Introduction

Productive HIV infection of cells begins with the binding of the virus to the CD4+ T cell helper cells. As the virus membrane virus is shed, the envelope of the virus contains proteins and two copies of single strand RNA (ssRNA), is released into the cytoplasm of the cell. Viral reverse transcriptase converts the ssRNA into DNA and then viral integrase inserts this DNA into the host cell genome. When this DNA is transcribed it will result in the production of copies of the virus that go on to infect more cells. Activated infected cells are somewhat quickly discovered by the immune system and destroyed, but infected resting CD4+ cells lead to the production of a latent reservoir of infected cells that the body does not destroy. Understanding the process through which this latent reservoir is formed brings key information to understanding of the HIV/AIDS epidemic. Before, it was generally accepted that the virus could not infect resting CD4+ cells, however recent in vitro studies have shown that co-cultural CD4+ T cells with endothelial cells (EC’s), the cells lining blood vessels, can increase viral infection rates without activating the CD4+ T cells. This study demonstrates the trends seen for in vitro infection of resting cells, it was shown that co-cultural CD4+ cells, when co-cultured with endothelial cells (EC), can be infected in vitro. The process and timing of the infection of resting, co-cultured, and activated cells was examined in this study. It was determined that infection rates were directly proportional to concentrations of virus added for all cell types until infection rates leveled off at high concentrations. Those highest concentration rates showed typically moderate infection rates for activated cells (about 10%), slightly lower for cells co-cultured with EC’s with interferon, and lower yet for EC’s without interferon. Resting cells were shown to have the lowest infection rates at less than 1%. Also the life of the GFP-HIV virus in culture was determined with a half-life of about 8 hours. Viability of CD4+ cells for in vitro experimentation was modeled using data on the lifespan of the cells after PBMC isolation, structural viability of dead cells, and proliferating time of activated cells determined in these experiments. These results combine to provide more information on the behavior of the cells and virus during in vitro experimentation.

Materials and Methods

Pseudotyped virus NL43-de-GFP:
The Env gene from laboratory HIV strain NL43 was replaced with the enhanced green fluorescence protein (EFP) gene including an endogenous reticulum retention sequence. This fluorescent protein allows for indication of infection using flow cytometry. Reporter virus was coated with an HIV envelope protein (using CXCR4 as a co-receptor) and only capable of single round infection. This is accounted for in the Results and Discussion section.

Cell isolation and In vitro infection:
Human umbilical vein endothelial cells (HUVEC) were purchased from PromoCell (Germany). 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.

Acknowledgement and References

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Conclusion

This study demonstrates the trends seen for in vitro GAP-HIV infection of CD4+ cells. It was further evidenced that EC’s do stimulate resting CD4+ infection, however concrete numbers cannot be determined because potency of viral infections has been shown to be dependent of both the batch of virus made and on the blood donor. Infection-induced cell death was shown to have little to no effect on cell viability in vitro. Infection rates were shown to increase with concentration of virus up to a certain point as concentrations hit a saturation limit. Also viral half-life in vitro was demonstrated to be between 7-9 hours. Further investigation is needed and expected to be done to provide even better information on the process and timing of the GAP-HIV viral infection in vitro, yet this is a good basis for future exploration.

Abstract

HIV has long been known to only productively infect activated CD4+ T cells in vitro experimentation because reverse transcription and integration are blocked in resting cells. However, activated cells would not form a latent reservoir in vivo as they would be eradicated by the immune system, so progressions to understanding how resting cells are infected in vivo has needed to be made through in vitro experimentation. Recently it has been shown that co-culturing CD4+ T cells, when co-cultured with endothelial cells (EC), can be infected in vitro. The process and timing of the infection of resting, co-cultured, and activated cells was examined in this study. It was determined that infection rates were directly proportional to concentrations of virus added for all cell types until infection rates leveled off at high concentrations. Those highest concentration rates showed typically moderate infection rates for activated cells (about 10%), slightly lower for cells co-cultured with EC’s with interferon, and lower yet for EC’s without interferon. Resting cells were shown to have the lowest infection rates at less than 1%. Also the life of the GFP-HIV virus in culture was determined with a half-life of about 8 hours. Viability of CD4+ cells for in vitro experimentation was modeled using data on the lifespan of the cells after PBMC isolation, structural viability of dead cells, and proliferating time of activated cells determined in these experiments. These results combine to provide more information on the behavior of the cells and virus during in vitro experimentation.

