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Current use of autologous bone marrow-derived stem cells (BMSCs) for equine joint injury and disease and comparison of immunomodulatory properties of equine allogeneic and autologous BMSCs

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Introduction

Arthroscopic surgery revolutionized our ability to treat joint injuries in the horse and return them to full athletic activity. However, limitations became recognized in certain situations to achieving this goal including acute articular cartilage loss, soft tissue injuries of intra-articular ligaments and meniscus as well as chronic disease leading to osteoarthritis (OA). This led to increasing the quest for better regenerative therapies. New biologic therapies that have increased our ability to rehabilitate joint injury and disease include protein therapies (autologous conditioned serum, platelet rich plasma) as well as cellular therapies. Cellular therapies have revolved around the principal of mesenchymal stem cells (MSCs) and, in particular, our clinical success has been achieved with the use of bone marrow-derived MSCs (BMSCs). These MSCs exert their effect in dual roles: 1) they can provide replacement units for expired cells and mesenchymal tissues and, 2) have trophic effects on cells in their vicinity without generating newly differentiated mesenchymal phenotypes.

Clinical use in the horse

The treatment of equine joint problems started with demonstration in a caprine model of OA that an intra-articular dose of 10 million autologous BMSCs suspended in HA compared to HA alone enhanced regeneration of medial meniscus tissue as well as decrease in degeneration of articular cartilage, osteophyte remodeling and subchondral sclerosis. This led to a clinical study with intra-articular BMSCs (approximately 20 million cells) injected intra-articularly into femorotibial joints showing a high rate of return to work and, in particular, 73% success where there was articular cartilage damage and diffuse change on the condyle and 85% returning to work even when full thickness cartilage damage/eburnation was also present in the articular cartilage[1]. The return after meniscal tears treated arthroscopically was significantly enhanced with BMSCs administered intra-articularly. We have also demonstrated augmentation of healing of full thickness microfractured defects with intra-articular BMSCs.

The potential for allogenic cells

We have done two studies at Colorado State University looking at the immunomodulatory effects of allogeneic BMSCs.[3,4] This was because of concerns being raised regarding the potential safety and effectiveness of allogeneic BMSCs including immunologic reaction to allogeneic cells. We conducted studies to assess the immunologic properties of equine allogeneic BMSCs compared with those of autologous BMSCs. Comparisons were made between BMSCs of matched and mismatched donors (Irish Thoroughbreds and Connemara Ponies) in a study in collaboration with Dr. Bea Ranera and Professor Frank Barry at the University of Galway in Ireland. Mismatched BMSCs inhibited proliferation of stimulated lymphocytes in a dose-dependent manner with the greater suppression occurring at 1:10 ratio of BM-MSCs to PBMCs. Proliferation of CD4+ and CD8+ sub population decreased in 1:10 co-culture with statistical significance in the case of CD8+ cells, while that of the CD4/CD8 double-positive population was similar to the phytohaemagglutinin control. These results demonstrate a dose-dependent immunosuppression of stimulated lymphocytes by mismatched equine BM-MSCs supporting their future application in allo-MSC clinical treatment.[3]

In a second study to assess inherent immunogenicity, the relative ability of allogeneic and autologous BMSCs to stimulate spontaneous proliferation of equine lymphocytes was compared[4]. The immunosuppressive activity of the two cells types was evaluated by adding autologous or allogenic BMSCs to activated lymphocytes and assessing suppression of lymphocyte proliferation and IFNγ production. Fifty-six allogeneic and 12 autologous combinations were evaluated. Studies were also done to elucidate mechanisms by which the MSCs suppress lymphocyte function. Potential mechanisms evaluated included production of prostaglandin E2 (PGE2), nitric oxide, transforming growth factor-β, indole amine, 2-3-dioxygenase.

We found that autologous and allogeneic BMSCs both inhibited mild but equivalent levels of spontaneous lymphocyte activation in vitro. In in vitro assays assessing the ability of BMSCs to suppress activated lymphocytes, both allogeneic and autologous BMSCs suppressed T-cell proliferation and IFNγ production to an equal degree. The primary mechanism of equine BMSC suppression of T-cells was mediated by PGE2. We concluded that allogeneic and autologous BMSCs are equivalent in terms of their immunomodulatory properties and stimulated peripheral blood mononuclear cells appear to trigger the immunosuppressive properties of MSCs. Therefore, both cell types appear to have equal potency in modulating inflammatory processes related to acute or chronic musculoskeletal injuries in the horse.[4]

In further ongoing work we are showing that the reaction to *in vivo* intra-articular injection of both autogenous and allogeneic BMSCs to be quite comparable with no significant increase of reactions in the allogeneic group.[3,4]

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