Do Mesenchymal Stromal Cells Abrogate The Host Immune Response In Massive Cortical Allograft Recipients?

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Introduction: Massive bone allografts are utilized to reconstruct large bone defects after tumor resection or trauma. Traditionally, allografts are obtained from deceased, non-related tissue donors, steriley harvested, stripped of soft tissues and frozen to diminish antigenicity. Due to lack of availability of matched tissue and the paucity of cellular material on the allograft, massive cortical bone allografts are not matched for major histocompatibility antigens with the recipient. Limb-salvage treatments utilizing massive allografts have a 60% failure rate at 10 years in human patients and a 50% failure rate at one year for canine patients. Previous studies have shown that the poor healing seen after mismatched large frozen cortical allograft transplants is partly immune-mediated, and can be contributed to T cell recognition of graft alloantigens. While current immunosuppressive drugs greatly decrease the risk of graft rejection, long-term use is not advantageous. The use of mesenchymal stem cells (MSCs) following major organ transplantation had been widely investigated in the hope of finding a new standard of immunosuppressive treatment. The objective of this study was to evaluate the cellular immune response against allograft bone versus autograft bone when delivered as a vaccine, and to determine whether or not the addition of adipose-derived mesenchymal stem cells (AD-MSCs) would dampen the immune response towards an allograft bone vaccine. To accomplish these objectives we developed a murine bone vaccine model to mimic the immune response seen following a limb-salvage procedure.

Methods: C57BL/6 mice were treated with an initial 0.1mL vaccine injection on Day 1, followed by a booster vaccine injection on Day 7. Mice (n=20) were randomly assigned to receive an ALP vaccine (n=5), an allograft vaccine (n=5), an autograft vaccine (n=5), or were non-vaccinated (n=5). A second set of mice (n=48) was randomly assigned to receive a vaccine with either intravenously delivered (IV) MSCs (n=24) or with subcutaneous MSCs (n=24). Recipients within each MSC delivery group received an ALP vaccine (n=8), an allograft vaccine (n=8), an autograft vaccine (n=8), or were non-vaccinated (n=8). Bones used for the allograft vaccine were obtained from the femurs of Balb/C mice and bones used in the autograft vaccine were obtained from C57BL/6 mice. Bones were smashed into a powder and the final concentration of bone powder in the vaccine was 10μg/mL. Final bone vaccines contained bone dust of the allograft bone or an autograft bone; a cationic liposomal DNA complex (CLDC) adjuvant; and sterile PBS. The allograft and autograft treatment groups were compared against a positive control vaccine containing alkaline phosphatase (ALP) at 10μg/mL that was re-suspended in sterile PBS and CLDC adjuvant. Spleens were harvested for the purpose of evaluating the cellular immune response to total bone antigens by measuring T cell proliferation present in the spleen cells. Spleen cells were stained with carboxyfluorescein succinimidyl ester (CFSE), cultured with a negative control, allograft bone protein, and autograft bone protein for 72 hours, and stained with a CD3 marker to evaluate T cell proliferation via flow cytometry. A difference in T cell proliferation between treatment groups was
determined using an ANOVA and a Bonferroni multiple comparison post-test. A p-value of less than 0.05 was considered statistically significant. The handling and treatment of animals followed a Colorado State University Institutional Animal Care and Use Committee approved protocol.

**Results:** Recipients of an allograft bone vaccine with IV MSCs and with subcutaneous MSCs had a significantly less potent T cell proliferation response to an allograft bone antigen compared to the response in non-vaccinated animals. No differences in T cell proliferation were observed between recipients of an allograft bone vaccine upon exposure to an autograft bone antigen (Figure 1). No differences in T cell proliferation were noted in recipients of an autograft bone vaccine with IV MSCs and with subcutaneous MSCs in response to an allograft bone antigen compared to the response found in non-vaccinated animals. The same result was observed in response to an autograft antigen (Figure 2).

**Discussion:** The role of T-cells in acute graft rejection has been examined as a key player in amplifying and propagating the graft-specific adaptive immune response. The novel bone vaccine model was used in this study in an attempt to mimic the immune response seen in a clinical setting following massive cortical allograft transplantation in order to investigate the cellular immune response towards a bone antigen. Evaluation of the MSC immunomodulation of the cellular immune response did reveal a suppressive effect on T cell proliferation levels in recipients of a vaccine with MSCs. This effect was most pronounced in recipients of an allograft bone vaccine and MSCs when spleen cells were exposed to an allograft bone antigen. T cell proliferation suppression by MSCs was less pronounced when cells were exposed to an autograft bone antigen. Additionally, this suppression was not as pronounced in recipients of an autograft vaccine when exposed to an allograft bone antigen or an autograft bone antigen. When considered as a whole, these data support the hypothesis that MSCs do suppress cellular immunity towards allograft bone antigens. An unexpected observation was noted during the analysis of the T cell proliferation following re-exposure to the bone antigens. Wherein one would normally expect re-exposure to an antigen via vaccine to enhance the immune response to a particular antigen, we noted that T cell proliferation towards the allograft bone antigen was dampened in animals previously vaccinated with an allograft bone vaccine. This effect was most profound when the allograft bone vaccine was given with MSCs. This suggests that vaccinating against a total allograft bone antigen may in fact decrease the immune response to the allograft, especially with the addition of MSCs.

**Significance:** The employment of a massive cortical allograft for limb-salvage is widely performed in clinical settings with little concern for the matching of donor and recipient MHC complexes. We developed a novel vaccine model to mimic the immune response seen towards a massive cortical allograft in a clinical setting. Data presented herein suggests a possible new avenue to explore a diminished immune response following massive allograft transplant through prior vaccination of the patient with the allograft. A future vaccine study looking at this potential mechanism is needed to determine if the suppression of T cell proliferation present in vaccinated animals is related to clinically improved graft incorporation. If successful, vaccination with an allograft bone antigen could yield a potential new therapeutic for increasing the success of a massive cortical allograft transplant.
Figure 1. T Cell Proliferation in Recipients of an Allograft Bone Vaccine.

Figure 2. T Cell Proliferation in Recipients of an Autograft Bone Vaccine.