Does HIV gp120 affect function of regulatory T cells?

Chintan Dave[1], Jason Fernandes[2], Jonathan B. Angel[1,2]

[1] Department of Biochemistry, Microbiology and Immunology, University of Ottawa, Ottawa, Canada

[2] Ottawa Health Research Institute, Ottawa, Canada

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 shown to cause impairment of mucosal barrier function. Both soluble and virion forms of gp120 have a direct effect on the PI3K/Akt signalling 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 T_{reg} cells.

Objectives

1) To differentiate T_{reg} cells from naive T cells and determine normal suppressive function.

2) Determine the effect of gp120 on differentiation and function of T_{reg} cells.

Methodology

Induction of $T_{\alpha CD3}$

CD4+ T cells + plate bound anti-CD3 —5 days→ IL-2 —2 days→ Fresh medium —5 days→ $T_{\alpha CD3}$

Assay for $T_{\alpha CD3}$ suppression

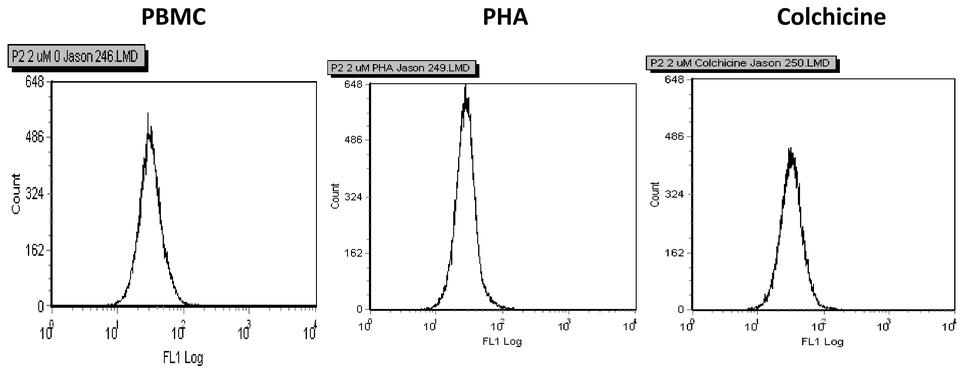
PBMC + $T_{\alpha CD3}$ + Soluble anti-CD3 —3 days→ Proliferation

Figure 1. The induction of regulatory T cells by anti-CD3 monoclonal antibody

For the optimization assay, PBMC (freshly-isolated and frozen) were stained with CFSE, with and without activation with anti-CD3 (1u/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).

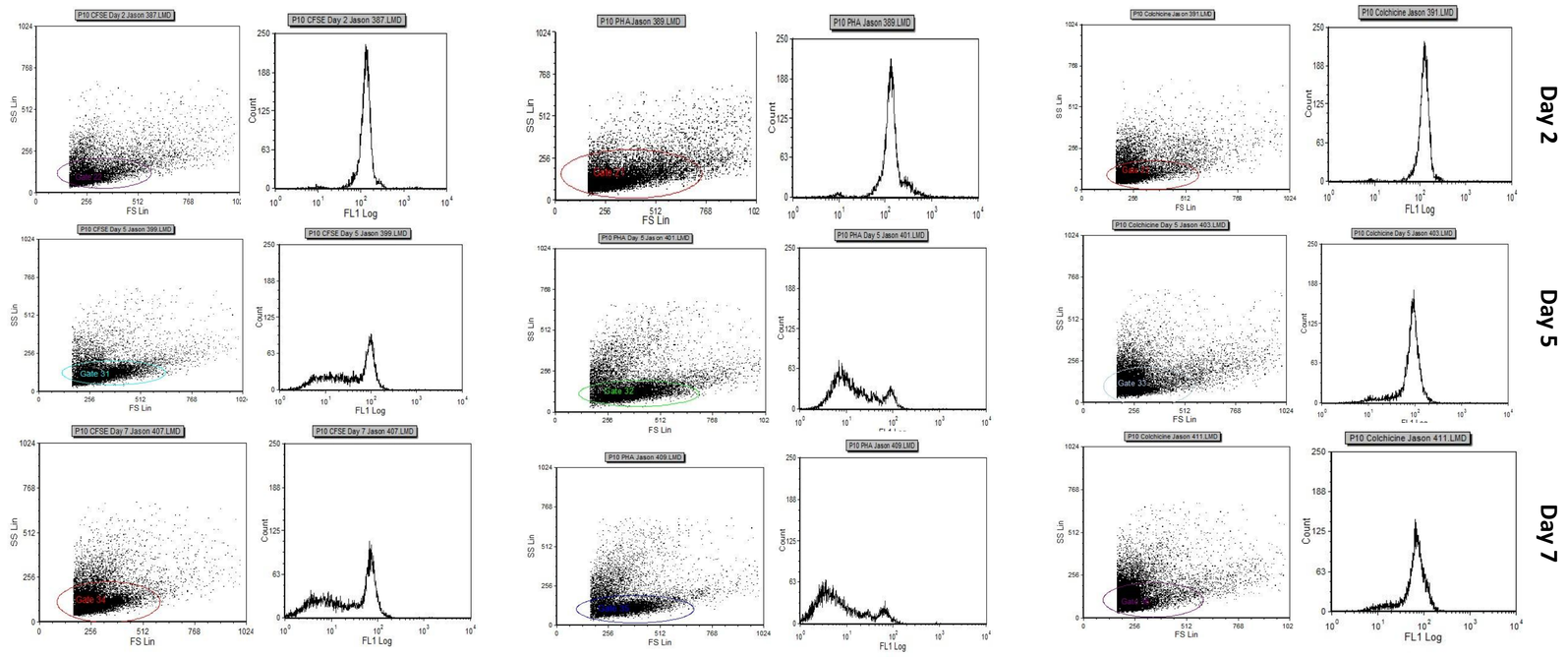
Results

Proliferation Assay:



CFSE-labelled PBMC (70 x 10^3) were cultured with varying amounts of T_{reg} cells (0-50 x 10^3) for 2 days and with PHA and colchicine serving as experimental controls.

Optimization Assay:



CFSE-labelled PBMC (70 x 10^3) were activated with anti-CD3 mAb (1μg/mL) and cultured for upto 7 days, where PHA and colchicine were used as controls.

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.

Acknowledgements

I would like to sincerely thank Dr. Jonathan Angel for providing me the opportunity to work in his lab. I would also like to personally thank Jason Fernandes for his continuous support and help throughout my time at the lab. I greatly appreciated the help and input from fellow labmates at Dr. Angel's lab and Dr. MacPherson's lab. Lastly, I extend my thanks to the donors who were gladly willing to donate their blood to make this research possible.
Thank You.