Equine Mesenchymal Stem Cell Interactions with CD4+ T cells

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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

Preliminary Results

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.

Co-incubation without contact
Intracellular Foxp3 (n=3)

PGE2 Blocked Co-incubation
Intracellular Foxp3 (n=2)

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.

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