Lymphoid Organ-chip as a Model to Study HIV Immunopathogenesis and Reservoir

222

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Lymphoid organ-chip has the potential to become a physiologically relevant, pre-clinical human system to study HIV immunopathogenesis, reservoir, and to evaluate novel HIV therapeutic and functional cure approaches.

BACKGROUND

A physiologically relevant human system is required to better understand HIV immunopathogenesis and reservoirs in secondary lymphoid organs. Recent studies demonstrate that lymphoid organ-chip, a microfluidic system in which high-density human peripheral blood mononuclear cells (PBMCs) cultured in a 3D collagen-matrigel extracellular matrix, can recapitulate key features of human lymphoid tissues. Therefore, we hypothesize that the lymphoid organ-chip my serve as a powerful human system to investigate HIV immunopathogenesis and reservoirs.

METHODS

Lymphoid organ-chips were generated by seeding high-density normal human PBMC within a collagen-matrigel extracellular matrix into the Emulate S1 Chip. Two HIV-1 infectious molecular clones, a CCR5-tropic transmitted/founder (T/F) virus CH058 and a CXCR4 tropic T/F virus 40700, were used to infect the lymphoid organ-chips 4 days after cell seeding. HIV-1 replication was assessed by measuring p24 concentration in the culture supernatants. Uninfected chips maintained under the same condition were used as controls. Cell phenotypes were determined by flow cytometry 15 days after HIV infection.

RESULTS

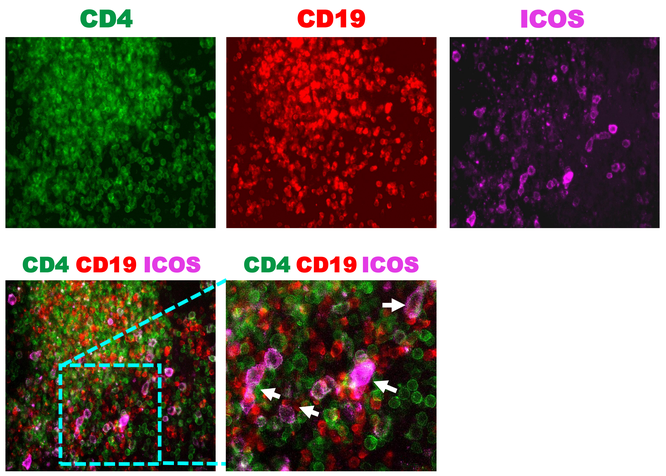


Figure 1. Recapitulation of B cell follicle formation in the lymphoid organ chip**.** Fluorescence microscopy image of a 4 days lymphoid organ-chip culture showing B cell follicle formation without exogenous cytokines stimulation. Within the follicle, activated ICOS $^{+}$ CD4^{+} T cells were observed in close contact with multiple B cells with immunological synapse formation (white arrows).

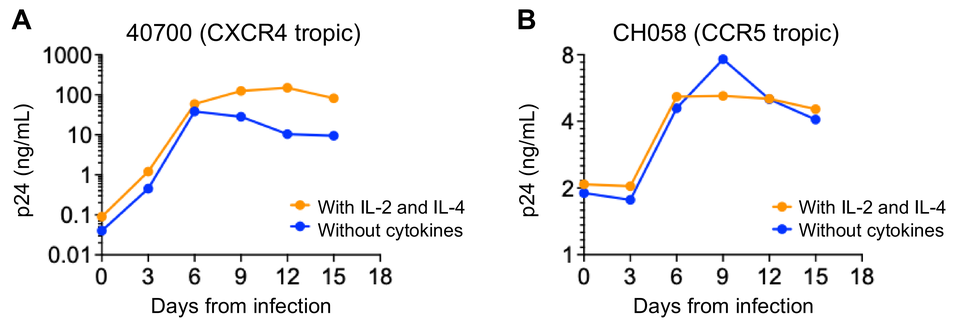


Figure 2. Replication kinetics of two infectious molecular clones (IMCs) 40700 **(A)** and CH058 **(B)** in the lymphoid organ chip with and without cytokine stimulations (IL-2 and IL-4). 40700 (X4) showed faster replication kinetics compared to CH058 (R5). Cytokines addition modestly enhanced and prolonged the replication of 40700.

