MHC I Knockout Through B2M Disruption to Enhance Allogeneic Feline Mesenchymal Stromal Cells Immunomodulation

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Introduction

- Mesenchymal stromal cells (MSCs) are of great interest due to their immunomodulatory functions; however, clinical inconsistencies in their efficacy remain present.[1,2] Major Histocompatibility Complex I (MHC I) allows immune cells to detect and ultimately destroy allogeneic (”non-self”) cells.[3] MSCs naturally express low levels of MHC I which was thought to provide them immunoprivilege.[3] However, it is also possible that a pro-inflammatory microenvironment may increase MHC I expression causing destruction of the MSCs.[4,5]
- In cats, MSCs have been shown by our group to be a curative treatment for the immune-mediated disease, Feline Chronic Gingivostomatitis (FCGS).[6,7] However, the use of allogenic MSCs provided a lower efficacy and took longer to induce its effects (Figure 1).

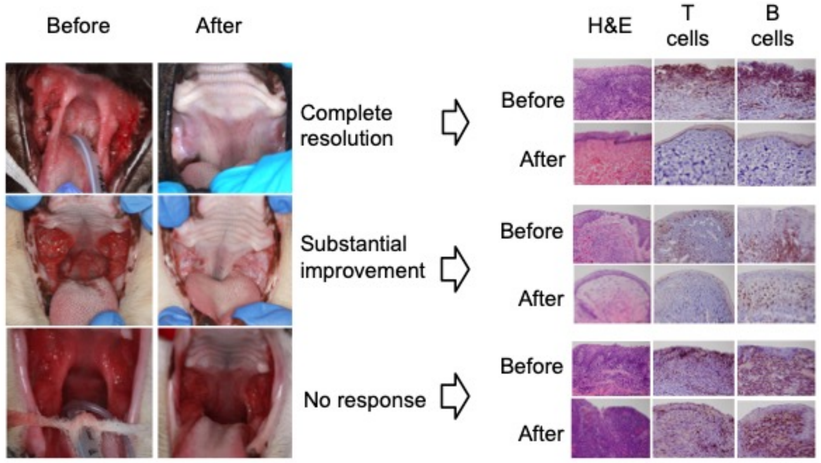
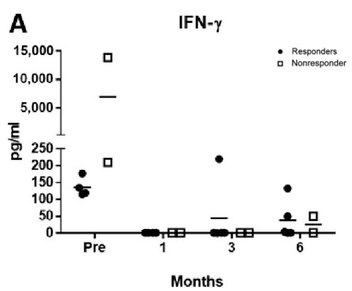


Figure 1:[6,7] Demonstration of the clinical effects of MSC treatment in patients with FCGS grossly and histologically

- Patients with FCGS also have increased levels of the pro-inflammatory cytokine INF- γ which we propose increases MHC I expression and increases detectability of MSC upon IV administration (Figure 2).



Thus, **we hypothesize** that allogeneic MHC I-null MSCs can be generated through the knockout of conserved subunit B2M using CRISPR/Cas9 gene editing in feline MSCs and that this KO will not compromise MSCs immunomodulatory capacity.

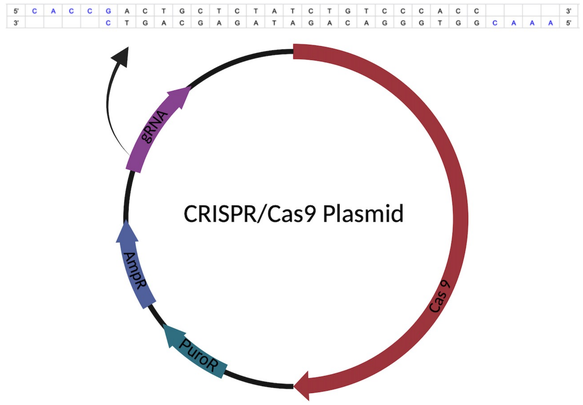
Figure 2:[6] Serum INF- γ concentrations demonstrating high levels in FCGS cats before MSC treatment

