Seeking stem cell efficacy – immunogenicity matters!

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Abstract

Despite hundreds of publications and registered clinical trials, definitive evidence of mesenchymal stem cell (MSC) efficacy does not exist. The notion that mesenchymal stromal cells (MSCs) are immune privileged is changing: MSCs constitutively express MHCI, can be induced to express MHCII and allo-immunization against allogeneic MSCs has been documented. Despite the clinical safety of allo-MSCs, which has been shown exhaustively without evidence for acute rejection reactions or local tissue inflammation, immune recognition and rejection occurs with a reduction in MSC efficacy, we have demonstrated localized tissue inflammation secondary to immune recognition of allo-MSCs. We have also demonstrated localized tissue inflammation secondary to immune recognition of fetal bovine serum contaminants to MSCs. This localized tissue inflammation supports the clinical relevance of allo-recognition of MHC mismatched MSCs and FBS contaminated MSCs, despite the lack of clinically apparent adverse reaction. These findings may explain why definitive proof of MSC efficacy has not been achieved.

Keywords: Equine, bone marrow, mesenchymal, stem, stromal, regenerative

Lameness is one of the leading reasons for suffering and ill health in the horse, and it is estimated that approximately 60% of lameness is due to osteoarthritis. Mesenchymal stem cells (MSCs) are a regenerative therapy used to mitigate chronic pain and slow the progression of osteoarthritis. In people, there are FDA non-cross matched allogenic (non-self) and autologous (self) MSC trials for joint disease and approximately half of all current MSC clinical trials in people are allogenic.[1] This is because allogenic MSCs have been long considered non-immunogenic, and are more easily commercialized. This remains true in horses as well, and several groups maintain the feasibility of allogenic MSC use.[2,3]

However, as clinical data accumulate, it appears there is greater variability in efficacy of allogeneic therapies as compared to autologous, causing the immune privileged status of allo-MSCs to be questioned.[4] In horse, published reports document a regenerative effect when autologous MSCs are used to treat joint disease, but anecdotal reports (where allogeneic MSCs are often used) are of minimal treatment effect. The lack of consistent lameness reduction is because allogeneic MSCs are recognized by the immune system, thus precluding the beneficial effects. This is in agreement with our previous work that showed in horse a significant adverse response when allogenic-MSCs were confined to the intra-articular environment compared to auto-MSCs.[5]

Long considered immune privileged, allogeneic (non-self, from one horse to another) mesenchymal stem cells (MSCs) have enhanced commercialization potential.[6,7] The immune privileged status of MSCs stems from absent MHCII expression, low MHCI expression, and immunomodulatory action by MSCs. Despite low expression of MHCI, we have recently demonstrated immune recognition of non-self MSCs in horses. This immune recognition may explain the greater variability in efficacy of allogeneic therapies that has been recognized in people and suggested in the horse.[3,4,8,9] Our recent work, along with data from equine researchers at NCSU demonstrating cytotoxic T cell responses in vitro after allogeneic MSC administration, provides sufficient evidence to question not only the efficacy but also the safety of allogeneic MSCs in the horse.[10,11]

Despite decades of mesenchymal stromal cell (MSC) therapy, definitive clinical evidence of efficacy does not exist, and it has been postulated that lack of efficacy is due to MSC preparation technique.[12,13] Supplementation of mesenchymal stromal cells (MSCs) with fetal bovine serum (FBS) has been a standard practice since MSCs were first described in 1970.[14] Since then, numerous veterinary and human medical patients have received MSCs supplemented with FBS for various medical conditions,

including osteoarthritis. Although clinical detection of adverse reactions appears to be rare, xenogen exposure to the MSC recipient because of FBS supplementation can cause local reactions,[15] anaphlyaxis,[16] and anamnestic responses,[17] which have been associated with MSC-non response.[18] While immune recognition and resultant adverse reactions are concerning, immune recognition and resultant cytotoxicity against MSCs may be a key factor in the elusive proof of MSC efficacy for most applications including osteoarthritis.[13,19]

Many blood derivatives including platelet products,[20] umbilical cord blood serum[21] and chemically defined media have been investigated to replace FBS supplementation of MSCs. Based on the ever-decreasing proportion of clinical.trials.gov registered MSC trials, with FBS supplementation of 80% of regulatory submissions in 2014[1] to 40% in 2019, it appears that the human regenerative medicine field agrees that FBS should be replaced. Surprisingly, the veterinary regenerative medicine community has not adopted this stance and FBS supplementation of MSCs remains the industry standard.[2,3] Similarly, FBS supplementation remains well-accepted in many preclinical MSC investigations in laboratory animals.[22] This may be because definitive evidence for immune targeting and cytotoxicity of FBS supplemented MSCs does not exist. Regardless of this disparity in human, veterinary and preclinical fields, it appears that no autologous supplement has replaced FBS with unaltered growth, characterization or clinical efficacy of MSCs.

We have previously shown that 48 hours of FBS depletion is sufficient to mitigate the mild local adverse reaction after repeated intra-articular injection of MSCs supplemented with FBS in horses.[5] We sought to develop an autologous, xenogen-free method for supplementation of MSCs that would obviate immune targeting of transplanted MSCs by the recipient, by removing FBS for the entire culture period. Our efforts with platelet lysate and platelet releasate, both autologous and pooled, were disappointing as has been previously reported.[23] With platelet products, isolation of MSCs was generally successful, but growth kinetics, cell surface markers, cell morphology and differentiation ability were more variable when compared to the response to FBS supplementation by the same donors.

By developing a replacement to fetal bovine serum supplementation of MSC culture, immune mediated targeting of culture expanded cells will be abolished. By evaluating immune reaction of matched and mismatched recipients we are developing a hierarchy of stem cell immunogenicity, which is a necessary step in pursuit of an acceptable allogeneic donor.

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