Adipose-derived mesenchymal stem cells as a potential therapy for equine recurrent uveitis

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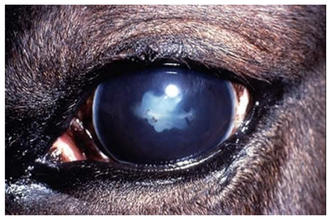
Introduction

Equine recurrent uveitis (ERU) is an immune-mediated, devastating disease that affects up to 15% of horses. ERU is currently the leading cause of equine blindness.



With their immunomodulatory properties, mesenchymal stem cells (MSCs) may be a promising therapy for ERU. Activated T cells are known to stimulate MSCs, which then secrete factors that 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 hypothesized that 1) horses with ERU would have a different immune cell phenotype than healthy horses and that 2) Equine MSCs would alter the T cell activation phenotype by decreasing IFNγ, and Foxp3 while increasing intracellular IL-10.**

Objectives

1. Determine if there are differences in immune cell phenotype between control and ERU horses *in vivo*.
2. Determine if MSCs alter T cell phenotype *in vitro* (CD25, CD62L, Foxp3, IL-10, and IFNγ).
3. Determine how MSCs alter T cell activation *in vitro* (cell-cell contact and/or soluble factors).

Materials and Methods

Phenotyping
- Whole blood was taken from 10 healthy control horses and 7 ERU horses
- Blood samples were centrifuged on a density gradient to isolate mononuclear cells
- Cells were labelled for CD3, CD4, CD8, CD21, CD25 and CD62L as well as the intracellular cytokines Foxp3, IFNγ, and IL-10 and assessed via flow cytometry

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 magnetic beads (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
- MSC- CD4+ T cell co-incubations were done with or without contact (transwells); for some experiments, prostaglandin (PGE2) was blocked with indomethacin

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

Acknowledgements

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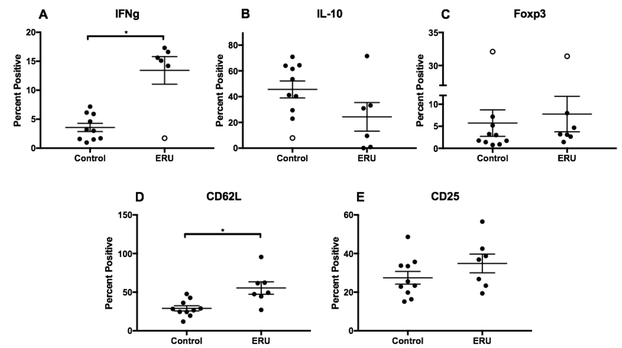
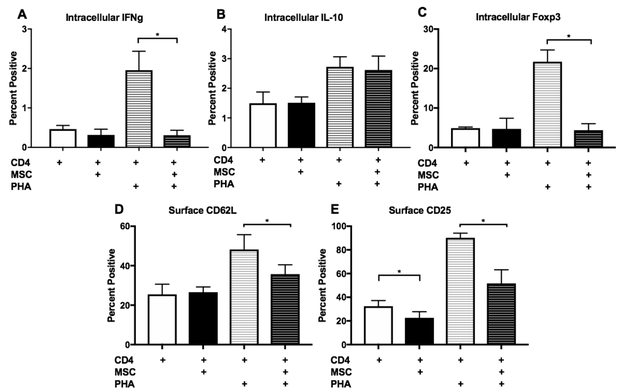
Results

CD4+ T Cell Phenotype



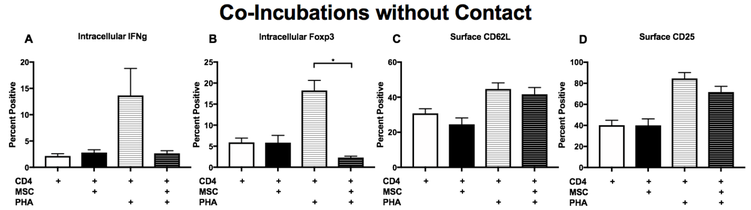
ERU horses have an activated CD4⁺ T cell phenotype. Horses with ERU had higher levels of IFNγ (p=0.01) and showed a trend towards expressing lower levels of IL-10 (p=0.07), indicative of a shift towards a Th1 activation phenotype. There was no difference in Foxp3⁺ expression of CD4⁺ T lymphocytes between ERU horses and control horses (p=0.32). ERU horses also expressed a higher percent of CD4⁺ CD62L⁺ T cells (p=0.01), but showed no difference in CD25 expression (p=0.17). Open dots represent outliers.