Methods

Generation of the CRISPR/Cas 9 Vector

- A segment (20bp guide sequence=sgRNA) of the feline B2M gene was amplified followed by insertion into the PuropSpCas9(BB)-2A-Puro (PX459) vector plasmid (Addgene).

gRNA sequence from B2M



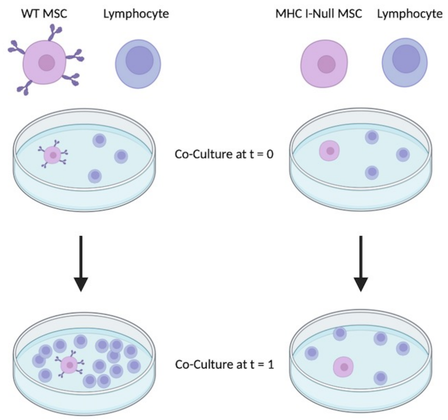
- The plasmid was amplified in bacteria, promising clones were collected, and DNA was isolated for PCR and Sanger sequencing to confirm insertion and correct orientation of the sgRNA.

Transfection

- 3 donor lines of feline MSCs were transfected with the generated plasmid to form MHC I-null MSCs.
- gDNA and RNA will be isolated in each cell line to allow for sanger sequencing and QT-PCR. Flow cytometry will be used to confirm protein level MHC I KO.

Lymphocyte Proliferation

- MSCs will be co-cultured with lymphocytes to determine if proliferation differs between wild-type and MHC I-null cell lines.



Results

MHC I Expression on MSCs

- We were able to demonstrate through flow cytometry that stimulation of feline MSCs with INF- γ upregulates MHC I expression on the surface of the MSC I (Figure 3).

Plasmid Vector Generation

- The plasmid vector was successfully generated and amplified in bacteria.
- Three MSC cell lines were transfected with the plasmid and currently are undergoing puromycin selection.

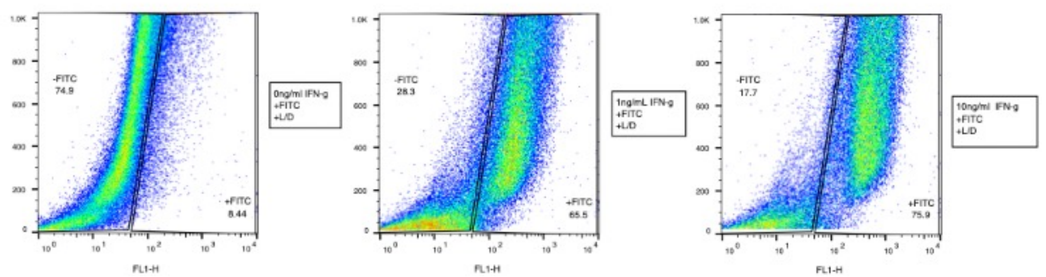


Figure 3: Flow cytometry results following stimulation of feline MSCs with 0ng/ml, 1ng/ml, and 10ng/ml INF- γ. MHC I was label with anti-body and FITC. This demonstrates that MHC I expression is upregulated following INF- γ stimulation at 1ng/ml and 10ng/ml.

Summary

- This study has demonstrated that feline MSCs increase MHC I expression upon INF- γ stimulation. Since INF- γ is increased in cats with FCGS, it may be the reason for reduced efficacy of allogenic MSCs (they are detected and destroyed by the recipient's immune system).[6,7]
- A CRISPR/Cas 9 plasmid vector was generated, amplified in bacteria, and confirmed to contain the B2M sequence in the correct location and orientation.

Future Directions

- Next, a temporal study of MHC I expression following INF-γ stimulation to determine how soon after INF- γ exposure MHC I expression changes will take place.
- Following successful KO of MHC I in 3 feline cell lines, a proliferation study will be completed to compare the effects of MHC I-null and wild-type MSCs on lymphocytes in co-culture.

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