Results and Discussion

• Cell Viability for GFP-HIV in vitro experiment
To test cell viability, CD4+ T cells were isolated from their PBMC (peripheral blood mononuclear cells) cultures and cultured in wells with 300,000 cells per well. EC’s: cells were co-cultured with the CD4+ resting cells while ACT and R cells were cultured by themselves. One plate of wells of each cell type was infected with GFP-HIV virus, at a concentration of 20μL of virus to 300μL of cells, to test differences in viability of infected and uninfected cells. After 2, 4, and 6 days of culturing, the cells were manually counted and analyzed using flow cytometry. Cells under flow cytometry with high forward side scatter (FSC) were deemed as alive. Those with low FSC were deemed as dead, while cells with extra large or small FSC were deemed as either debris from disintegrating cells or other cells such as EC’s, and these were ignored. The total population of uninfected cells (live and dead) was shown to decrease 10-20% to debris over the course of six days for CD4+ resting cells and those with EC co-cultures. Further, EC+ cells were shown to increase at a significantly triple the population in four days and then start decreasing on day six. All unactivated cells were shown to transition from live to dead by about 50% by day six, with EC+/ co-cultured cells making that transition slightly slower than purely resting CD4+ cells (Figure 1). For infected cells (Figure 2), viability was relatively similar to the uninfected cells, showing little to no effect of infection-induced cell death. For EC+ cells, this was different. For ACT and R cells, CD4+ cell viability dropped slightly, possibly hinting at infection-induced cell death for populations with higher infection rates. Rates of infection, in terms of total number of infected cells divided by the total number of cells were shown to be .025, 3, and .6% for Resting, EC+, and EC- cells. This represents the possible null effect of infection-induced cell death at such low infection rates of resting and EC- cultured cells. Infected ACT cells were similar again to the uninfected versions, but highest cell counts (due to proliferation) were shown at day six rather than day four.

• Mathematical Modeling of Cell Viability of Uninfected Cells
To model cell death and disintegration, typically exponential equations are used. Instead, a delayed-death assumption was made in this case because dead cells were shown only to rise dramatically in population when infected. ACT cells were cultured with EC- and R cells. Infected cells were shown to rise dramatically at high concentrations. This was caused by the MOI of the virus being below one, so when virions infect resting cells, high concentrations of virus led to infection rates leveling off around 10% for ACT and below 1% for resting cells. EC- co-cultured cell infection rates tended to vary within the range of ACT and resting, with EC+ being higher than EC-. The leveling off at high concentrations shows a saturation effect on the virus, exemplified by the linear increase in infection rates with low concentrations of virus before the plateau of infection rates at high concentrations (Figure 3). A relationship of the fraction of infected cells to the concentration of virus can be modeled using the following equation.

\[ \frac{dN_{infected}}{dt} = \frac{dN_{total}}{dt} \times \left(1 - \frac{N_{infected}}{N_{total}}\right) \]

This assumes m as the MOI, which is directly proportional to the virus concentration. This also assumes a saturation effect, \( P_{infected} \), as indicating that some of the cells are not able to be infected. The model is used as the lines in figure 3, where the \( P_{infected} \) was fitted to the data.

• Virus Viability
To determine a half-life for the GFP-HIV virus, the virus stock was incubated for 0, 1, 2, 3, and 4 days at 37°C before being used to infect cell cultures. For each cell type, infection rates were relatively constant for the first couple days of incubation and then declined exponentially for the last days (Figure 4). This is caused by the MOI of the virus being below one, so when virions are lost, infection rates do not drop at first. A model, showing the decay of the virus stock, was made by relating the number of infected cells to the maximum number of infected cells based on the virus concentration as it approaches 0 over the course of the decay.

\[ \frac{dN_{infected}}{dt} = \frac{dN_{total}}{dt} \times \left(1 - \frac{N_{infected}}{N_{total}}\right) \]

Here, \( N_{infected} \) is the number of infected cells based on virus incubation time. \( N_{total} \) is the maximum number of infected cells of the virus concentration and the half-life of the virus is the half-life the virus was determined through the data (Figure 4), and was shown to be between 7-9 hours.