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IN VIVO ADMINISTRATION OF U0126 TO THE SHEEP FETUS BLOCKS ACUTE AND CHRONIC CARDIAC ERK PHOSPHORYLATION.

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We have shown that the MAPK signaling protein ERK is phosphorylated in the ventricles of chronically hypertensive fetuses. We do not know if ERK activation is responsible for cardiomyocyte proliferation, enlargement or terminal differentiation occurring in these fetuses. As a preliminary step towards determining the role of ERK in fetal cardiac growth, we sought to determine if ERK phosphorylation could be inhibited acutely and chronically in the fetal heart using the MEK inhibitor U0126. **Methods:** Near-term fetal sheep were surgically instrumented with vascular catheters and allowed to recover. U0126 was dissolved in DMSO at 1mg/ml for intravascular administration then diluted in Krebs-Henseleit buffer to 5μg/ml. To determine if U0126 administration blocked acute fetal cardiac ERK phosphorylation, 0.2mg/kg/hr of U0126 or vehicle was infused for 24 hours. Twenty minutes following injection of 6μg/kg angiotensin II (angII), fetal hearts were excised and expeditiously frozen in liquid nitrogen. To determine if U0126 administration blocked chronic fetal cardiac ERK phosphorylation, 0.2mg/kg/hr of U0126 or vehicle was infused for 4 days. These fetuses also received an infusion of plasma protein (~13g/day), which we have shown to cause arterial and venous hypertension, cardiomyocyte growth, and cardiac ERK phosphorylation. At the conclusion of the experiment, a biopsy of the left ventricle (LV) was freshly prepared for protein analysis. Levels of protein expression and phosphorylation were measured by Western blot analysis. Data were normalized as phosphorylated-to-total protein. **Results:** U0126 did not block the fetal arterial blood pressure or reflex heart rate responses to angII. U0126 blocked acute ERK phosphorylation following stimulation with angII. U0126 also blocked chronic ERK phosphorylation during arterial and venous hypertension induced by plasma infusion. Furthermore, isolated cardiomyocytes from the U0126-infused fetus did not display the same level of ERK phosphorylation as the vehicle-infused fetus after *ex vivo* stimulation with angII. **Discussion:** U0126 does not alter fetal pressor response to angiotensin II or plasma infusion. Despite the poor solubility of U0126 in aqueous solutions, this MEK inhibitor can be successfully administered to the sheep fetus via intravascular catheters to block acute and chronic cardiac ERK phosphorylation.

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DECREASED NEUROTROPHIN EXPRESSION IN CONGENITAL DIAPHRAGMATIC HERNIA HUMAN LUNG TISSUE.

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Background: Neurotrophins [nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3)] are growth factors that function as mediators for the development, survival and differentiation of neurons and other types of cells. During normal tissue morphogenesis, neurogenesis and myogenesis proceed as tightly linked processes. In the condition, congenital diaphragmatic hernia (CDH), there is failure of the diaphragm muscle to develop with consequent pulmonary hypoplasia and pulmonary hypertension. The probability that impaired neurogenesis, or coordination of neuro and myogenesis, underlies the disrupted development of the diaphragm muscle in CDH, needs to be explored. **Objectives:** To determine the expression of neurotrophic factors (NGF, NT-3 and BDNF) in neonatal lung disease; specifically congenital diaphragmatic hernia (CDH), persistent pulmonary hypertension (PPHN) and chronic lung disease (CLD) vs. normal lung tissue. **Methods:** Immunohistochemical studies for neurotrophin proteins (specific antibody staining using polyclonal rabbit anti-human NGF, BDNF and NT-3) were applied to human neonatal lung tissue samples. The samples included a control group of 18 samples ranging from 23wk EGA to term, a CDH group of 15 samples, a PPHN group of 6 samples and a CLD group of 12 samples. The tissue samples were studied and 4 representative slide fields of aveoli/saccules and 4 of bronchioles were recorded from each sample. These slide fields were then graded (from 0-3) by several blinded observers for intensity of staining. **Results:** BDNF, NGF and NT-3 immunostaining intensity scores were significantly less in the CDH lung tissue samples (n 15) compared to normal neonatal lung tissue (n 18) ($p<0.001$). Similarly, PPHN lung tissue (n 6) scored less than normal lung tissue, although not statistically significant; likely due to few samples studied. Chronic lung disease tissue (n 12) did not appear to differ in their neurotrophic staining intensity compared to normal neonatal lung tissue. **Conclusion:** Neurotrophin expression is decreased in CDH lungs. The decreased expression of neurotrophins in CDH lung tissue suggests they contribute to the abnormality in this condition. Further studies are needed to explore the role of deficient neurotrophin expression in the development of CDH.

