

Defining Human Mesenchymal Stem Cell Efficacy *In Vivo* in Asthma

Tracey L. Bonfield, Mary T. Koloze, Donald P. Lennon, Brandon Zuchowski, Brian Jennings, Sung Eun Yang, Arnold I Caplan. Department of Pediatrics and Skeletal Research Center, Department of Biology Case Western Reserve University, Cleveland, Ohio 44106-4948

Allogeneic human mesenchymal stem cells (hMSCs) can suppress graft versus host disease (GvHD) and can have profound anti-inflammatory and regenerative capacity in the case of stroke, infarct, spinal cord injury, meniscus regeneration, tendinitis, acute renal failure, and heart disease in human and animal models of disease. The challenge in hMSC based therapy is defining the efficacy of hMSCs *in vivo*. Models which may provide insight into hMSC bioactivity *in vivo* would provide an alternative means to distinguish hMSCs for clinical utility. Further, hMSC's can avoid host immune response, providing xenographic applications. MSC function has been described as both regenerative and trophic through the production of bioactive factors. The secreted factors generated by the hMSCs are specific and provide signatures for activity and potential responses *in vivo*. To study the *in vivo* immuno-regulatory effectiveness of hMSCs, we used the ovalbumin challenge model of acute asthma. This is a short-term *in vivo* pulmonary inflammatory model which can provide readily accessible ways of measuring effectiveness of hMSCs. Mice were sensitized with ovalbumin, rested for 14 days and then lung-challenged daily with ovalbumin or sham saline for 5 days. Mice were sacrificed and the lungs were processed for inflammation with bronchoalveolar lavage (BAL) or histology without BAL. Ovalbumin challenged mice had a significant increase in inflammatory cells, 4.8×10^5 cells/ml compared to saline challenged mice, 2.3×10^5 cells/ml ($n=4$, $p=0.04$). The inflammation was significantly decreased by 23 ± 7 % with hMSCs therapy to 3.7×10^5 cells/ml ($n=4$, $p=0.05$). Treatment of the acute asthma mice with hMSCs resulted in increased production of macrophages and decreased production of neutrophils and eosinophils consistent with airway inflammation attenuation. Histologically, the epithelial lining of the bronchiolar airways appeared to have less thickening and hyperplasia when compared with animals not treated with hMSCs. To evaluate the effectiveness of hMSCs on chronic asthma, we utilized the same model but challenged the animals with the ovalbumin every-other day for 4 weeks, followed by hMSC and harvest 7 days later. The hMSCs dramatically decreased chronic airway inflammation with less goblet and epithelial hyperplasia (lung sections below). Our studies show the potential of hMSCs to improve airway inflammation in the mouse model of acute and chronic asthma demonstrating the effectiveness of using these models to measure hMSC effectiveness. Further, the results from these studies verify the *in vivo* immuno-modulator effectiveness of hMSCs and support the potential therapeutic alternative of hMSCs for the treatment of airway inflammation associated with asthma. The funding for this research was graciously provide by the David and Virginia Baldwin Fund, Center for Stem Cell and Regenerative Medicine and the Case Western Reserve Vision Fund.

