pressed 25 micrograms/mL of hAAT. **Conclusions:** uPA administration results in a 100-fold increase in transplanted hepatocyte engraftment and function. This model offers a method to confer positive selection for liver engraftment and yields a quantifiable increase in the hepatocyte-specific function of transplanted cells. This data establishes a basis to compare the kinetics of engraftment and function of cellular transplants from various sources after acute liver injury.

P107. **Syngeneic Reconstitution Rescues Mice That Develop Severe GVHD After Allogeneic Reconstitution.** G. Rofaiel, M.B.B.C.H., D. Giangiacomo, K. Gandy, J. Domen, Ph.D. Duke University Medical Center.

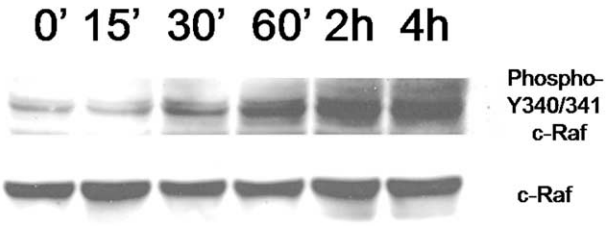
Background: Our Laboratory has been investigating donor specific hematopoietic reconstitution for the purpose of tolerance induction, a therapy currently limited by GVHD. This study investigates methods by which such GVHD can be managed. **Methods and Results:** We have established an acute GVHD mouse model by coinjection of T-cell depleted whole bone marrow (TCD-WBM) with $CD4^+$ and $CD8^+$-enriched splenocytes into lethally irradiated recipients. T cell depletion (TCD) was performed by extraction of $CD3^+$, $CD4^+$, $CD8^+$, and $NK1.1^+$ cells using magnetic beads. 25 Balb/c mice were given allogeneic 5×10^6 TCD-WBM plus 8×10^4 $CD4^4/CD8^+$-enriched splenocytes. 5 mice were given 5×10^6 TCD-WBM alone. At day 9, 15 mice were rescued using 2, 5 and 10×10^6 syngeneic TCD-WBM, 5 mice in each group. GVHD was monitored by observing total body weight, body habitus, coat condition, and survival. At 42 days, survival of mice given TCD-WBM alone was 100% compared to 40% for mice TCD-WBM and $CD4^+/CD8^+$ splenocytes. Survival of mice given 2, 5, and 10×10^6 TCD-WBM was 60%, 60%, and 80% respectively. Weight data correlated as well. By day 16, the group which was given TCD-WBM alone was 10% below the start up weight compared to 41% for the group given TCD-WBM/splenocytes. Autologous rescue with 2, 5, and 10×106 TCD-WBM resulted in weights of 28. 28, and 22% of start-up weights, respectively. Peripheral blood of mice in all experimental groups was more than 98% donor-derived at 12 weeks. The group of mice given TCD-WBM/splenocytes and rescued with 2×10^6 autologous rescue cells did not survive beyond 9 weeks, but 99% of their peripheral blood cells was donor derived at 4 weeks. Conclusion: Such data demonstrate that acute GVHD can be alleviated without reverasal of donor-derived reconstitution using syngeneic rescue. These data suggest that such a method might prove useful for treatment of GVHD in methods of tolerance induction which utilize hematpoietic reconstitution.

PEDIATRICS ORAL POSTER SESSION

P108. **Raf-Dependent Apoptotic Response to Lipopolysaccaride (LPS) in IEC-6 Enterocytes.** A. Grishin, Ph.D., J. Wang, D. Hackam, M.D., J. S. Upperman, M.D., R. Zamora, Ph.D., H. R. Ford, M.D. University of Pittsburgh.

