Abstract

In vitro, to effectively infect resting CD4+ T cells with HIV-1 is very difficult. But stimulation of resting T cells with endothelial cells (EC) can effectively achieve this and can lead to latent infection of HIV. In previous studies, it was discovered that no direct contact is required between CD4+ T cells and EC cells for both productive and latent infection to occur. This suggests that soluble factors were involved. In this study, IL-6 was found to induce infection in resting T cells without activation of the T cells. Additionally, IL6 tends to have a larger effect on RA naïve T cells than on RO memory T cells. Anti-IL6 antibody was only partially mitigate the infection rates, that there is an additional cytokine or other molecule, i.e CD2 are involved.

Introduction

HIV is difficult to treat because the virus is known to form latent reservoirs in vivo through CD4+ T cells (1). Not much is known about the mechanism involved with latency formation in infected resting CD4+ T cells. Choi et al. (2,3) discovered that CD4+ T cells can be infected by HIV-1 while remaining a resting phenotype when they were stimulated by endothelial cells (EC). This is further accentuated when Shen et al. (4) later showed that after stimulation by EC, latent infection as well as productive infection happened in resting CD4+ T cells. Unfortunately, not much is known about this interaction between EC and CD4+ T cells. In order to understand the mechanisms and key players that play a role in this stimulation and latency formation process, we looked into soluble factors that were up-regulated after EC stimulation. By understanding more about how IL6 plays a role in this interaction, we can gain more understanding on how resting T cells are infected and latent reservoirs are formed.

Conclusion

This study found that 1) IL6 Stimulation increases infection without increasing activation in the CD4+ T cells (Fig 1.), IL6 stimulation increases infection disproportionaly between RA Naïve cells (Fig 2.) and RO Memory T cells (Fig 3.); the addition of Anti-IL6 antibody partially mitigates infection (Fig 4.) and that the additive effects of Anti IL6 and Anti CD2 antibodies do not completely diminish infection (Fig 5.), suggesting that there are additional intercellular molecules involved. Consequently, further work must be done to wholly understand what other soluble factors are involved in the latent infection of CD4+ T cells.

Methods

Endothelial cells and human resting CD4+ T cells preparations:
Human umbilical vein endothelial cells (HUVEC) were purchased from PromoCell (Germany). EC were pre-treated with IFN-γ (50ng/mL) for 3 days to induce the expression of MHC II (EC+) or not treated (EC−) prior to addition of resting T cells. Resting CD4+ T cells was isolated from PBMC via negative depletion using Miltenyi Microbeads.

Pseudotyped reporter virus NL43-dE-GFP:
The env gene from laboratory HIV strain NL43 was replaced with the enhanced green fluorescence protein (EGFP) gene. Reporter virus was coated with an HIV envelope protein (using CXCR4 as a co-receptor) and only capable of single round infection.

Stimulation of T Cell with IL-6:
Resting T cells were first treated with recombinant human IL-6 (1ug/mL, Biolegend) with similar concentrations to EC cultures that was determined through ELISA assay. The cells were allowed to incubate for 1 day before they were infected with a reporter virus. The levels of cytokine was refreshed 1, 3 and 5 days after infection. LEAF Purified IL-6 antibody (1mg/mL, Biolegend) was added for an hour on plated EC. Then resting CD4+ T cells were added, and the anti-IL-6 antibodies were refreshed 1 and 3 days after infection. On the 6th day, the cells were examined for levels of infection (GFP expression).

Figures and Results

Figure 1. IL6 stimulation increases infection without increasing activation in CD4+ T cells.

Figure 2. A. IL6 stimulation increases infection in RA naïve and RO memory T cells. RA Memory T cells (RA) and RO Memory T cells (RO) were separated via negative column separation. After culturing for a day, the cells were infected with a virus expressing GFP. GFP was examined on day 6 post infection. Resting Naïve T cells and resting Memory cells were used as control. IL6 increases infection in both RA and RO T cells, but as a much great affect on RA Naïve cells than RO memory T cells.

Figure 3. IL6 Titration

Figure 4. Anti IL6 antibody partially mitigates infection in endothelial cells (EC). Anti-IL6 antibody (IL6) and CD2 antibody (CD2) were added to ECs+ for an hour before addition of resting T cells in direct contact. After culturing for a day, the cells were infected with a virus expressing GFP. GFP was examined on day 6 post infection. Resting CD4+ T cells cultured alone or treated with isotype antibody served as controls (R).

Figure 5. CD2 in conjunction with IL6 does not completely diminish infection. Anti-IL6 antibody (IL6) and CD2 antibody (CD2) were added to ECs+ for an hour before addition of resting T cells in direct contact. After culturing for a day, the cells were infected with a virus expressing GFP. The antibodies were refreshed on days 1 and 3 post-infection. GFP was examined on day 6 post infection. Resting CD4+ T cells cultured alone or treated with isotype antibody served as controls (R). CD2 was found to affect infection rates with EC+ but have no effect on infection rates with EC−.

References + Acknowledgements

We thank our blood donors for contributing to our research. We thank Mary J. Dekker and Lori Keen for her invaluable technical and managerial support. We also want to thank Calvin College and NIH (R15A906991) for financial support.