Transfer of HIV-1 from HIV-1 latently infected $CD34^+$ haematopoietic progenitors to $CD4^+$ T cells

AKL Cheung *, Y Huang, M Chen, ZW Chen

KEY MESSAGES

1. HIV-1 can infect $CD34^+$ progenitor stem cells and become latent.
2. Latent HIV-1 can affect the CD34 cell to halt homeostasis.
3. HIV-1–infected $CD34^+$ progenitors may be a vehicle for viral dissemination.

Hong Kong Med J 2019;25(Suppl 7):S37-40
HMRF project number: 13120612

AKL Cheung, Y Huang, M Chen, ZW Chen

AIDS Institute, Department of Microbiology, LKS Faculty of Medicine, The University of Hong Kong

* Principal applicant and corresponding author: akcheung@hkbu.edu.hk

Introduction

It remains controversial whether HIV-1 can infect $CD34^+$ haematopoietic stem cells in the human host. HIV-1 can establish a latent reservoir in vitro and in vivo. This study aims to elaborate on latency of HIV-1 infection in $CD34^+$ haematopoietic stem cells. During latency, HIV-1 remains quiescent and viral replication is very limited with no consequence of immune clearance with declining CD4 counts. Although activation of memory CD4 T cells leads to HIV replication, this does not occur in detectable levels during the latency phase of HIV infection. Latency in the primary site $CD4^+$ T cells can occur within 1 week after primary infection. However, attempts to purge latency have not been successful, despite using different agents to reactivate latent HIV-1 genome in $CD4^+$ T cells.[1] Nonetheless, HIV-1 genome is preferentially present in long-lasting central memory $CD4^+$ T cells and T cells with stem cell-like properties.[2] Latency in $CD4^+$ T cells has been reported,[3] but $CD34^+$ haematopoietic progenitor as a latent reservoir of HIV remains poorly understood.[4] These progenitor cells can be latently infected by HIV-1 without active virus production unless reactivation is induced. Furthermore, in HIV-1 patients, 0.025% to 0.4% of integrated HIV-1 genome is found in $CD34^+$ cells in the bone marrow. The underlying mechanism and how HIV-1 modulated immune response in $CD34^+$ progenitor cells after latent infection remains elusive.

Methods

Healthy human peripheral blood mononuclear cells (PBMCs) were isolated from buffy coats obtained from Hong Kong Red Cross, and 2×10^8 PBMCs were used for magnetic bead separation and cultured in StemPro34 serum-free media (Invitrogen) with 25 ng/mL GM-CSF, 100 ng/mL SCF, 50 ng/mL IL-3 (Peprotech) at a density of 2×10^4 cells/0.2 mL per well in a U-bottom 96-well culture plate for up to 14 days with one-third media change every 3-4 days.

HIV-1 viruses (strains NL4-3 and JRFL) were propagated using PHA/IL2-activated healthy human PBMCs, and virus titre was assessed using p24 ELISA kit according to manufacturer instructions (ZeptoMatrix). HIV-1 infection was performed using 20-50 ng p24 per mL per 5×10^6 cells. To construct pseudoviruses, Red-Green-HIV (RGH) plasmid and plasmid-encoding envelope proteins from X4-tropic HxB2 (ie, X4-RGH) and R5-tropic JR-FL (R5-RGH) were used to co-transfect 293T cells. After 2 days, supernatants were collected and virus concentrated by ultracentrifugation against sucrose gradient. Infected cells were monitored for fluorescent signals using confocal microscopy.

Digital PCR was performed using primers and probes described previously[5] in the QuantoStudio 3D digital PCR system or ViiA7 real-time PCR instrument (Life Technologies). Flow cytometry was performed to examine the expression of HLA-DR, HLA-A,B,C, CD4, CD34, CD33, and p24 (KC57; Becton Dickinson).

