Introduction

In productive HIV infection, viral RNA is first converted into cDNA by a viral reverse transcriptase. Then the viral cDNA is integrated into the host genome. From the integrated provirus, new virions are produced. Activated T cells that become infected can be destroyed and HIV will not spread. Resting T cells that become infected go unnoticed and cannot be destroyed by the immune system or by anti-retro viral drugs and form a latent infection reservoir. A further understanding of the process to form the latent reservoir in resting T cells could help the scientific community progress to being able to eradicate HIV. It is generally understood that HIV can only productively infect CD4+ T cells that are activated and proliferating, and infection of resting CD4+ T cells by HIV is blocked prior to viral integration. However, in vivo studies have shown HIV can infect resting CD4+ T cells (ref 3). This is largely due to resting T cells coming in contact with external signals and antigen-presenting cells (APCs). Endothelial cells (ECs) can act as these APCs both in vivo and in vitro. Previous studies (ref 1 and 2) have found that resting CD4+ T cells co-cultured with Human Umbilical Vein Endothelial Cells (HUVEC) can be productively infected by HIV. ECs (HUVEC) can also induce latent infection in resting T cells (ref 4). However, HUVEC cells are not the exact endothelial cell type that interact with T cells and HIV in vivo. A better representation would be lymphatic endothelial cells (LEC). In this study, we used a LEC – resting T cell co-culture system and pseudotyped virus to investigate the effect of LECs on productive and latent HIV infection of resting T cells.

Materials and Methods

**Pseudotyped virus NL43-dE-GFP:**

The env gene from laboratory HIV strain NL43 was replaced with the enhanced green fluorescence protein (EGFP) gene including an endoplasmic reticulum retention sequence. Reporter virus was coated with an HIV envelop protein (using CXCR4 as a co-receptor) and only capable of single round infection.

**Cell isolation and In vitro infection:**

Human umbilical vein endothelial cells (HUVEC) were purchased from PromoCell (Germany). Lymphatic endothelial cells (LEC) were purchased from ScienCell. Resting CD4+ T cells was isolated via negative depletion using Miltenyi Microbeads. When indicated, ECs were pre-treated with IFN-γ (50ng/mL) 3 days prior to addition of resting T cells. Resting T cells were co-cultured with ECs for 1 day prior to overnight infection. Expressions of GFP was examined various days post infection using flow cytometry.

**Flow Cytometric sorting:**

Used for sorting GFP negative cells at 8 days post infection. Sorting was done by Louis King and his associates at Michigan State University.

References and Acknowledgements

We thank our blood donors for contributing to our research. We thank Lori Keen for her invaluable technical and managerial support. We also thank Robert Siliciano’s lab for providing plasmids and advice. Lastly we thank Calvin College and NIH (R15AI096991) for financial support.
Abstract

HIV could only infect proliferating CD4+ T cells in vitro because viral reverse transcription and integration are blocked in resting T cells. However, in vivo resting T cells are productively infected, due to interactions with other cell types and cytokines. Previous studies showed that resting CD4+ T cells stimulated by endothelial cells (HUVEC) could be infected by HIV. In this study we examined the roles of lymphatic endothelial cells (LEC) in HIV infection of resting CD4+ T cells. LEC are derived from lymphoid tissue and have more relevance in in vivo interactions with T cells. We found that LEC were able to induce both productive and latent infection, though to a lesser extent compared with HUVEC. Similar to HUVEC stimulation, LEC stimulation of resting T cells does not require direct contact. Such results confirmed the importance of endothelial cells in HIV infection of resting T cells, and in latency formation.

