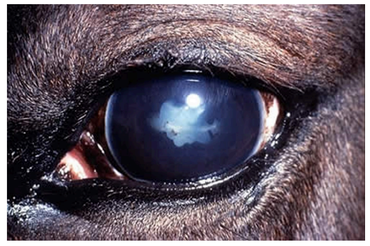
Equine Mesenchymal Stem Cell Interactions with CD4+ T cells

Laurel Saldinger, Kaitlin Clark, Naomi Walker, Dori Borjesson
Department of Pathology, Microbiology, & Immunology, UC Davis School of Veterinary Medicine

Introduction

Equine recurrent uveitis (ERU) is a devastating disease known to affect up to 15% of horses, and is the leading cause of blindness in horses. ERU is an immune-mediated disease that develops through activation of T cells. Previous data from the Borjesson Lab has shown horses with ERU to have differences in T cell phenotype, with raised CD62L+ and IFNγ and lowered IL10 expression compared to normal horses.



With their immunomodulatory properties, mesenchymal stem cells (MSCs) may be a promising therapy for ERU. Activated T cells are known to stimulate MSCs, which in return secrete factors that can decrease T cell activation. MSCs have previously been used to treat autoimmune uveitis, and a clinical trial treating ERU with MSCs has already begun. **We hypothesize that co-incubation with equine MSCs will alter the CD4+ T Cell activation phenotype by decreasing CD25, IFNγ, and Foxp3 while increasing intracellular IL-10. MSCs will also increase CD4+ T cell effector memory cells by decreasing CD62L.**

Objectives

1. Determine if MSCs alter T cell phenotype *in vitro* looking at surface CD25 and CD62L and intracellular Foxp3, IL-10, and IFNγ.
2. Determine if phenotype changes occur through contact and/or soluble factors.

Materials and Methods

MSCs
- MSCs were adipose-derived and were previously collected and expanded by the Borjesson Lab

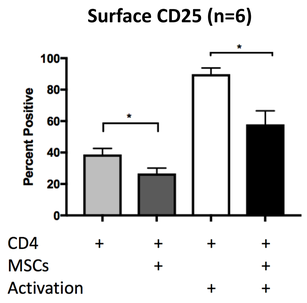
CD4+ T cells
- Whole blood samples were collected from the jugular vein of horses housed at the Center for Equine Health
- Blood samples were centrifuged on a density gradient to isolate mononuclear cells
- CD4+ T cells were positively selected for using a LS Column (Miltenyi Biotec)

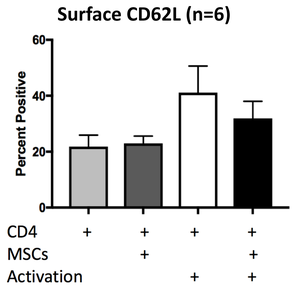
Co-incubation
- MSCs were co-incubated at a ratio of 1:5 with CD4+ T cells for 4 days
- Four conditions were assessed: CD4+ T cells alone, CD4+ T cells with MSCs, activated CD4+ T cells alone, and activated CD4+ T cells with MSCs
- Co-incubations were done with contact between MSCs and CD4+ T Cells, without contact (transwell), and PGE2 blocked (using indomethacin)

Flow Cytometry
- After co-incubation, surface expression of CD25 and CD62L and intracellular expression of Foxp3, IFNγ, and IL-10 were assessed

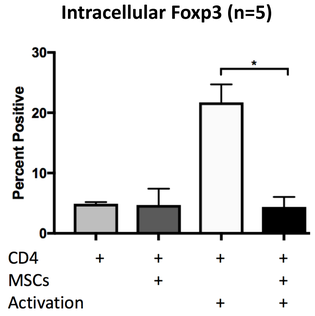
Results

Co-incubations with Contact

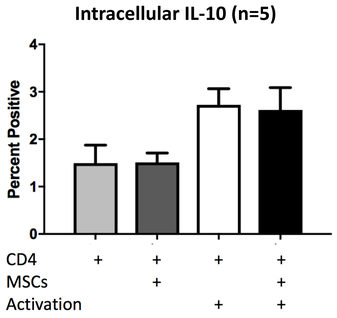


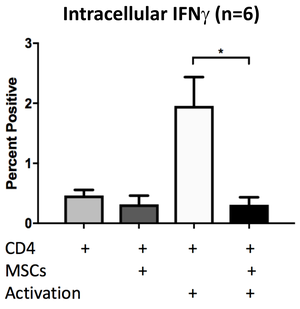


MSC co-incubation decreased surface CD25 expression both with and without activation of the CD4+ T cells. MSCs did not cause a change in surface CD62L expression.



Activation increased CD4+ expression of Foxp3, which was decreased when co-incubated with MSCs.



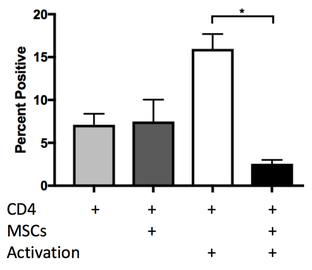


MSCs did not change intracellular IL-10 expression of CD4+ T cells. MSCs did reduce IFNγ expression in activated CD4+ T cells.

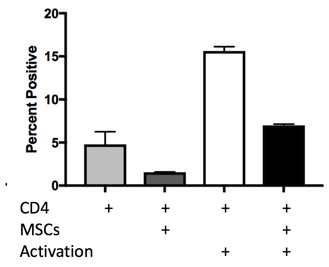
*Bars with an asterisk indicate significant differences in mean values with $p<0.05$

Preliminary Results

Co-incubation without contact



PGE2 Blocked Co-incubation



Preliminary data show that co-incubation with MSCs resulted in an 84% decrease in Foxp3 expression from activated CD4+ T cells even in the absence of cell-cell contact.

When PGE2 was blocked, co-incubation with MSCs reduced Foxp3 expression by 55%.

*Bars with an asterisk indicate significant differences in mean values with $p<0.05$

Conclusions

- Co-incubation with MSCs reduced CD4+ T cell expression of CD25, Foxp3, and intracellular IFNγ when T cells were activated
- CD25 expression is also reduced in the absence of activation
- MSCs may have an effect on CD62L expression
- MSCs have no effect on IL-10 expression
- Preliminary data shows that the reduction in Foxp3 expression is not mediated through contact, but may be partially PGE2 mediated

Overall, MSCs were seen to decrease the CD4+ T cell activation phenotype, which continues to be a promising indicator for treatment of CD4+ T cell mediated diseases. Future directions for this research include continuing the no contact and PGE2 blocked co-incubations, as well as continuing clinical trials using MSCs to treat horses with ERU.

Acknowledgements

Thank you to the STAR Program and the NIH for supporting this research and the Center for Equine Health for funding this project.