Results

HIV-1 infection of PB-CD34 cells

We performed a series of experiment including qPCR, dPCR, and p24 detection to determine if HIV-1 can infect PB-CD34 cells. First, we analysed HIV-1 integration into PB-CD34 cell genome. To increase the sensitivity of detection of proviral HIV-1 DNA, we conducted the TaqMan assay using digital PCR (QS3D, Life Technologies) that allows absolute quantification of DNA copy number without a reference gene. To accurately determine the number of host DNA copies found in the input template, we further used the CCR5 assay.[5] Combing the results,

© 2019 Administering Institution and Hong Kong SAR Government

we found that in 500 cells, there were approximately 25 copies of proviral HIV-1 DNA (~5% infection) in X4- or R5-tropic virus infection of PB-CD34 cells (Fig 1).

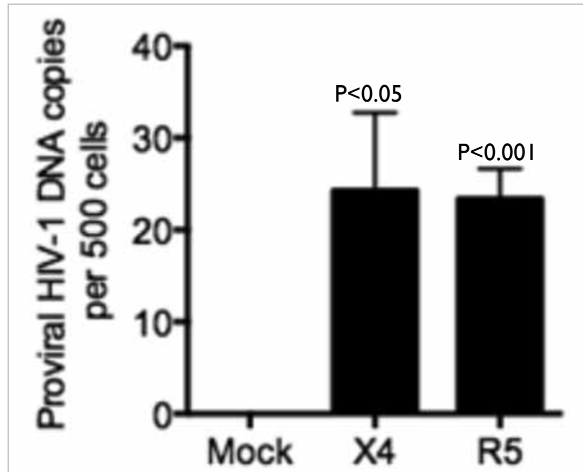

FIG 1. DNA copy number in HIV-1 infection of PB-CD34 cells: At day 1 following mock, X4-tropic, or R5-tropic HIV-1 live virus infection, cells were harvested for DNA extraction. TaqMan assays were performed for dPCR for HIV-1 p17. qPCR for CCR5 was performed to determine number of cells in the template.

Dual-reporter pseudovirus infection of PB-CD34 cells

To further illustrate that PB-CD34 cells can support HIV-1 infection, we used the dual reporter construct RGH. RGH differentiates between silent latent infection and active infection based on Gag-eGFP (green) and Nef-mCherry (pink) fluorescences, such that early eGFP detection indicates active infection, mCherry indicates latent infection, and dual positive represented by co-localised yellow fluorescence depicts *de novo* Gag expression and integrated latent virus (Fig 2a). Pseudoviruses with X4-tropic HxB2 (X4-RGH) and R5-tropic JR-FL (R5-RGH) envelopes were generated. The infectious ability of the RGH pseudovirus was first tested in GHOST(3)-CXCR4$^+$CCR5$^+$ cells. At day 2 post-infection, eGFP and mCherry signals can be detected on GHOST cells and some cells have co-localised signals (yellow arrows) [Fig 2b], suggesting that integration and replication can be detected using X4-RGH. Similar results were obtained for R5-RGH (data not shown) and validates that the pseudoviruses generated can be used. Next, PB-CD34 cells were infected with X4- or R5-RGH pseudoviruses. After 2 days, confocal microscopy was used to determine fluorescence signals. PB-CD34 cells infected with X4- or

