Comparison Of The Immunosuppressive Properties Of Allogeneic And Autologous Equine Bone Marrow Derived Mesenchymal Stem Cells

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Introduction: Bone marrow derived mesenchymal stem cells (BMDMSCs) have shown promise in the treatment of musculoskeletal injuries in the horse. However, the use of culture expanded autologous stem cells requires a critical, two to three week expansion period after individual bone marrow aspiration. This delays treatment and renders it costly. If allogeneic BMDMSCs are found to be non-immunogenic, then the use of allogeneic cells will provide an alternative, readily available and potentially less costly treatment. The initial step in ensuring the safety of intra-articular injection of allogeneic stem cells requires assessing their ability to cause an immune response or hasten the immune response in vitro. Exposing one horse’s lymphocytes to mesenchymal stem cells of a different (allogeneic) horse allows for analysis of the T cell response to BMDMSCs. Flow cytometry using CFSE stain provides an optimal method of measuring lymphocyte cell division and, therefore, immune reaction. An immune response can be measured by an increase in lymphocyte proliferation; alternatively, immune suppression is documented by a decrease in lymphocyte division. If these cells are determined to be non-immunogenic or even immune suppressive then further in vivo work is warranted. The first objective of the study was to determine if allogeneic BMDMSCs caused an immune response or, alternatively, created immune suppression. After completing the first phase of the in vitro study, the second objective was to determine the mechanism by which BMDMSCs may cause immune suppression.

Methods: Initially, BMDMSCs from 8 horses were compared for their ability to stimulate proliferation of equine lymphocytes in vitro, using both allogeneic and autologous lymphocyte samples. Equine autologous and allogeneic BMDMSCs were cultured in vitro with activated (concanavalin-A ) equine T lymphocytes at ratios of 1:10, 1:100, and 1:1000 (MSC: lymphocyte). Peripheral blood mononuclear cells (PBMCs) were labeled with CFSE, stimulated with concanavalin-A and incubated with BMDMSCs for 90 hours. After incubation, intracellular IFNγ staining was completed prior to flow cytometry. Dual staining flow cytometry was utilized to assess BMDMSCs effects on lymphocyte proliferation by the CFSE dye dilution method and intracellular IFNγ production. Results were compared to a positive control of CFSE labeled, concanavalin-A stimulated PBMCs. Data was analyzed using a student T-test with significance set at p<0.05. To determine the mechanism used by BMDMSCs for immunosuppression of PBMCs, MSC and PBMCs were cultured at a ratio of 1:10 (MSC:lymphocyte) and percent of reversal of immunosuppression of the PBMCs was measured by reversing pathways that may be responsible for
immunosuppression. PGE2, reactive oxygen species, TGFß, and Indoleamine 2,3-dioxygenase were inhibited after stimulation with concanavalin-A. PGE2 was inhibited with indomethacin. Indoleamine 2,3-dioxygenase was inhibited with 1-MT. Reactive oxygen species was inhibited with N-acetylcysteine, and TGFß was inhibited by SD208.

**Results:** Autologous BMDMSCs suppressed proliferation by 22%, while allogeneic BMDMSCs suppressed proliferation by 29% from the positive control (Figure 1). A student T-test revealed no difference between proliferation suppression by autologous versus allogeneic MSC (p= 0.11). A dose-dependent suppression of INFγ production by T cells occurred when autologous or allogeneic BMDMSCs were added to the activated cultures (Figure 2). Preliminary data suggests the strongest reversal of suppression was noted with indomethacin, the reversal agent of PGE2 (Figure 3). Continued research is focused on identifying the possibility of multiple mechanisms of suppression.

**Discussion:** Decreased lymphocyte proliferation and a decrease in intracellular IFNγ production indicate allogeneic BMDMSCs to be non-immunogenic, and suggest an immune suppression of equine PBMC by allogeneic and autologous BMDMSCs. Preliminary work to identify the mechanism of immune suppression, suggests allogeneic BMDMSCs may utilize PGE2 as a major mechanism of immune suppression. The study suggests that BMDMSCs do not elicit an immune response. In contrast, they may cause immune suppression demonstrated by a decrease in lymphocyte proliferation and a decrease in intracellular IFNγ production, serving as an anti-inflammatory treatment. Preliminary work to identify the mechanism of immune suppression, suggests allogeneic BMDMSCs may utilize PGE2 as a mechanism of immune suppression. Further research is warranted to assess the in vivo response to intra-articular injection of allogeneic BMDMSCs and their potential clinical application.

**Significance:** The study suggests that allogeneic BMDMSCs are non-immunogenic, and identifies a potential mechanism of immune suppression by BMDMSCs. If allogeneic BMDMSCs are non-immunogenic, these cells may provide a more affordable, timely and potentially effective treatment for musculoskeletal diseases in the horse.
Figure 1. Effects of autologous versus allogeneic BMDMSC on proliferation of normal equine lymphocytes.

Figure 2. Effects of autologous versus allogeneic BMDMSC on IFNγ production by normal equine lymphocytes.
Figure 3. The figure shows the percent proliferation of PBMCs when a potential mechanism of immune suppression is reversed.