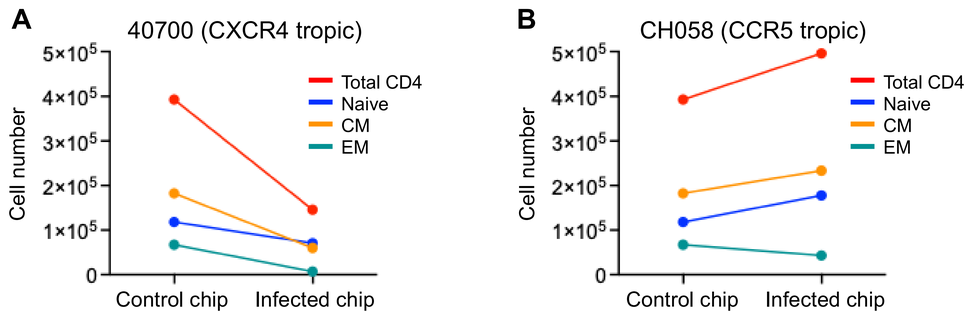


Figure 3. Differential depletion of CD4^{+} T cell subsets by 40700 **(A)** and CH058 **(B)**. After 15 days of HIV infection, cells were recovered from the infected and control chips (without HIV infection) and analyzed by flow cytometry. 40700 depleted all CD4 subsets compared to the control chip, CH058 only showed modest depletion of the EM CD4 subset.

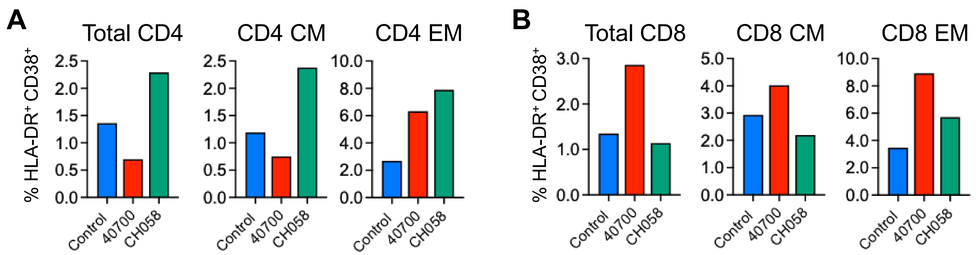


Figure 4. Immune activation of CD4^{+} **(A)** and CD8^{+} **(B)** T cells was determined by HLA-DR and CD38 co-expression. Compared to the control chip, CH058 led to enhanced immune activation of total CD4, central memory (CM) and effector memory (EM) CD4, while 40700 led to enhanced immune activation only in EM CD4. Conversely, 40700 led to elevated immune activation of total CD8, CM and EM CD8, while CH058 led to increased activation only in EM CD8.

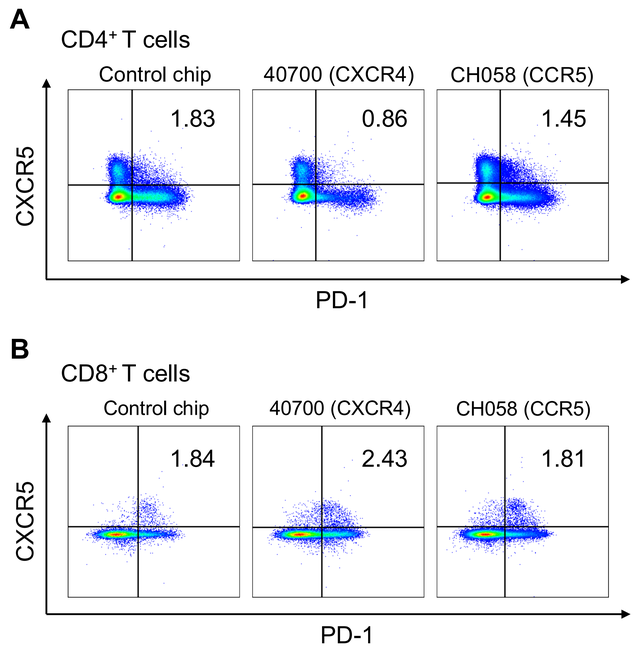


Figure 5. Frequency of follicular CD4^{+} T cells (Tfh) **(A)** and follicular CD8^{+} T cells (Tfc) **(B)** in the lymphoid organ chip after 40700 and CH058 infection. The frequency of CXCR5 and PD1 double positive CD4^{+} and CD8^{+} T cells were determined by flow cytometry 15 days after HIV infection. In comparison to the control chip (without HIV infection), 40700 led to more pronounced depletion of the Tfh CD4^{+} T cells compared to CH058. No obvious depletion of the follicular CD8^{+} T cells (Tfc) were observed after 40700 and CH058 infection in comparison to the control chip.

CONCLUSIONS

Lymphoid organ-chip model supports replication of both CCR5 and CXCR4-tropic HIV-1 without the need for exogenous T cell activation (for example, by anti-CD3/CD8 antibodies or PHA). Key HIV immunopathogenesis events, including CD4^{+} T cells depletion and immune activation, can be recapitulated in this system. It holds promise as a physiologically relevant, pre-clinical human model to study HIV immunopathogenesis, reservoirs, and to evaluate novel HIV therapeutic and functional cure approaches.