production of identical progeny, 2) appearance early in development and persistence throughout life, and 3) long-term proliferation and multipotency.

The purpose of this study was to explore potential gender differences on transplantation efficiency. Specifically, we sought to determine whether there is a difference in 1) the regeneration efficiency of male- versus female-derived MDSCs and 2) the receptiveness of the male and female mdx hosts to transplantation.

Our studies found that a donor population of MT (male, three-weeks donor) did not have the same level of engraftment as FT (female, three-weeks) in terms of dystrophin delivery to mdx animals. Female MDSCs are more efficient than male MDSCs in facilitating dystrophin delivery and muscle regeneration in the murine muscular dystrophy model While we recognize there is large variability among engraftments, we found overall that the average regeneration index for the female populations was 516 dystrophin positive myofibers per 10^5 donor cells, and 135 fibers/10^5 cells for male populations ($p<0.05$). Several in vitro characteristics were explored to understand the in vivo differences. While both populations were isolated by the preplate technique and were capable of extended replicative lifetime, there were differences in proliferation rate, desmin expression and CD34 expression which may help to explain the differences in regeneration efficiency.

Examination of the host receptiveness also revealed that female MDSC were able to achieve a much larger engraftments in female mdx hosts as compared to their performance in male mdx hosts. High engraftment was observed for female cells into female host (RI= 556 fibers/10^5 cells), which supported previous results. However, these same cells have a lower engraftment when injected to age-matched male hosts (RI= 276 fibers/10^5 cells) (p=.064).Our results imply that gender-related differences play a role in the transplantation efficiency of MDSCs. We are conducting an ongoing analysis in an attempt to better understand the mechanism by which female MDSCs support higher engraftment than male MDSCs. In addition, we are exploring possible immune responses that the male host may have to female donor cells.

247. Expressing Full-Length Dystrophin in 50% Cardiomyocytes Corrects Cardiomyopathy in the Mdx Mouse Model for Duchenne Muscular Dystrophy

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Cardiomyopathy is a major determinant of the clinical outcome in Duchenne and Becker muscular dystrophy (DMD, BMD). Nearly every DMD and BMD patient suffers from some degree of cardiomyopathy. More then one tenth of DMD patients eventually die of heart failure. Clinical success of DMD gene therapy will depend upon functional improvement in both skeletal and cardiac muscle. Substantial progress has been made in DMD skeletal muscle disease gene therapy. However, few studies have been done in DMD cardiomyopathy gene therapy. We recently reported that micro-dystrophin was equally efficient in restoring the dystrophin-glycoprotein complex and maintaining sarcolemma integrity in the mdx heart (Yue et al *Circulation* 108:1626,2003). The minimal number of dystrophin expressing cells needed for cardiomyopathy therapy has not been determined however. In this study, we used female heterozygous mice (F1 from BL10 and mdx crossing) as an experimental model to evaluate whether dystrophin expression in half of the cardiomyocytes was enough to improve heart function in mdx mice. Consistent with the random X-chromosome inactivation theory, we found that 51.22% and 55.40% of the heart cells were expression dystrophin in maternal and paternal heterozygous mice respectively. The mdx mouse hearts were heavier than the BL10 hearts. Interestingly, weights of the heterozygous mice hearts were similar to those of the BL10. In contrast to previous reports of the benign histology in the mdx hearts, we detected fibrosis in 85.71% of the mdx hearts (N=42). More than half of the fibrosis was in the range of medium-to-large size. Only 43.59% of the heterozygous mice had hearts that contained fibrous regions, and the majority of the fibrosis was localized to small areas. To determine whether full-length dystrophin expression in half of the cardiomyocytes can protect the heart from mechanical-stress induced injury, we challenged the hearts with the inotrope b-isoproterenol. After administrating a vital dye, Evans blue (EBD), we found that 11.26 ± 3.40 % of the heart area was EBD positive in mdx mice. In the heterozygous mouse hearts, the EBD positive area was reduced to 2.37 ± 0.70 %. This result suggests that a significant improvement in cardiomyocyte sarcolemma integrity has been achieved in the heterozygous mouse hearts. In summary, our results suggest that a 50% correction in the mdx heart is sufficient to ameliorate cardiomyopathy in mdx mice.

248. cFLIP or IKBSR Inhibition of NF-κB Activation in Mouse Myoblast Cells: Potential Application of Gene Therapy for Cancer Cachexia

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Muscle wasting, a syndrome characterized by the disproportional loss of skeletal muscle, is a frequent complication in patients with cancer or other chronic diseases. Recent studies *in vitro* and *in vivo* have demonstrated that tumor cells produce and secrete cytokines such as TNF-α that are responsible for inhibition of differentiation of myoblast cells into multinucleated myotubes and thereby may prevent muscle repair in cancer patients. Activation of NF-κB triggered by TNF-α interaction with its receptor on muscle cells, is considered a major pathway through which cytokines lead to muscle wasting in cancer patients. Gene therapy offers a potential solution by modulating downstream events within muscle cells. *In vitro* models of muscle wasting can provide an important resource to understand which pathways to modulate and to test potential therapies. In our *In vitro* studies, we found that application of TNF-α results in apoptosis of primary C57 mouse myoblast cells at 3 days after treatment. IFN-γ, IL-1β and IL-6 do not lead to apoptosis of myoblast cells, but result in inhibition of myoblast differentiation at 20 ng/ml. In addition, the conditioned media from human prostate cancer cell lines (PC-3 and DU145P) and a human melanoma cell line (Mel) significantly inhibit the differentiation of myoblast cells. NF-κB activity was activated in treated myoblast cells compared to untreated or 293 cell culture supernatant-treated myoblast cells. However we found that myoblasts stably transfected with IKBSR, a mutant IKBα, or cellular FLICE/Caspase-8-inhibitory protein, cFLIP, inhibited NF-κB activation after treatment of myoblast cells with cytokines (TNF-α, IFN-γ, IL-1β or IL-6) or tumor cell conditioned media (PC-3, DU145P or Mel). Inhibition of NF-κB activation was associated with amelioration of the inhibition of muscle cell differentiation. To our knowledge, this is the first report that cFLIP, an important caspase-8-inhibitory protein is capable of inhibiting NF-κB activation and reducing inhibition of myoblast cell differentiation treated with PC-3, DU145P and Mel tumor media. The results encourage us to generate gene transfer virus vectors expressing IKBSR or cFLIP or both, and to determine whether IKBSR or cFLIP or both can ameliorate muscle wasting.