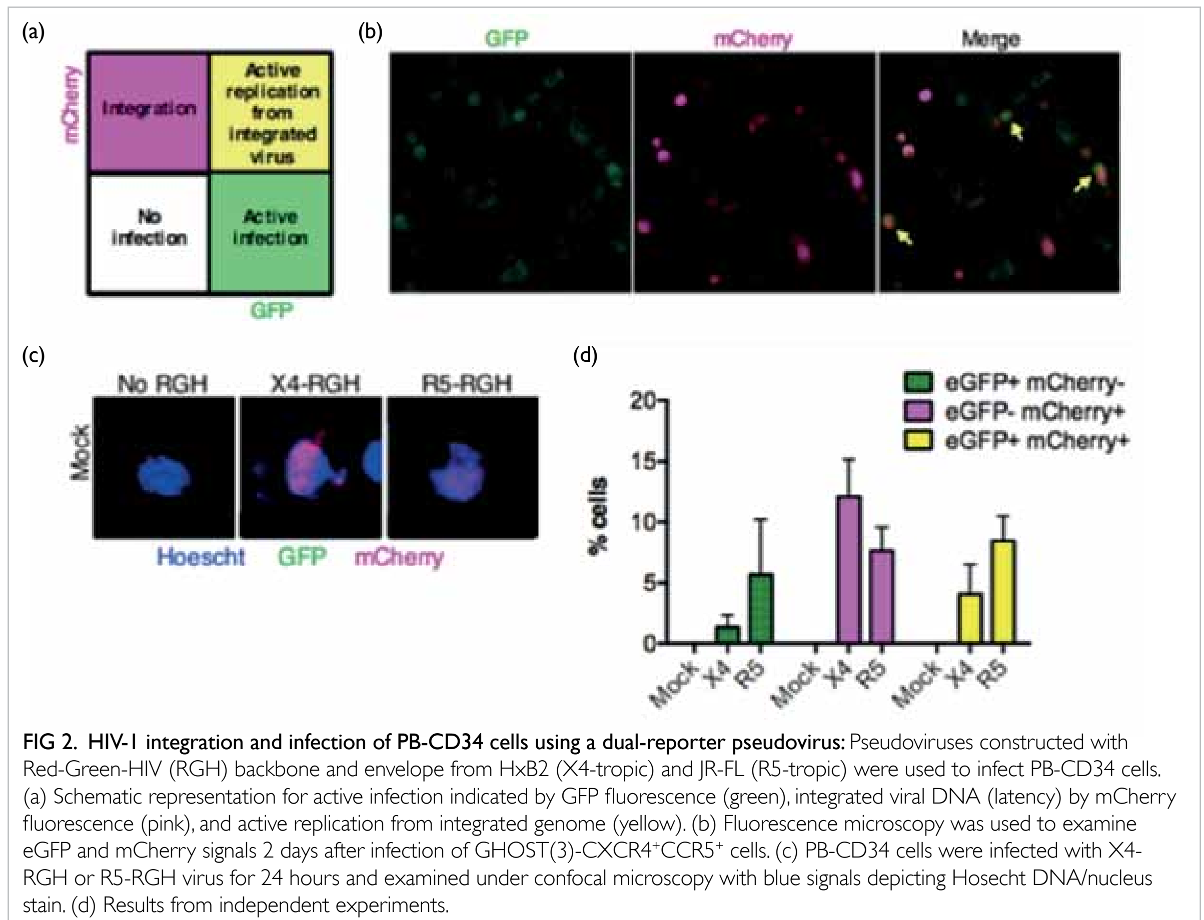

FIG 2. HIV-1 integration and infection of PB-CD34 cells using a dual-reporter pseudovirus: Pseudoviruses constructed with Red-Green-HIV (RGH) backbone and envelope from HxB2 (X4-tropic) and JR-FL (R5-tropic) were used to infect PB-CD34 cells. (a) Schematic representation for active infection indicated by GFP fluorescence (green), integrated viral DNA (latency) by mCherry fluorescence (pink), and active replication from integrated genome (yellow). (b) Fluorescence microscopy was used to examine eGFP and mCherry signals 2 days after infection of GHOST(3)-CXCR4$^+$CCR5$^+$ cells. (c) PB-CD34 cells were infected with X4-RGH or R5-RGH virus for 24 hours and examined under confocal microscopy with blue signals depicting Hosecht DNA/nucleus stain. (d) Results from independent experiments.

R5-RGH viruses had mainly red signals (Fig 2c, 2d), suggesting that latent infections were established by the pseudovirus, albeit a low level of subsequent replication. However, for R5-RGH, a slight but significant increase in active infection was observed and most cells still retained HIV-1 latency (Fig 2c, 2d). Therefore, using the dual-reporter pseudovirus system, CD34+ cells can be successfully infected with characteristic of integration and replication and confirms that CD34+ cells can be infected by HIV-1.

