Presence of Immunosuppressive Drugs Affect Innate Immune Response and Monocyte Differentiation in Lung Transplant Patients

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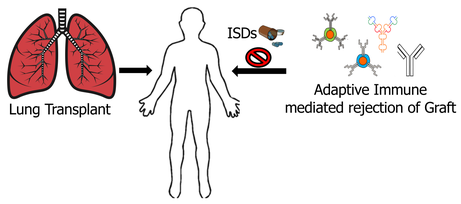
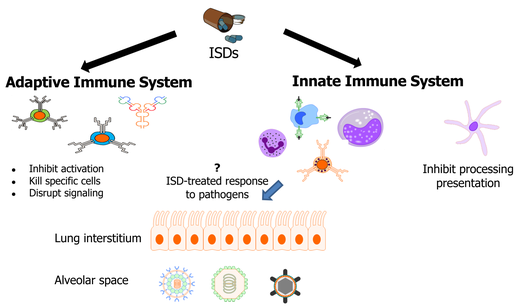
Introduction

Lung Transplant



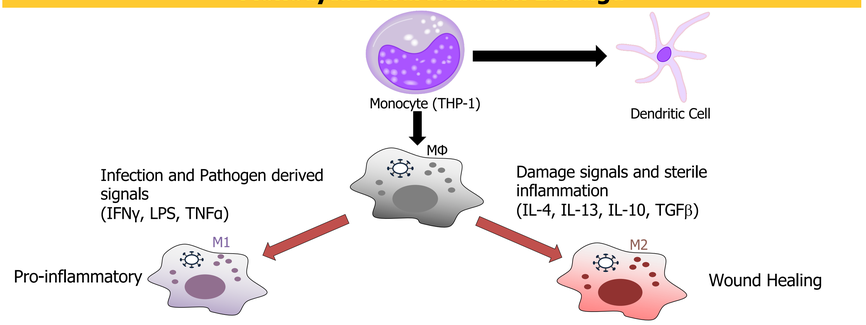
- Lung Transplants are a unique challenge as these organs require prevention from rejection but must still be able to defend against exposure to outside pathogens
- Patients are treated with Immunosuppressive Drugs (ISDs) to prevent transplant rejection
- ISDs prevent rejection AND inhibit immune response to pathogenic microbes (1).

Immune Response to Immune Suppression



- Immunosuppressants prevent rejection by acting against the adaptive immune system response to the graft (2).
- Immunosuppressants suppress the presentation function of dendritic cells
- Exposure to pathogens in the lung occurs in the alveolar space

Monocyte Differentiation Lineage



Experimental Question:
How do ISDs affect Innate Immune Response to Pathogens?

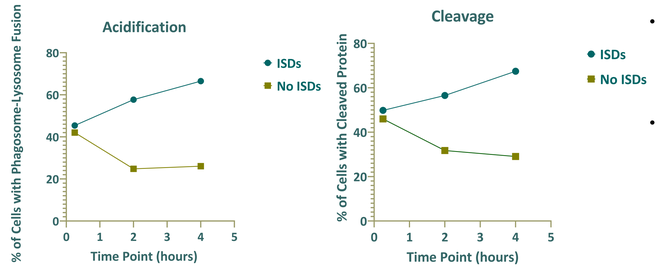
Methods

- Human Monocyte Cell Lineage (THP-1s) were treated with Immunosuppressive regimen used in Lung Transplants which included **Cyclosporin A, Azathioprine, and Prednisone**.
- Cells were harvested at different time points (15min, 2 hours, 4 hours) to check for the percent of cells with completed antigen presentation through Flow Cytometry.
- THP-1s were treated with PMA to allow for differentiation into macrophages.
- Differentiated THP-1s were then treated with course of ISDs followed by transfection or treatment with different viral mimics as well as LPS and CpGA (bacterial mimics).
- Cells were harvested the following day and RNA from cells was extracted.
- RNA from cells was then synthesized into cDNA to allow for running on the PCR.
- cDNA then treated with SYBR green PCR Mix, Primer mix, and Water to prep for PCR, and then PCR conducted.

Experimental Design

Results

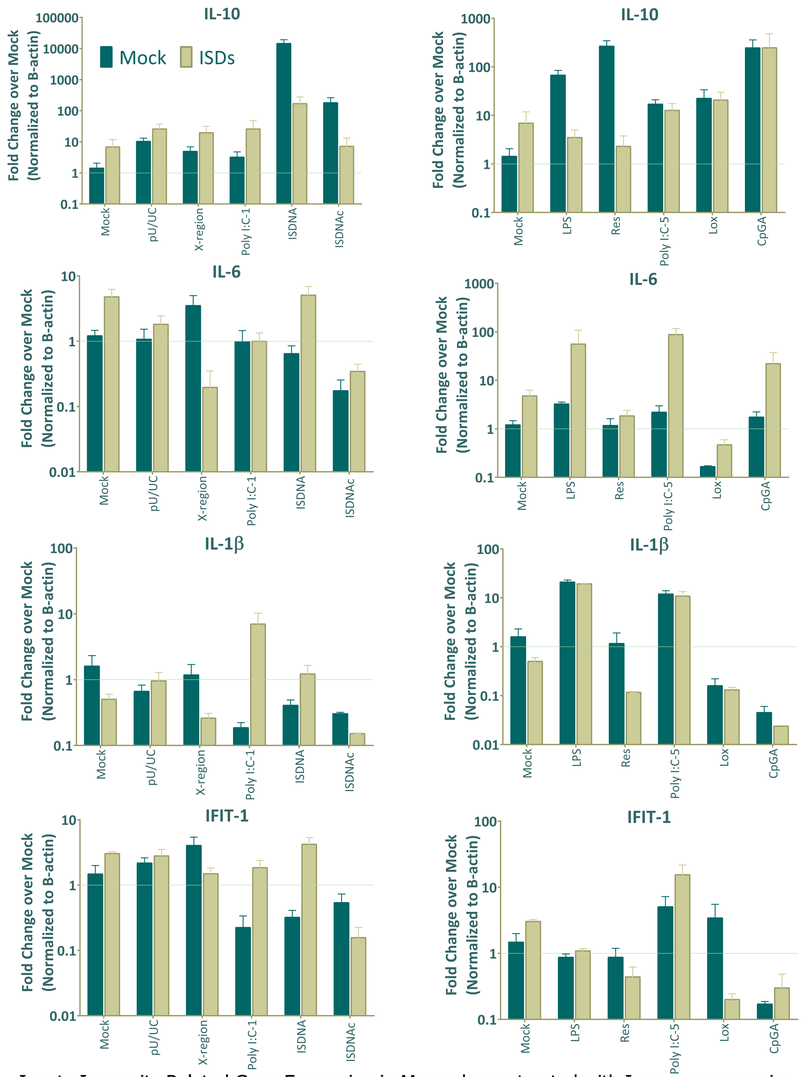
Figure 1: ISD effect on Monocyte Differentiation into Macrophages



