

## **IN VIVO IMMUNOMODULATION AND MOBILIZATION OF ENDOTHELIAL-LIKE CELLS FOLLOWING INFUSION OF MARROW STROMAL CELLS (MSC)**

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Marrow stromal cells (MSC) expanded from aspirated marrow, have been used clinically for several indications with some success, however the mechanism by which any beneficial effects are achieved is not clear. We hypothesized that MSC mediate their effect by activating an endogenous cell population which can modulate the immune response and/or enhance the repair process in damaged tissues. To test this hypothesis immortalized and cloned populations of canine MSC were generated to provide a consistent product for in vivo testing.

One MSC line, DS-1, was infused into 3 normal dogs and blood samples obtained through day 28. Following infusion there was no consistent change in the number of WBC; however by day 3 there was a significant, but transient, decrease in the level of FoxP3 expression in blood-derived CD3+ T cells ( $p < 0.001$ ), in contrast there was no significant difference in IL-10 and TGF $\beta$  expression in these cells. At autopsy on day 28, CD3+ cells in lymph nodes showed a decrease in IL-10 ( $p = 0.06$ ); whereas CD3+ cells in spleen showed a decrease in both IL-10 ( $p = 0.06$ ) and TGF $\beta$  ( $p = 0.05$ ). There was no significant difference in FoxP3 expression in T cells in these tissues. Quantitative gene expression analysis of peripheral blood mononuclear cells (PBMC) indicated a transient upregulation of endothelial cell and activated monocyte markers including CD133, Tie-2, MARCO and LOX1/OLR1. The percentage of monocytes and the expression levels of CD14, CD68, CD45, and CD105/Endoglin, however, were constant at all time points. These data indicate that a single infusion of DS-1 cells into normal dogs results in activation of circulating monocytes and changes in immune competent cells possibly related to a transient marginalization of Tregs.

DS-1 cells were then infused into 2 dogs undergoing lung allograft rejection at 19 days post transplant. Although imaging studies of indium<sup>111</sup> labeled MSC indicated that the MSC did not reach the rejecting lung, pulmonary function tests and radiography indicated, in one dog, prolonged graft survival and normal lung function which persisted for at least 60 days with no further treatment. Efforts to associate circulating cells with this beneficial effect identified recipient fibroblastoid adherent cells that were negative for macrophage markers (CD14, CD45, MARCO and LOX1/OLR1) but positive for endothelial markers (CD34, KDR, vWF, Tie2, CD31, and VE-cadherin). These cells formed cord-like structures when cultured on matrigel. In the second dog that did not show improvement, DS-1 cell infusion did not induce mobilization of the endothelial-like cells.

Taken together these data indicate that infusion of DS-1 cells in normal dogs results in transient qualitative changes in PBMC. We speculate that in association with lung allograft rejection, DS-1 infusion may induce mobilization of endogenous endothelial cells that may contribute to vascular repair of the rejecting lung.