**Lymphatic Endothelial Cells Interact Differently with Memory and Naïve Resting CD4+ T Cells to Promote HIV-1 Infection**

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**Introduction**

HIV, the virus which leads to AIDS, currently infects nearly 40 million individuals worldwide (2). While the virus can be controlled using highly active antiretroviral therapy, it will rebound if the medication is discontinued due to stable latent reservoirs of virus inside the body (3). CD4+ T cells comprise a large portion of this latent reservoir and previous studies have shown that human umbilical vein endothelial cell (EC) stimulation of resting CD4+ T cells results in increased productive and latent infections (1). In this study, we used a more physiologically relevant type of endothelial cell derived of lymphoid tissue (LEC) and compared the infection rates of resting CD4+ T cells stimulated by LEC to those stimulated by EC. We also compared infection rates among memory and naïve resting CD4+ T cells cocultured with EC and LEC.

**Methods**

**Cell Preparation**

- Human umbilical vein endothelial cells (EC) were purchased from PromoCell and Lymphatic endothelial cells (LEC) were purchased from ScienCell Research Laboratories.
- The cells were either pre-treated with IFN-γ (50 ng/ml) for three days to induce the expression of MHC II (EC+ and LEC+) or not treated (EC- and LEC-) prior to being cocultured with the T cells.
- Resting CD4+ T cells, memory T cells, and naïve T cells were each isolated from PBMC via negative depletion using Miltenyi microbeads.

**Infection**

- The virus used was a pseudotyped strain of HIV capable only of single-round infection.
- The virus was introduced to the cells one day after coculturing.
- Infection rates were measured 3-8 days after infection using flow cytometry to detect GFP.

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**Abstract**

Previous research has shown that human umbilical vein endothelial cell (EC) stimulation can render resting CD4+ T cells permisssible to HIV-1 infection. We expanded upon this research by using endothelial cells derived of lymphoid tissue (LEC) which have more relevance in vivo and comparing the results. We found LEC stimulation significantly increased HIV infection rates in resting T cells and had later peak of infection than EC stimulation. Upon LEC stimulation, memory (RO) T cells had much more infection than naïve (RA) T cells, but the pattern is different than EC stimulation.

**Results and Discussions**

- Resting CD4+ T cells stimulated by Lymphatic Endothelial Cells (LEC) show later increases in infection rates than those stimulated by Human Umbilical Vein Endothelial Cells (EC)
- Resting CD4+ T cells stimulated by Lymphatic Endothelial Cells (LEC) show later increases in infection rates than those stimulated by Human Umbilical Vein Endothelial Cells (EC) (Fig. 2). In this experiment, LEC-, LEC+, EC-, and EC+ cells were each plated separately into wells on a 24-well plate. Following an incubation period of about four hours, 300,000 CD4+ resting T cells were added to each well and the cells were infected the next day. Infection rates were measured on days 3, 6, and 8 post infection. While infection rates in resting CD4+ T cells cocultured with EC increased or plateaued after day six post infection, the infection rates in resting CD4+ T cells continued to rise leading up to day eight after infection. Also, cells cultured with LEC showed lower infection rates overall than those cultured with EC. These differences suggest that the mechanisms by which the LEC stimulate the T cells differ from those used by the EC, an observation important to our research as the LEC have more relevance in vivo.

**Conclusions**

- For resting CD4+ T cells, stimulation by LEC results in greater infection rates than in T cells alone, similar to EC.
- T cells cocultured with LEC show a later peak of infection than T cells cocultured with EC.
- For naïve T cells, LEC+ stimulation has a greater effect than LEC- stimulation, a trend also observed in EC.
- Interestingly, memory T cells cocultured with LEC- show greater infection rates than those cocultured with LEC+ which differs from the pattern exhibited by memory T cells and EC. This discovery suggests that the LEC are interacting with the memory T cells differently than the EC.

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