Resting CD4+ T Cells cannot normally be infected successfully with HIV-1 in vitro but when co-cultured with Endothelial Cells (ECs), a high rate of infection is achieved. Using flow cytometry, we characterized various aspects of this co-culture system in the context of HIV infection. We first compared the R5 strain of the virus to the X4 strain of the virus. We found that the R5 strain showed low infection rates. Our EC/Resting T cell co-cultures showed medium infection rates which were higher than the resting CD4+ T cells, but lower than the activated CD4+ T cells. We also investigated the minimum incubation time required to achieve maximum infection in our co-cultures. We found that maximum infection was only reached when the resting T cells were removed from the ECs after a 5 day co-culture but not before. Glut-1 is a transport molecule that is shown to increase productive HIV infection. We found that in our co-cultures Glut-1 expression did not correlate with infection rates. Lastly we found that when resting T cells were placed in transwells with ECs, infection rates were not as high as when resting T cells were in direct contact with ECs, even though the infection rates were still higher than resting T cells cultured alone.

### Results and Discussion

**Abstract**

**Introduction**

In vitro, resting CD4+ T cells show very low levels of HIV-1 infection but in vivo, productive infection occurs. When these resting cells are co-cultured with human endothelial cells (ECs), they show high amounts of infection, sometimes even greater than that of activated CD4+ T cells. Although this system is not yet extensively understood there are some known factors contributing to its phenomenon. One of those factors involved the transfer of cytokines and soluble factors between the ECs and resting T cells. Also, ECs are antigen-presenting cells (APCs) and can be treated with IFN-γ to induce MHC-II expression (EC+). Exploring this co-culture in detail will reveal some of the factors affecting resting cell infection, something that occurs frequently in vivo.

**Materials and Methods**

**Virus Preparation:** Pseudotype viruses were created by removing the env gene from HIV and replacing it with enhanced green fluorescent protein (EGFP). The virus is only capable of single round infection.

**T Cell Isolation and Cell Plating:** Resting CD4+ T cells were isolated via negative bead depletion from HIV negative blood donors. ECs were plated in a normal cell culture plate. The supernatant was removed and new, fresh media was added. Baskets were placed on top and the resting T cells were added. The cells were allowed to incubate for 1 day before infection.

**Transwells:** ECs were plated in a normal cell culture plate. The supernatant was removed and new, fresh media was added. Baskets were placed on top and the resting T cells were added. The cells were allowed to incubate for 1 day before infection.

**Measuring Maximum Incubation Time:** ECs were plated in transwells and resting T cells were added. They were infected with the pseudotype virus and removed at days 1, 3, 5, 7 and 10 of infection. GFP was measured on the flow cytometer at day 7.

**Measuring Protein Expression:** GLUT-1 was measured using an anti GLUT-1 antibody and a secondary, IgG-2a antibody. GFP+ cells were fixed using 2% formaldehyde. Analysis was done immediately on the flow cytometer.

**Results and Discussion**

**Figure 1: Comparison of R5 and X4 Infection Rates.** Comparing the two viral strains showed different patterns. The R5 virus shows lower infection rates, the co-cultures show lower infection than the activated cultures, but higher than resting alone.

**Figure 2: Maximum Incubation Time for Co-cultures.** Cells were incubated in co-cultures and removed at different days post infection. Infection rates increase until around day 5 and plateau afterward.

**Figure 3: Comparison of Glut-1 Expression and Infection Rates.** The Glut-1 levels for resting and EC+ cultures are similar, while EC+ levels are slightly higher. Expression of Glut-1 in activated cells is very high, even higher than infection rates.

**Figure 4: Comparisons of Co-cultures in Transwell versus Contact.** For each cell type, infection rates are higher when full contact is available. EC+ show the biggest difference between transwell and contact.

**R5 virus shows different infection rates than X4 virus**

R5 virus shows, in general, lower infection rates than the X4 virus (figure 1). In the co-cultures the rates of X4 infection are higher than that of the activated cells, but for the R5 virus the co-cultures have substantially lower infection rates than the activated. With both strains of virus, the infection rates for the EC+/+ cultures is significantly higher than the resting CD4+/+ T cells alone.

**Minimum incubation time for co-culture is around 5 days**

Infection rates for both of the EC+/+ co-cultures increase with further incubation, but seem to level off around day 5 (figure 2). Past day 5, the differences in infection are not significantly different.

**Glut-1 expression does not correlate with HIV infection rates**

There seems to be no correlation between Glut-1 expression and infection rates (figure 3). For the resting, EC- and EC+ the Glut-1 levels are similar, but the infection rates are substantially different. For the activated cells Glut-1 expression is very high, but the infection rates are not higher than that of the co-cultures.

**Co-cultures in transwells show higher infection rates than normal resting T cells, but not as high as co-cultures with direct contact**

We found that when cells had cell to cell contact the infection rates were much higher (figure 4). The implications of this are not conclusive. One major possibility is that there is freer soluble factor interaction when the cells are not in transwells. The biggest difference in infection occurs with the EC+ co-culture. This seems logical, knowing that EC+