HIV-1 can transmit from infected PB-CD34 cells to autologous CD4+ T cells

HIV-1–infected PB-CD34 cells were shown to be capable of eliciting an immune response by upregulation of type I IFNs and MHC-I/II molecules. We then sought to determine if HIV-1 transmission can occur to CD4+ T cells. First, PB-CD34 cells were mock or infected by HIV-1NL4-3 for 7 days. By day 6, naive CD4+ T cells were isolated from the same donor PBMCs using magnetic beads, and left in media or stimulated with PHA or anti-CD3/anti-CD28 antibodies for 24 hours. To note, ELISA did not detect any p24 in the supernatant of PB-CD34 cells at day 7 post-infection. On day 7, co-culture occurred at a ratio of one PB-CD34 cell to five CD4+ T cells. One day later, cells were subjected to intracellular immunostaining for p24 following surface CD34, CD4, and activation marker CD25 staining, and analysed by flow cytometry. Following gating on CD34- cells, CD4 cells were analysed for p24 signals among CD25+ and CD25- cells. Following co-culture, p24 signals can be detected in naive CD4+ T cells without stimulation but upregulated for CD25 (Fig 3). PHA or anti-CD3/anti-CD28 stimulated cells did not display p24+ cells. This is in stark contrast to HIV-1–free virus infection of CD4+ T cells where activated cells are required for efficient infection. When a transwell was used to separate PB-CD34 cells and CD4+ T cells, no viral p24 signals was detected on CD4+ T cells (data not shown), suggesting that direct cell-cell contact is required for viral transmission. Interestingly, the up-regulation of CD25 following co-culture among naive CD4+ T cells suggest that upon interaction with infected PB-CD34 cells, the naive T cells are activated in a certain level, probably due to the unregulated type I IFNs and MHC-I/II antigen presentation molecules.

