IL6 and CD2 involvement in HIV infection of resting CD4+ T cells stimulated by Lymphatic Endothelial cells.

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Abstract

Infection of resting CD4+ T cells can be stimulated by endothelial cells. Previous research correlates IL6 levels in resting CD4+ T cell and HUVEC cocultures to increased infection rates. In this study, we observed the effects of anti-IL6 and anti-CD2 antibody as well as measured IL6 concentrations in LEC cocultures. Anti-CD2 antibody had little effect on infection rates while anti-IL6 antibody significantly, but not completely, reduced infection rates in LEC. We also saw a clear correlation between IL6 concentrations and LEC passage number.

Introduction

Since its emergence, human immunodeficiency virus (HIV) has refused to yield to modern medicine. Highly active antiretroviral therapy (HAART) can suppress the virus to undetectable levels; however, if medication is discontinued, viral latent reservoirs will allow the infection to rebound (1). We know this phenomenon arises from the infection of resting CD4+ T cells, which forms the latent reservoir. Morris et al. demonstrates that infection of resting CD4+ T cells can be induced by endothelial cells (2). Experiments with human umbilical vein endothelial cells(HUVEC) show a positive correlation between resting CD4+ T cell infection and concentrations of soluble factor interleukin-6 (IL6) (3). We utilized human lymphoid endothelial cells (LEC) to better mimic in vivo conditions and focused on the effects of IL6 and CD2. By studying the interactions between resting CD4+ T cells and LEC, we can gain a better understanding of the mechanism behind latent reservoir formation.

Methods

Endothelial cells and human resting CD4+ T cells preparation.
Lymphatic Endothelial Cells (LEC) were purchased from PromoCell (Germany). LEC were pre-treated with IFN-γ (50 ng/mL) for 3 days to induce the expression of MHC II (LEC+) or not treated (LEC-) prior to addition of resting T cells. Resting CD4+ T cells was isolated from PBMC via negative depletion using Miltenyi and Biolegend Microbeads.

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

Detection of IL-6 using ELISA
Supernatants from cell culture wells were collected and frozen in -80°C. ELISA kits for IL-6 were purchased from BioLegend, and experiments were performed according to manufacturer's instructions. 100µL of supernatants were used from each sample in duplicates.

Stimulation of T cells with anti-IL6 Ab
LEAF Purified anti-human IL-6 antibody (BioLegend) was added to wells at concentrations of 10µg/mL and 5µg/mL immediately after introducing resting CD4+ T cells to LEC/EC.

Stimulation of T cells with anti-CD2 Ab
LEAF Purified anti-human CD2 antibody (BioLegend) was incubated with resting CD4+ T cells for at least an hour before being cultured with the LEC/EC. Anti-CD2 Ab was added in concentrations of 5µg/mL and 2µg/mL

Results & Discussion

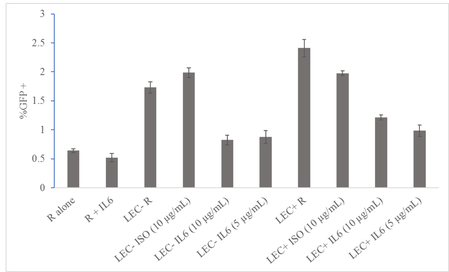

Figure 1. Resting CD4+ T Cells cocultured with LEC show lower infection rates when blocked my anti-IL6 Antibody. Infection rates of resting CD4+ T cells increase when they are cocultured with LEC. When T cells are cocultured with LEC and anti-IL6 Ab, infection is significantly, but not totally, reduced. This tells us that IL6 has a significant role in the spread of HIV. However, there are other factors contributing to increased HIV-1 infection.

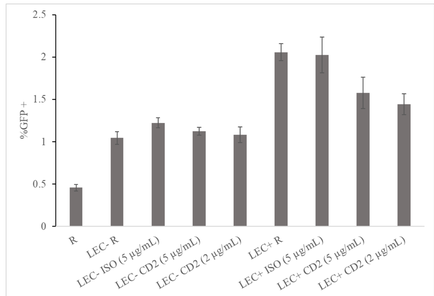

Figure 2. Resting CD4+ T Cells cocultured with LEC show little change infection rates when blocked my anti-CD2 Antibody. When T cells are cocultured with LEC- and anti-CD2 Ab, there is little to no effect of infection rates. When cocultured with LEC+ and anti-CD2 Ab, there is a small drop in infection rates. We can conclude that CD2 plays little to no role in HIV infection of resting CD4+ cells.

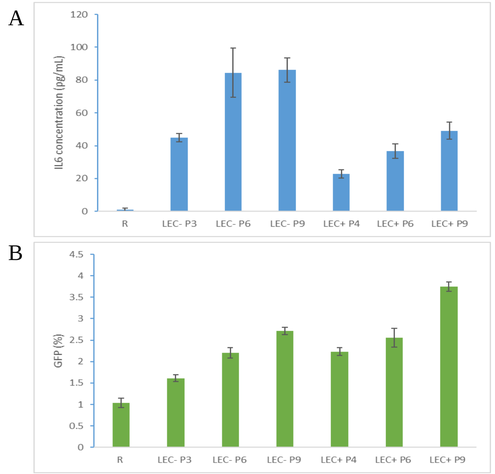

Figure 3 A. IL6 concentration of Supernatant from T-cells cocultured with varying passages of LEC. In LEC-, IL6 concentrations increase as cell passage number and the year increases. In LEC+, IL6 concentrations decrease as cell passage number increases.

Figure 3 B. Percent GFP expression T Cells cocultured with varying passages of LEC. In both LEC+ and LEC- cocultures, we see a significant correlation between T-cell infection rates and cell passage number.

There is a significant correlation between IL6 concentrations and infection rates. In LEC+ cells, even though IL6 concentration is significantly lower compared to the LEC- cultures, the infection rates are comparable. This further supports that there are other factors contributing to increased HIV-1 infection rates in cocultured T cells.

Conclusions

- CD2 blocking had very little effect on the infection of resting CD4+ T cells
- IL6 blocking greatly reduces infection in LEC. However, the reduction is incomplete, suggesting other factors also play a significant role
- Later passages of the LEC correlated with increased IL6 concentrations and increased infection rates

References & Acknowledgements

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