cellular reservoirs, particularly in microglia, the resident macrophages of the central nervous system (CNS)[2]. These cells serve as long-lived viral sanctuaries, contributing to viral persistence, drug resistance, and the pathogenesis of HIV-associated neurocognitive disorders (HAND). Targeting HIV-infected microglia is thus critical for achieving a functional cure.

To address this challenge, we aim to develop a human microglial HIV-1 latency reporter cell line using the well-authenticated HMC3 cell line[3] (ATCC® CRL-3304). This model will enable mechanistic studies of viral latency in the CNS, facilitate drug screening, and provide a platform to investigate HAND. Furthermore, the latently infected microglia can be incorporated into microglia-containing human brain organoids (MC-HBOs), a 3D in vitro system that recapitulates key features of the human brain, will be a valuable tool to study HIV-CNS interactions, and the efficacy of new therapeutic and curative strategies on the CNS viral reservoir.

While we have successfully established the first HIV-1 infected microglia HMC3 cells, current infectivity levels remain low. Ongoing efforts are focused on enhancing infectivity and establishing latent HIV-1, with GFP reporter expression under viral promoter control enabling real-time monitoring of latency reversal and reactivation dynamics.

Methods

NL4.3 ADA-GFP-IRES-Nef virus. An infectious clone (**Fig. 1**) was used to generate purified virus. Additionally, a dual envelope approach was employed, incorporating VSG to enhance the viral entry. The virus contains the CCR5-tropic ADA Env and co-expresses Nef and GFP from a single bicistronic RNA by IRES.

Overview Establishment HIV-1 latent reporter microglial cell line.

HMC3 stably expressed CD4 cells will be infected with an ADA-GFP reporter. At 2–5 dpi, GFP⁺ cells will be isolated via FACS and plated into 96-well plates. Sorted cells will be monitored for 15–30 days to confirm the establishment of latency, as indicated by diminished GFP expression . Following expansion, The infected cells will be treated with TNF-α to reactivate HIV-1. Clones exhibiting the highest GFP induction (indicating viral reactivation) will be selected, cryopreserved, and subsequently used for screening of latency-reversing agents (LRAs) (**Fig.2**).

Figure 1. Genetic Structure of HIV-1 ADA-GFP Reporter (HRP-20367)

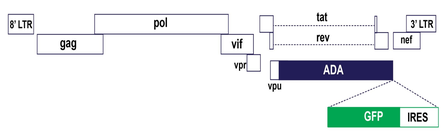
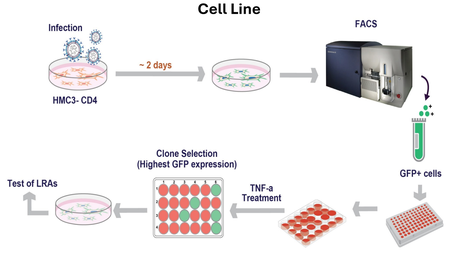


Figure 2. Establishment of an HIV-1 Latent Reporter Cell Line



Results

Generation of HMC3-CD4 Stable Cell Line

Due to the lack of the HIV CD4 receptor, HMC3 was engineered for stably expression via lentiviral transduction with a CD4-expressing vector. Several stable clones were generated, and based on moderate CD4 expression levels, a stable cell line (Clone 9) was selected. Flow cytometry analysis confirmed the expression of CD4 as well as the co-receptors CCR5 and CXCR4 in the HMC3-CD4 clone 9 stable cells. Additionally, antibody titration was performed to determine the optimal antibody concentration for cell sorting (**Fig 3**).

Figure 3. Expression of HIV-1 receptor and co-receptors in HMC3-CD4 clone 9

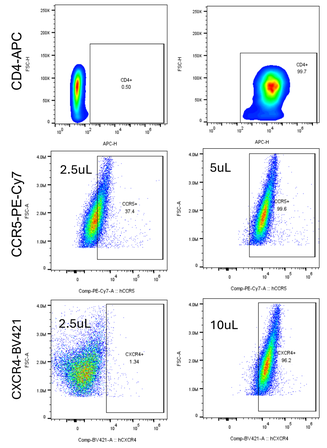
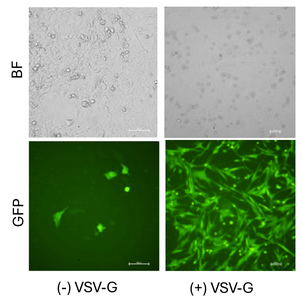


