Combined MSC-antiviral treatment for T cell injury in cats with spontaneous feline infectious peritonitis (FIP)

Rachel W. Qiao, Patrawin Wanakumjorn, Diego Castillo, Amir Kol

Department of Pathology, Microbiology, and Immunology, School of Veterinary Medicine, University of California, Davis

UC DAVIS VETERINARY MEDICINE
Students Training in Advanced Research

Background

- Feline infectious peritonitis (FIP) is a common and lethal disease of cats caused by infection with an enteric coronavirus that mutates to infect macrophages and cause severe systemic inflammation[1]
- Lymphocyte apoptosis and T cell exhaustion are suspected contributors to FIP pathogenesis, resulting in lymphopenia, lymphoid tissue atrophy, and further disease progression[2-4]
- GS-441524 is an experimental antiviral that has successfully treated FIP.[5] However, its ability to regenerate damaged lymphoid tissue and restore normal immune function post-infection is unknown
- Multipotent stromal cells (MSCs) have immunomodulatory and regenerative properties[6,7]
- We are interested in exploring the use of MSCs as a combined therapeutic with GS-441524 in order to support long-term recovery from FIP

Hypothesis and Aims

Hypothesis: FIP induces T cell injury that results in cell loss and exhaustion, and a combined MSC-antiviral therapy will support restoration of depleted lymphocyte populations and rejuvenation of exhausted T cells to a greater extent than antiviral therapy alone

- **Aim #1:** Determine lymphocyte subset proportions (B, T, CD4, CD8) upon presentation and over the course of treatment
- **Aim #2:** Determine the frequency of polyfunctional T cells upon presentation and over the course of treatment

Methods

Double-blinded and randomized clinical trial with 10 client-owned cats with effusive FIP

- Treatment groups (each n=5)
  1) Combined antiviral-MSC
  2) Antiviral-saline (placebo)
- All cats received GS-441524 PO daily for 11 weeks
- MSC/placebo infusions administered at weeks 1 and 3
- Blood collected at weeks 0, 1, 3, 7, 11
- 5 cats from a specific pathogen free (SPF) colony as healthy controls

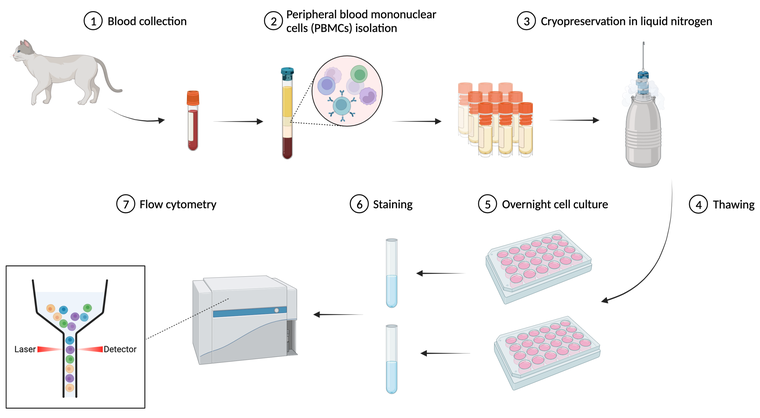


Figure 1. Preparation process of blood samples for flow cytometry

Methods (continued)

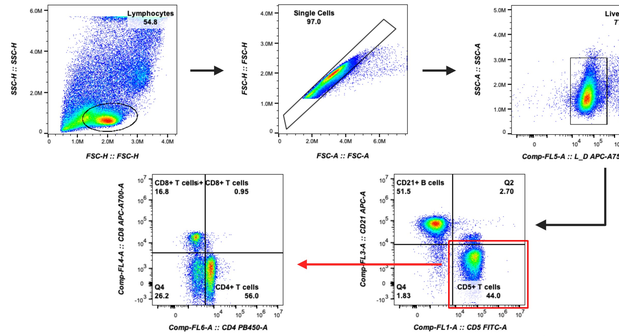


Figure 2. FlowJo gating strategy for Panel 1 (surface markers)