Introduction: Necrotizing Enterocolitis (NEC) is a leading cause of premature infant mortality. Bacteria and bacterial by-products such as lipopolysaccaride (LPS) in the gut lumen may play a pivotal role in the gut barrier failure. We hypothesized that LPS may contribute to gut barrier failure by inducing enterocyte apoptosis through a Raf-dependent pathway. **Methods:** IEC-6 cells (young & old passage) were exposed to LPS (0–25 ug). Apoptosis was measured by DNA laddering and morphology. Mitochondrial membrane potential was tested with Mitotracker™. Cytochrome c, caspase expression and tyrosine phosphorylation was measured by SDS-PAGE. Raf activation was inhibited with GW5074. **Results:** LPS stimulated IEC-6 cells undergo time- and dose-dependent apoptosis. The induction of apoptosis was observed in the young (passage 5–20) but not in the old (passages beyond 30) cultures. The LPS-induced apoptosis was associated with the loss of mitochondrial membrane potential, release of cytochrome *c* from mitochondria into cytoplasm, activation of caspases, and cleavage of caspase substrate proteins. Inhibition of Raf, the MEKK kinase upstream of ERK, abrogated the LPS-induced apoptosis. MEK1 and MEK2 inhibitors had no effect on apoptosis. Phosphorylation at tyrosines 340/341 was associated with Raf activation and activation increased in the presence of LPS. (Figure) **Conclusion:** Thus, stimulation of apoptosis by LPS in a Raf-dependent manner may contribute to enterocyte death and the development of gut barrier failure in NEC.

Figure. LPS-induced Raf phosphorylation



P109. **Wound Splinting Modulates Wound Cell Proliferation.** M. A. Carlson, M.D., M. T. Longaker, M.D., J. S. Thompson, M.D., University of Nebraska Medical Center.

Introduction: Rigid anchorage of the extracellular matrix is important for fibroblast survival and proliferation *in vitro.* We hypothesized that rigid matrix anchorage in a wound splinting model would modulate wound cell proliferation *in vivo.* **Methods:** Male rats (age = 3 months; N = 12) were excisionally wounded (4 cm^2 square of dermis + panniculus from the dorsum), and a square stainless steel splint was sutured to the wound edge. The splint was removed on day 5 from 6 rats. The animals were injected with BrdU 18 hr later, and then the granulation tissue with surrounding dermis was excised 24 hr after desplinting. BrdU- and propidium iodide-labeled nuclei were quantified on frozen sections of granulation tissue, cut at three different levels. **Results:** A total of 201 microscopic sections were counted (about 16 per rat), representing about 37,000 nuclei. The rate of BrdU-positive nuclei in the splinted vs. desplinted animals was 6.15 ± sd 2.45 vs. 3.03 ± 1.58%*, and the total number of nuclei per microscopic field was 175 ± 27 vs. 197± 38*, respectively (*$p < 0.001$, unpaired t-test). Wound cross-sectional area decreased approximately 50% after desplinting (data not shown). **Conclusions:** Removal of the rigid wound splint decreased the rate of BrdU-labeled cells in the granulation tissue by 50%; there was a slight increase in the cell population density, which may be explained by the contraction which occurs after desplinting. Wound cell proliferation is modulated by anchorage of the wound edge.

P110. **Deletion of Fibroblast Growth Factor Receptor 2b (Fgfr2b) Gene Results in Anorectal Malformation in Mouse.** T. J. Fairbanks, M.D., R. C. Kanard, M.D., S. P. De Langhe, F.G. Sala, K. D. Anderson, M.D., D. Warburton, M.D., S. Bellusci, Ph.D., R. C. Burns, M.D. Childrens Hospital, Los Angeles.

Introduction: Anorectal malformations occur in 1 per 4,000 live births, and represent a surgical challenge. *Fgfr2b* is known to serve a key role in the development of other organ systems including parts of the gastrointestinal tract (GIT). We sought to evaluate the role of *Fgfr2b* in the development of normal anorectal structures. **Methods:** *Fgfr2b* expression in wild type (Wt) embryos was evaluated using whole mount in-situ hybridization. Wt and $Fgfr2b^{-/-}$ embryos were harvested from timed pregnant mothers at E10.5 through E18.5 and were analyzed for anorectal phenotype. **Results:** FGFR2 is expressed throughout the structures of the hindgut (data not shown). $Fgfr2b^{-/-}$ mutants