Comparing the Immunosuppressive Capacity of Human Mesenchymal Stromal Cells to Prescription Drugs in a Dose Dependent Manner Using Principal Component Analysis

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Background

The purpose of this study was to develop a quantitative immunosuppression assay and compare clinically relevant immunosuppression drugs to human mesenchymal stromal cells (hMSCs). Many immunosuppressive drugs have been known to target T-cell function by inhibiting certain signals that lead to activation of T-cells, while hMSCs have gained significant attention due to their anti-inflammatory and immunomodulatory properties. In this work, a parallel dose response study was performed using three FDA approved drug treatments (Cyclosporin; Mycophenolic acid (MPA); Rapamycin) and two hMSC lines (BM2893; RB14), and the immunomodulatory properties of the drug treatments and cell lines were quantitatively compared.

Introduction

Traditionally, immunosuppression assays have measured decreases in T-cell proliferation in response to an immunosuppressive treatment. To receive a more nuanced idea of a treatment's effect on a group of T-lymphocytes, one could use this canonical method of measurement and measure proliferation at different doses to the treatment being applied. As technology advances, methods that examine multiple parameters and produce more nuanced datasets provide a potentially more complete view of immunosuppression than just measuring T-cell proliferation alone. As measuring proliferation at multiple doses gives a more complete idea of how the treatments effect the cells, measuring multiple parameters at different treatment dosages would present a more complete explanation of how the cells are responding to a particular treatment.

In this work, we measured 7 different parameters of T-cells (PBMCs) exposed to either drug treatments or hMSCs. In addition to the canonical measurement of proliferation, we stained the treated PBMCs for viability, TNFα, INFγ, and CD25, as well as group the various PBMCs into populations of CD4+ and CD8+ cells. In order to analyzed this information, we used principle component analysis (PCA) to reduce the dimensionality of the data.

Principle Component Analysis and T-Cell Proliferation

Prescription Drugs

hMSC Lines

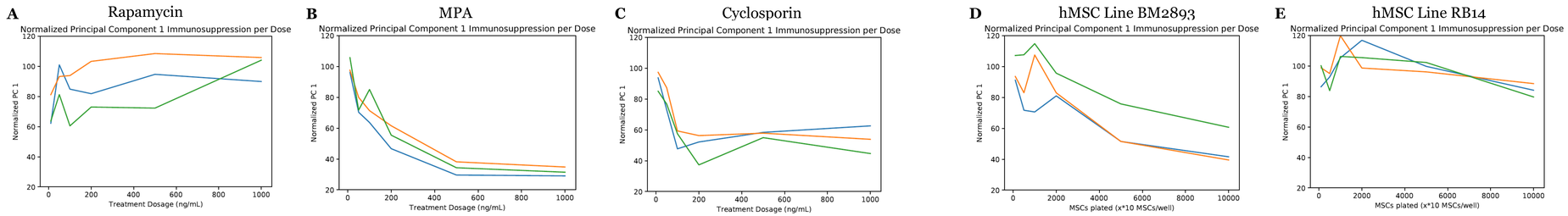

Figure 2. Principal component analysis reduces multiple parameters indicative of T-cell immunosuppression. Principal component 1 of the PCA as it changes in response to the concentration of A: Rapamycin; B: MPA; C: Cyclosporin; D: BM2893 hMSCs; E: RB14 hMSCs. Data shown is from PBMCs from three different donors – Blue Donor 1, Orange Donor 2, Green Donor 3.

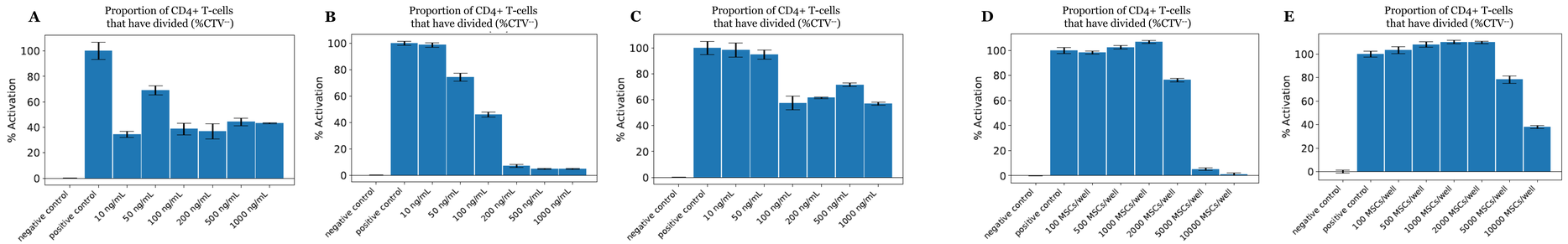

Figure 3. CellTrace Violet Staining of CD4+ T-cells Shows Different Patterns of Immunosuppression. The canonical measurement of immunosuppression showing the proliferation of T-cells when treated with A: Rapamycin; B: MPA; C: Cyclosporin; D: BM2893 hMSCs; E: RB14 hMSCs. Data shown is from PBMC donor 1.