Contact Co-Incubations



Co-incubation with MSCs decreased CD4⁺ T cell activation phenotype. Activated CD4⁺ T cells co-incubated with MSCs showed decreased levels of IFNγ (p=0.01), Foxp3 (p=0.02), and CD62L (p=0.05). MSCs did not change activated CD4⁺ T cell expression of IL-10 (p=0.567). Expression of CD25 by CD4⁺ T cells decreased both with and without activation (p=0.02, p=0.01) when co-incubated with MSCs.

Co-Incubations without Contact

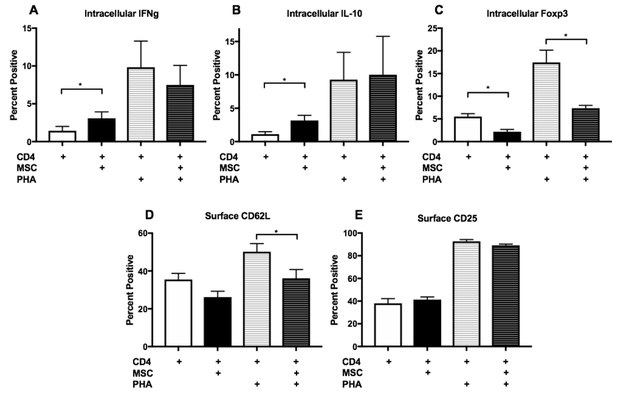


MSCs use a soluble mediator to decrease CD4⁺ T cell expression of Foxp3 and IFNγ. Without contact, MSCs still showed a tendency to reduce activated CD4⁺ T cell expression of IFNγ (p=0.08) and showed a reduction in Foxp3 similar to co-incubation with contact (p=0.01), indicating a soluble mediator was responsible for these changes. MSCs reduced expression of CD62L in 4 out of 6 lines without contact and reduced expression of CD25 in 5 out of 6 lines without contact. Overall, the reduction of CD62L (p=0.8) and CD25 (p=0.12) in activated CD4⁺ T cells was not significant.

*Bars with a single asterisk represent differences in mean value with p<0.05

Results

Prostaglandin Blocked Co-Incubations



Prostaglandin signaling reduces non-activated CD4⁺ T cells response to MSCs. In the absence of prostaglandin signaling, non-activated CD4⁺ T cells increased their IFNγ (p=0.02) and IL-10 (p=0.01) secretion when co-incubated with MSCs.

Prostaglandin is required for MSC reduction of CD25 and IFNγ expression in activated CD4⁺ T cells. When prostaglandin signaling was blocked, MSCs showed reduction of IFNγ in activated CD4⁺ T cells in 3 out of 5 lines (p=0.41), as opposed to 5 out of 5 lines when prostaglandin signaling was occurring. Co-incubation without prostaglandin also showed increases in IL-10 in activated CD4⁺ T cells in another 3 out of 5 lines (p=0.75), though there was no significant difference overall. MSCs were able to reduce Foxp3 (p=0.01 and p=0.01) and CD62L (p=0.06 and p=0.02) expression in both non-activated and activated CD4⁺ T cells without prostaglandin signaling. However, MSCs were not able to reduce CD25 expression (p=0.15) in the absence of prostaglandin.

Conclusions

- ERU horses show an activated CD4⁺ T cell phenotype, with increased levels of IFNγ and decreased levels of IL-10, and are likely skewed towards a Th1 response.
- MSCs reduce CD4⁺ T cell activation by reducing levels of IFNγ, Foxp3, CD25, and CD62L in activated T cells.
- MSCs rely on a soluble mediator to decrease intracellular IFNγ and Foxp3, and may rely on a soluble mediator to reduce CD62L and CD25.
- Prostaglandin signaling is required to reduce non-activated CD4⁺ T cell reaction to MSCs, as seen by increases in IFNγ and IL-10 when co-incubated with MSCs in the absence of prostaglandin.
- Prostaglandin signaling is also required to reduce CD25 and IFNγ expression in activated CD4⁺ T cells, as contact co-incubations with MSCs were able to reduce expression of both CD25 and IFNγ, but co-incubations without prostaglandin were not.