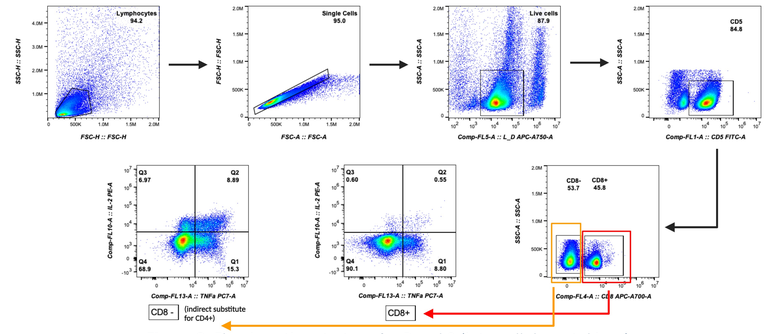
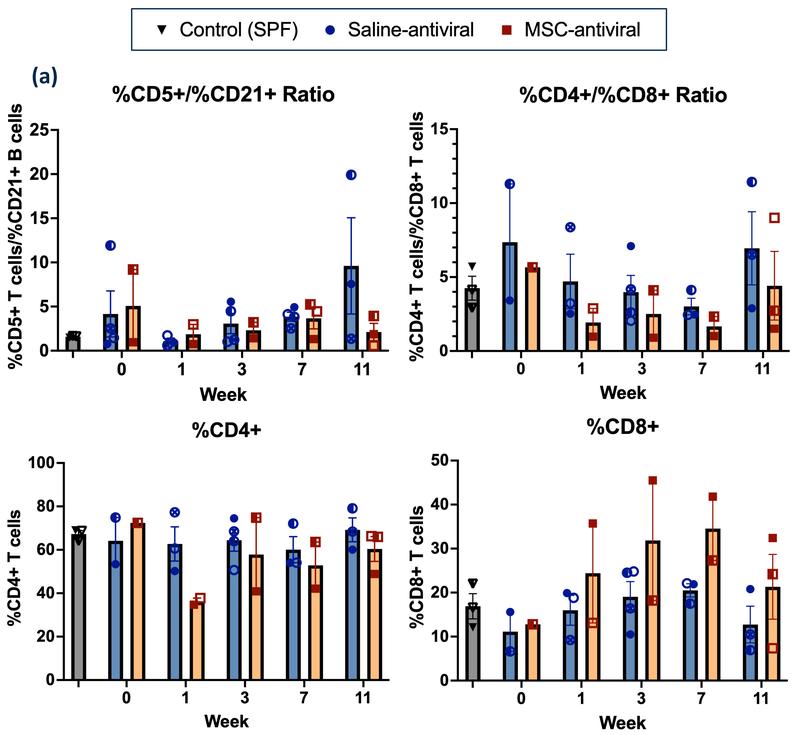


Figure 3. FlowJo gating strategy for Panel 2 (intracellular cytokines)

Results



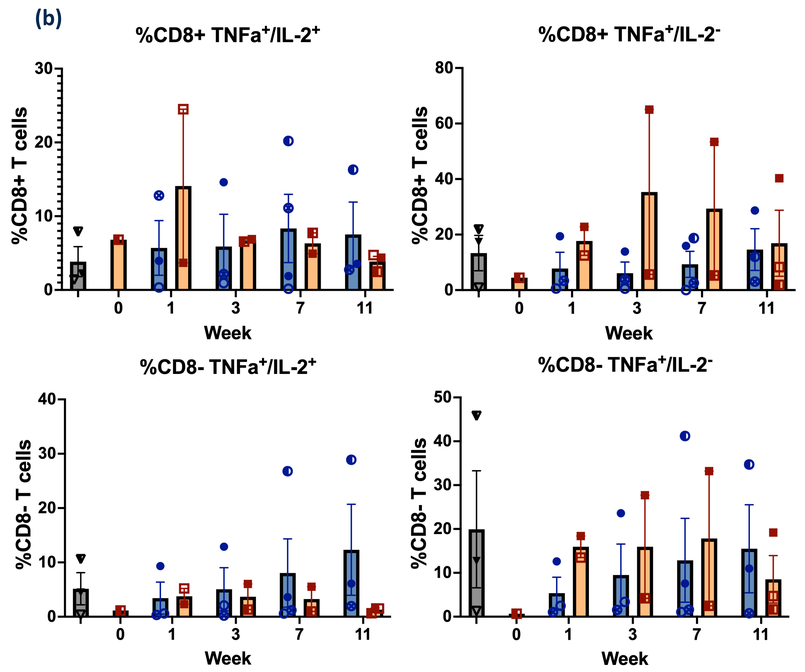


Figure 4. (a) Lymphocyte subset proportions and **(b)** cytokine production levels over time

Discussion

- Completed Panels 1 and 2: 7 FIP cats (3 MSC, 4 saline) and 3 SPF cats
  - Some data points not viable
- Cats with FIP have a markedly increased proportion of events within the lymphocyte gate that are both CD5- and CD21- upon presentation
- Cats with FIP have highly variable CD5+/CD21+ ratios upon presentation, some of which are markedly increased compared to healthy controls
- Cats with FIP have increased variability in CD4+/CD8+ ratios upon presentation and over the course of both treatments
- No apparent difference in CD4+ or CD8+ proportions between healthy and FIP cats upon presentation
- No apparent difference in CD4+ proportions following both treatments
- Both treatment groups showed an upward trend in CD8+ proportions over time
- Future directions
  - Continue data analysis
    - Correlation of flow results with hematology, cytokine, and clinical data
  - Acquire data for remaining 3 FIP and 2 SPF cats
  - Panel 3: exhaustion markers
    - L/D, CD5, CD4, CD8, CTLA-4, EOMES, FAS, TOX

Acknowledgements

Funding for this study was provided by National Institute of Child Health and Human Development 1R21HD106027-01

Financial support was provided by the Students Training in Advanced Research (STAR) Program through National Institutes of Health T35-OD010956

Thank you Amir Kol, Pat Wanakumjorn, Diego Castillo, Krystle Reagan, Brian Murphy, Tamar Cohen-Davidyan, Jully Pires, Ehren McLarty, and Terza Brostoff

References