Materials and Methods

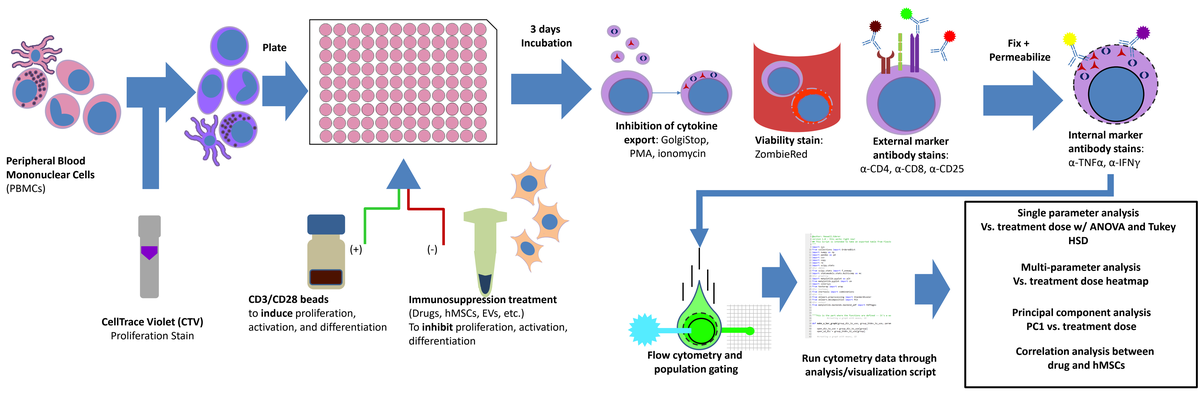

Figure 1. Schematic of the workflow for the immunosuppression assay and analysis. PBMCs are activated with CD3/CD28 beads and immunosuppressive treatments are added to the plate at different concentrations to suppress the activation from the beads. After incubation with the immunosuppressive treatments, the PBMCs (both CD4+ and CD8+ cells) are stained for cell viability (ZombieRed), proliferation (CellTrace Violet), cytokine production (TNF-α and INF-γ), and activation (CD25). Flow cytometry is subsequently used to measure each parameter.

Drug and hMSC Treatment Correlation

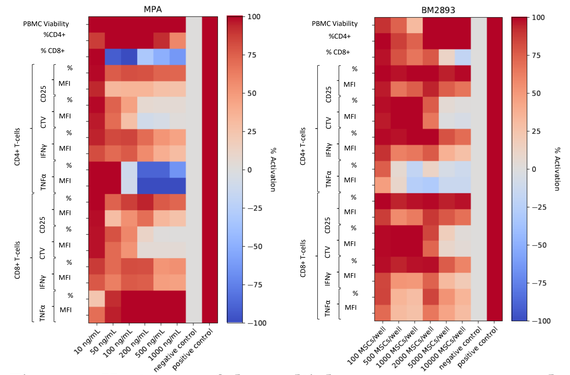

Figure 4. Heatmaps of the multiple parameters measured and analyzed for MPA and BM2893. Color indicates magnitude of the decrease in parameters, with positive control (activated/no treatment) normalized to 100 and negative control (unactivated) normalized to 0. Measurements are either Median Fluorescence Intensity (MFI) or percentage of activated cells. Data shown is from PBMC donor 1.

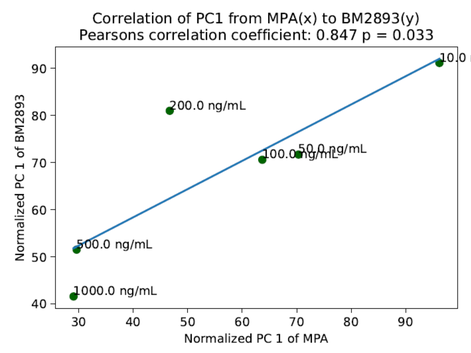

Figure 5. MPA and BM2893 are correlated. Comparing similar doses of MPA to the hMSC line BM2893 shows a high correlation between the principal components of the data from the two treatments.

Acknowledgements

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