Figures

Figure 1: Infection of resting T cells co-cultured with EC and LEC. Resting CD4+ T cells cultured alone, resting T cells alone with the cytokine IL-6, and resting CD4+ T cells co-cultured with ECs (with (+) and without (-) IFN-γ), were infected with the pseudo virus NL43-dE-GFP. Two types of ECs were used; HUVEC (EC- and EC+) and Lymphatic EC (LEC- and LEC+). GFP expression was measured eight days post infection. Resting T cells alone were included as negative control.
Co-cultured LEC- and LEC+ induce higher latency than resting T cells alone. Eight days after infection, GFP negative cells were sorted by flow cytometry. Half of the sorted cells from each sample were induced with PMA/Ionomycin and Raltegravir. Then the expression of GFP was compared between the induced and un-induced populations. Graph A shows the total infection rates of each induced and un-induced sample. Graph B shows the difference of infections of the populations induced with PMA/Ionomycin and Raltegravir of each type minus the un-induced populations of the same type.
Infection of resting T cells co-cultured with EC in transwells. Resting CD4+ T cells cultured alone, resting T cells alone with the cytokine IL-6, and resting CD4+ T cells co-cultured with ECs (with (+) and without (-) IFN-γ), were infected with the pseudo virus NL43-dE-GFP. Two types of ECs were used; HUVEC (EC and EC+) and Lymphatic ECs (LEC and LEC+). GFP expression was measured six days post infection. Resting T cells alone were included as negative control.

Results and Discussion

- **T cells co-cultured with LECs have increased infection rates.**

  In order to find out how LECs affect the infection rates of resting T cells, resting T cells were co-cultured with LECs. LECs or ECs were placed in the well first, then T cells were placed on top of the LECs or ECs. We evaluated each type of EC with and without IFN-γ and the infection rates of resting T cells with the cytokine IL-6. As seen in Figure 1, both LEC- and LEC+ showed higher infection rates than resting T cells alone. The infection rates with LEC- and LEC+ was close to the infection rates of the resting T cells also containing the cytokine IL-6. However, the infection rates of EC- and EC+ was significantly higher than the infection rates of the LEC- and LEC+. The LEC- and LEC+ more accurately represents the in vivo interactions that occur between endothelial cells, T cells, and the HIV virus. This demonstrates that LECs increase the productive infection of resting T cells.

- **Latent viral infection is increased in resting T cells in contact with LECs.**
In order to access whether in vitro infection of resting T cells co-cultured with EC could result in post-integration latent infection, at day 8 post infection (long enough for most unintegrated viral DNA to decay), GFP- cells were sorted and half of each sample were induced with PMA and Inomycin for 2 days (with Raltegravir to block new viral integration). PMA is known to activate T cells and reactivate latent HIV from T cells. Then looking at the difference in GFP of the induced cells and the un-induced cells the latent infection can be found. As shown in Figure 2A, the induced cells (with PMA) had increased infection over the un-induced cells. Latent infection was found in resting T cells alone, along with the LEC cultures. Figure B shows the difference in infection of the induced cultures and the un-induced cultures. LEC- had the highest latent infection, followed by LEC+. This study shows that latent infection occurs in resting T cells and that LECs likely play a role in developing the latent reservoir in CD4+ resting T cells.

- **The influence of LEC in productive infection is due to soluble factors.**

In order to see if the LECs act on T cells directly to increase productive infection, LECs and ECs were cultured with resting T cells without direct physical contact using transwell plates. T cells were placed above the LECs and ECs inside the transwell, and the LECs and ECs were plated on the bottom. As seen in figure 3, all the LEC cultures and the IL-6 had increased infection rates over resting T cells alone. However unlike the EC cultures, the LEC transwell cultures had similar infection rates to the non-transwell LEC cultures. This suggests that direct physical contact is not responsible for the increase in productive HIV infection in resting T cells when cultured with LECs even though it plays a large role in the productive infection when cultured with ECs.

- **Conclusion and discussion.**

This study has shown that LECs increase productive infection in resting T cells. The effect of LECs on infection of resting T cells is not as large as the effect of ECs, but is similar to the effect of the IL-6 cytokine. This study has also demonstrated that LEC- and LEC+ could induce more latent infection than resting T cells alone. However, LEC- increased latent infections more than LEC+. Finally, this study has shown that direct contact between the LECs and the resting T cells is not needed to increase infection. The influence that LECs have over T cells is mediated by soluble factors without direct contact seeming to play much of a role in the LEC- and with LEC+ direct contact may play a small role in the infection rates of resting T cells. This study showed the importance of LECs in productive and latent HIV infection and additional research must be done to further understand the mechanisms of HIV.