- The presence of ISDs acidified and cleaved a higher percent of cells compared to the non-ISD control cells
- Presence of ISDs seems to allow processing of antigen (% cleaved cells) but prevents full degradation of FITC marker (seen in the normal non-ISD treated cells at different time points)

Figure 2: Innate Immunity Related Gene Expression

Cytosolic Sensors	Toll-Like Receptors

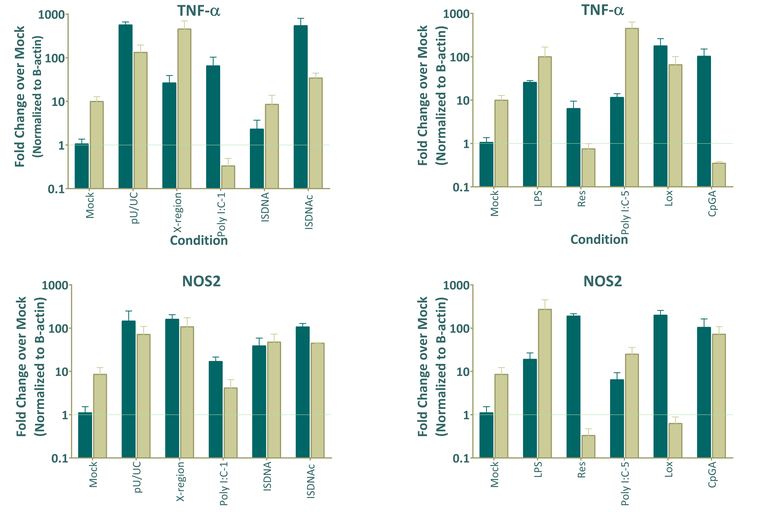


Innate Immunity Related Gene Expression in Macrophages treated with Immunosuppressive Drugs in the presence of Viral Mimics (and some bacterial).

Results

Figure 3: M1 associated Gene Expression

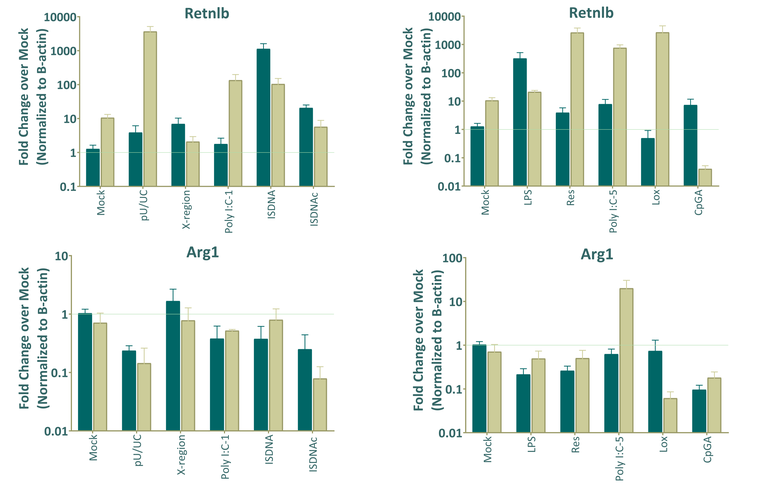
Cytosolic Sensors	Toll-Like Receptors



M1 (Pro-inflammatory) Related Gene Expression in Macrophages treated with Immunosuppressive Drugs in the presence of Viral Mimics (and some bacterial).

Figure 4: M2 Associated Gene Expression

Cytosolic Sensors	Toll-Like Receptors



M2 (Wound Healing) Related Gene Expression in Macrophages treated with Immunosuppressive Drugs in the presence of Viral Mimics (and some bacterial).

Future Direction

1. Assess dendritic cells in response to ISDs:
   - Gene expression
   - Differentiation capabilities
   - Functional innate response to viral mimics
   - Chemotaxis
2. Conduct experiments that test the mechanism of action of Immunosuppressive Drugs that prevent Monocyte differentiation into Macrophages
3. Assess what property of specific ISDs allows some viral and bacterial mimics to prevent an immune response vs. illicit an immune response even in the presence of Immunosuppressive Drugs.
4. Reconduct ISD response to Viral Mimic exposed Macrophages experiment

References

1. Scheffert JL, Raza K. *J Thorac Dis.* 2014 **2.** LaRosa DF, Rahman AH, Turka LA. *J Immunol.* 2007

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