Introduction

During acute HIV infection, T cells are selectively depleted and many become dysfunctional. Proteins associated with HIV infection alter function and growth of specific T cell subsets through various mechanisms. Naturally arising CD4+CD25+ regulatory T (Treg) cells are essential for maintenance of immunological self-tolerance and immune homeostasis; the depletion of which produce a variety of autoimmune diseases. In context of HIV, Treg cells are shown to have simultaneous effects; in one of which the Treg cells decrease chronic immune activation, thereby slowing disease progression, while another study showed that they might inhibit immune responses, thereby hastening disease progression. Recent literature suggests the importance of Th17/Treg balance in the maintenance of mucosal integrity. During HIV infection, this balance is lost due to the selective depletion of Th17 cells, which indicates an innate mechanism that slows the infection of Tregs. Gp120, main envelope protein of HIV, is known to cause impairment of mucosal barrier function. Both soluble and virion forms of gp120 have a direct effect on the PI3K/Akt signaling pathway, a critical pathway in development of suppressive function of Tregs.

The suppressive function of Tregs was characterized with staining of PBMC with Carboxyfluorescein succinimidyl ester (CFSE) dye, which allows staining of lymphocytes and thus can be used to monitor proliferation of PBMC through Flow Cytometry possible.

**HYPOTHESIS:** Gp120 interferes with the immunosuppressive function of Treg cells.

Objectives

1) To differentiate Treg cells from naive T cells and determine normal suppressive function.

2) To determine the effect of gp120 on differentiation and function of Treg cells.

Methodology

For the optimization assay, PBMC (freshly-isolated and frozen) were stained with CFSE, with and without activation with anti-CD3 (1µg/mL), which is an antibody that binds to the CD3 receptor on the surface of T cells. Proliferation of PBMC was measured by Flow Cytometry on Days 0, 2, 5 and 7 (when necessary).

Conclusion

Due to restriction in time, the experiment procedure was only completed once (n=4). The ineffectiveness of the experimental controls disallowed a proper conclusion to be reached. The optimization assay yielded a novel protocol for efficient detection of change in proliferation of CFSE-stained PBMC activated with anti-CD3 (1µg/mL). This protocol will be used to carry out the experimental procedure again and for future experiments pertaining to this project.

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