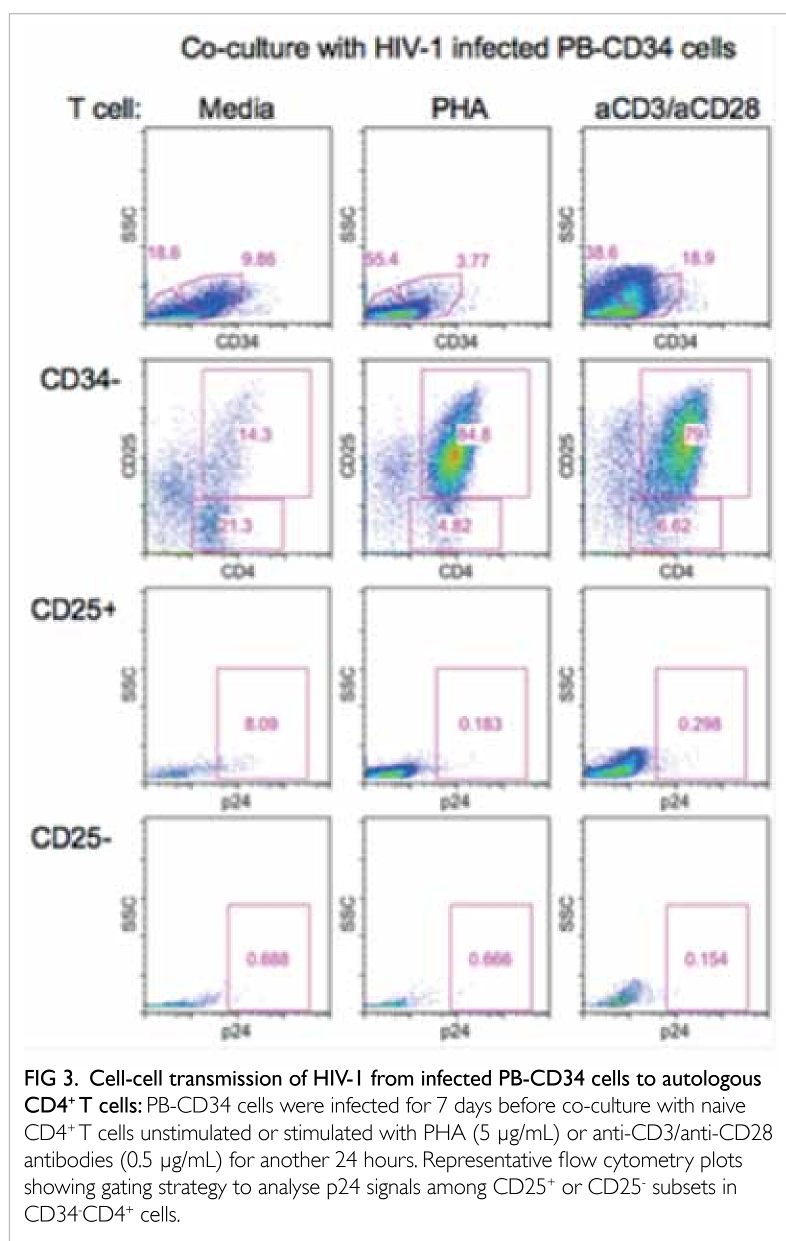

FIG 3. Cell-cell transmission of HIV-1 from infected PB-CD34 cells to autologous CD4+ T cells: PB-CD34 cells were infected for 7 days before co-culture with naive CD4+ T cells unstimulated or stimulated with PHA (5 μg/mL) or anti-CD3/anti-CD28 antibodies (0.5 μg/mL) for another 24 hours. Representative flow cytometry plots showing gating strategy to analyse p24 signals among CD25+ or CD25- subsets in CD34-CD4+ cells.

Discussion

Infection of CD34+ cells by HIV-1 remains controversial in two aspects: (1) whether CD34+ cells can be infected by HIV-1, and (2) whether a latent reservoir is established. The difficulty of addressing these issues mainly lie at the lack of a robust cell-based model of study. Thus, this study provided a robust peripheral blood-derived CD34+ cell culture model that can support HIV-1 infection, integration, and replication, and a mean of viral transmission by cell-cell spread to CD4+ T cells.

The importance of CD34+ cells as a latent reservoir has been neglected. We showed that CD34+ cells can act as effectors of innate immune response through expression of PRRs and inducible type I IFNs, as well as expression of antigen presenting MHC-I/II molecules. Notwithstanding that the HSCs infected by HIV-1 have a higher tendency towards the myeloid lineage likely due to recognition of HIV-1. HIV-1 could be transmitted through cell-cell contact; these cells along with dendritic cells and macrophages contribute to CD4+ T cell depletion. However, whether this occurs in the bone marrow or the periphery remains to be investigated.

Acknowledgements

This study was supported by the Health and Medical Research Fund, Food and Health Bureau, Hong Kong SAR Government (#13120612). We thank Miss Yiru Huang, Ms Hau-yee Kwok, Dr Min Chen, Mr Lijun Ling.

References

1. Shan L, Deng K, Shroff NS, et al. Stimulation of HIV-1-specific cytolytic T lymphocytes facilitates elimination of latent viral reservoir after virus reactivation. Immunity 2012;36:491-501.
2. Buzon MJ, Sun H, Li C, et al. HIV-1 persistence in CD4+ T cells with stem cell-like properties. Nat Med 2014;20:139-42.
3. Redel L, Le Douce V, Cherrier T, et al. HIV-1 regulation of latency in the monocyte-macrophage lineage and in CD4+ T lymphocytes. J Leukoc Biol 2010;87:575-88.
4. Carter CC, Onafuwa-Nuga A, McNamara LA, et al. HIV-1 infects multipotent progenitor cells causing cell death and establishing latent cellular reservoirs. Nat Med 2010;16:446-51.
5. Malnati MS, Scarlatti G, Gatto F, et al. A universal real-time PCR assay for the quantification of group-M HIV-1 proviral load. Nat Protoc 2008;3:1240-8.