The Roles of Chemokine receptors and intracellular factors in HIV-1 Infection of Resting CD4+ T Cells Stimulated by Endothelial Cells

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Abstract

Resting CD4+ T cells are very resistant to HIV infection in vitro. However, various factors including stimulation by endothelial cells (EC) may render them permissive to HIV infection. Previously, we have found IL6 and CD58 to be involved in the stimulation, but their effects were not complete. After an RNAseq experiment, we found potential cytokines that were over-expressed in EC. In this study, we measured the expression of the receptors for these cytokines on T cells with Flow Cytometry. We also looked for intracellular factors that were potentially up-regulated in resting CD4+ T cells after EC stimulation in the RNAseq experiment. The expressions of these genes were also measured before and after EC stimulation. We found that the expression of chemokine receptor CXCR3 on resting T cells correlates with HIV infection in EC stimulated resting T cells, whereas CXCR2, GITR (TNFRSF18), CX3CR1 were not expressed on T cells with or without EC stimulation. Intracellular factors SOCS3 and KLF10 were expressed by resting T cells, but their levels did not change after EC stimulation.

Introduction

The infection of resting CD4+ T cells with HIV-1 in vivo leads to the formation of the latent reservoir. This makes the virus difficult to eliminate, as antiretroviral therapy has little to no effect on the reservoir (1). It has been previously found that EC secrete cytokines that contribute to both productive and latent infection (2,3). Lymphatic endothelial cells (LEC) are closer to in vivo conditions than EC, and studies have determined the cytokines they secrete (4). Some of the cytokines are similar to those secreted by EC. A previous RNAseq experiment revealed both cytokines that were overexpressed by EC and genes that were overexpressed in CD4+ T cells after exposure to EC. The goal of this study is to find out both if some of those cytokines’ receptors are expressed on CD4+ T cells and if they influence the HIV infection of these T cells. We also looked at some intracellular factors to see their effect on HIV infection of CD4+ T cells.

Methods

Extracting CD4+ T cells and co-culture with LEC:
LEC and EC were cultured and treated with interferon-γ to induce the expression of MHC-class II (LEC+/EC+) or not treated with IFN-γ (LEC−/EC−). After magnetically separating CD4+ T cells from peripheral blood mononuclear cells using a kit from Miltenyi, they were transferred into plates where LEC and EC had been cultured earlier. Some experiments called for the separation of the T cells into memory (RO) and naïve (RA) cells, also separated using a Miltenyi kit, which were then transferred to plates with LEC and EC similarly. One day after the co-culture, the plates were infected using a pseudotyped delt4 virus with a green fluorescent protein (GFP). Infection was allowed for usually 6 days before analysis.

Extracellular staining of receptors:
Six to eight days after infection, the infection rates were analyzed using a Flow Cytometer that would read the amount of GFP expressed by the T cells. The cells were also stained to see how expression of a receptor correlated with infection.

Intracellular Staining of SOCS3 and KLF10:
Because SOCS3 and KLF10 are genes, staining a receptor was not sufficient to get the right information. To stain SOCS3, a primary and secondary antibody were used. To stain KLF10, a different primary and secondary antibody were used. The cells were fixed and permeated prior to staining.

In this study, we were able to conclude that CXCR3 levels correlate with infection. We have also been able to see more of what is, and what is not, expressed by CD4+ T cells. SOCS3 and KLF10 are both expressed by CD4+ T cells and have levels that are unchanged after co-culture with LEC. CXCR2, GITR (or TNFRSF18), and CX3CR1 were not expressed on CD4+ T cells either with or without exposure to LEC in coculture. Further work must be done to determine why CXCR3 levels correlate with infection, if SOCS3 and KLF10 are involved in infection, and if other soluble factors influence HIV-1 infection of resting T cells.

Results

Figure 1. Infection rates differ between CXCR3+ and CXCR3− cells, as well as RO and RA T cells. In this experiment, memory (RO) and naïve (RA) T cells were separated prior to coculture with LEC. This was important to do because RO cells have more CXCR3 than RA cells. Six days post-infection, the RO and RA T cells were tested for both levels of GFP (infection) and stained for CXCR3. As shown here, there tends to be a higher rate of infection in both RO and RA T cells that express CXCR3. It can be concluded that the levels of CXCR3 on RO and RA T cells correlate with infection, with a higher amount of CXCR3 meaning an increased rate of infection. *Student t-test: p < 0.05.

Figure 2. Expression of GITR, CX3CR1, and CXCR2 is consistent with or without coculture with LEC. In this experiment, CD4+ T cells were plated as a coculture with LEC− and LEC+ one day prior to infection. Six days after infection, the cells were stained for either GITR, CX3CR1, or CXCR2 to allow expression levels to be seen. The expression of the three cytokines was under 1% in the cells, and levels of expression did not change with or without the LEC coculture. It can then be determined that GITR, CX3CR1, and CXCR2 are not expressed by CD4+ T cells, and the minute amount of expression does not change with or without LEC coculture.

Figure 3. (A) KLF10 is expressed in CD4+ T cells. (B) SOCS3 is expressed in CD4+ T cells. In these experiments, CD4+ T cells were plated in coculture with LEC− or LEC+ one day prior to infection. Four (SOCS3) to six (KLF10) days after infection, the CD4+ T cells were stained using primary and secondary antibodies to show expression levels. Analysis determined that while KLF10 and SOCS3 are expressed in CD4+ T cells, the level of expression does not change after coculture with LEC.

Conclusions

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