Figure 4. HMC3-CD4 clone 9 infection by HIV-1 ADA-GFP virus



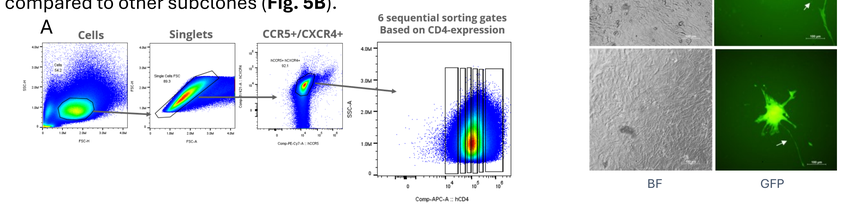
Infection of HIV-1 Reporter Virus in HMC3-CD4 Clone 9

To assess the infectivity of HMC3-CD4 stably expressed cells, the cells were infected with ADA-GFP virus. Interestingly, the cells were successfully infected, as indicated by GFP expression, though the infection level was low (1–2%). To further confirm viral infectivity, we used a dual-envelope virus (+ VSV-G), which showed a significantly higher infection rate (~ 90%). These data suggest that CD4 expression levels may influence viral infection efficiency (**Fig. 4**).

Minimal Enhancement of ADA-GFP Infectivity Observed in Higher CD4+ HMC3 subclone

Due to the low infectivity observed in HMC3-CD4 Clone 9 cells, we further sorted these cells based on CD4 expression levels using FACS. The cells were first gated for coreceptors, CCR5⁺/CXCR4⁺, populations and then sorted into six distinct subclones based on CD4 expression (**Fig. 5A**). Each subclone was infected with ADA-GFP, and infectivity was monitored via GFP expression. Notably, Subclone 9-6, which exhibited the highest CD4 expression, showed only a minimal (~5%) enhancement in infectivity compared to other subclones (**Fig. 5B**).

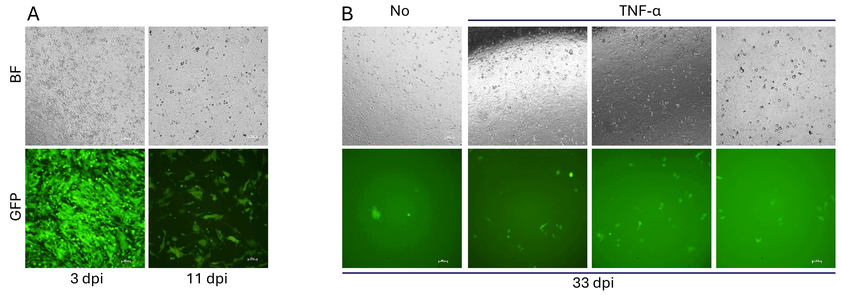
Figure 5. A. Sorting of HMC3-CD4 Clone 9 cells for CD4+ cells by FACS. B. ADA-GFP infection



Proof of Concept: Establishment of HIV Latency in HCM3-CD4 Cells

As a proof of concept to demonstrate that HMC3-CD4 Clone 9 cells can support HIV latency, the cells were infected with a dual-envelope ADA-GFP reporter virus to enhance viral entry. By 3 dpi, ~ 90% of cells were infected, as determined by GFP expression. However, by 11 dpi , a reduction in GFP expression was observed (**Fig. 6A**). The cells were passaged for 33 days , after which only 1–2% retained detectable GFP expression. To confirm whether the remaining GFP-negative cells harbored latent HIV, they were treated with TNF-α for 24 hr, followed by GFP monitoring. As shown in **Fig. 6B**, TNF-α treatment reactivated latent HIV, leading to GFP expression. These data clearly demonstrate that HMC3-CD4 cells can establish HIV latency. Current efforts are focused on generating a single-cell clone of a latent reporter cell line for further studies.

Figure 6. Establishment and Reactivation of Latent HIV in HMC3-CD4 clone 9-6 Cells



Conclusions

- ❖ Human microglia HMC3 cells were successfully constructed for stably expression of HIV CD4 receptor.
- ❖ We demonstrate for the first time that HMC3 cells can support HIV-1 infection, though with low efficiency (~1-2%).
- ❖ Higher CD4 expression minimally enhanced infectivity, as the highest-expressing HMC3-CD4 subclone (9-6) showed only ~ 5% increased infection, implying additional factors regulate HIV in these cells.
- ❖ HIV latency was successfully established in HMC3-CD4 cells, and current efforts are focused on creating a clonal latent reporter cell line for mechanistic studies and drug screening.

Acknowledgements

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References

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