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TEMPORAL EXPRESSION OF TLR2 ON HEMATOPOIETIC STEM CELLS IN MURINE FETAL LIVER.

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Recently Toll like receptors (TLRs) have been shown to play an essential role in innate immunity, recognizing a specific component of foreign pathogens. TLR2 recognizes gram-positive bacterial cell wall molecules such as peptidoglycan (PG), whereas TLR4 recognizes gram-negative bacterial lipopolysaccharide. TLRs are expressed on various immunological cells such as neutrophils, monocyte/macrophages, dendritic cells and B cells, however, their expression pattern during hematopoietic development/differentiation is not well known. We examined expression of TLR2 or 4 on hematopoietic stem cells (HSCs) in fetal liver. Fetal liver cells at 12.5-16.5 days postcoitum (dpc) were collected and analyzed by flow cytometry. TLR2 and 4 were detected in the murine $CD34^+c\text{-}kit^+Sca\text{-}1^+lineage^-$ (34KSL) population of the12.5 d.p.c fetal liver. 34KSL cells were separated into 3 fractions depending on the expression of TLR2 and 4; $TLR2^{bright}TLR4^+$ cells (7.3%), $TLR2^{dim}TLR4^-$ cells (84.3%), and $TLR2^-TLR4^-$ cells (3.5%). $TLR2^{dim}TLR4^-$ and $TLR2^-TLR4^+$ cells had high colony-forming ability but $TLR2^{bright}TLR4^+$ cells formed very few colonies. In 14.5dpc fetal liver, 90% of 34KSL cells were $TLR2^{dim}$, however, in 16.5dpc fetal liver, most of the 34KSL cells were TLR2 negative. Interestingly, this TLR2 expression on fetal liver HSCs was synchronized with the expression level of Mac1. When $TLR2^{bright}$, $TLR2^{dim}$ or $TLR2^-$ 34KSL cells of the 12.5 dpc fetal liver from Ly5.1 C57BL/6 mice were transplanted into lethally irradiated adult Ly5.2 C57BL/6 mice respectively, $TLR2^{dim}$ 34KSL cells were confirmed to reconstitute the recipient hematopoietic system. To examine the function of TLR2 on fetal liver HSCs, $TLR2^{dim}$34KSL cells were cultured with/without PG in the presence of SCF and TPO. After 7 days of incubation, cells were collected and analyzed by colony assay and surface markers. Colony forming ability was maintained in both groups but cells differentiated into different lineages. We conclude that TLR2 is temporary expressed on fetal liver HSCs but the role of TLR2 in hematopoiesis is unclear.

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ECTOPIC EXPRESSION OF GATA2 NORMALIZES ABERRANT MYELOMONOCYTIC DIFFERENTIATION INDUCED BY ACTIVATING *PTPN11* MUTATIONS.

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Purpose of Study: Juvenile myelomonocytic leukemia (JMML) is a myeloproliferative disorder characterized by overproduction of myelomonocytic cells. Activating mutations of *PTPN11*, which encodes the protein tyrosine phosphatase, Shp-2, are found in 35% of JMML patients. Murine bone marrow low density mononuclear cells (LDMNCs) expressing activating Shp-2 mutants preferentially undergo myelomonocytic differentiation despite being subjected to conditions that select for mast cell development (Chan *et al*., Blood *106*, Abstract #3519, 2005). Consistently, GATA-2 expression is dramatically reduced in cells expressing activating Shp-2, suggesting that Shp-2 gain-of-function mutants alter hematopoietic lineage-specific transcription factors and thus shift myeloid differentiation toward the myelomonocytic lineage. We hypothesized that ectopic GATA2 expression would normalize the aberrant myelomonocytic differentiation induced by activating Shp-2 mutants. **Methods Used:** To address this hypothesis, we utilized retroviral co-transduction of LDMNCs to generate four experimental groups: 1) pMIEG3-Shp-2WT plus pCD4 (empty vector); 2) pMIEG3-Shp2D61Y plus pCD4; 3) pMIEG3-Shp-2WT plus pCD4-GATA2; and 4) pMIEG3-Shp2D61Y plus pCD4-GATA2. Cells were stained with anti-human CD4-APC, sorted for the EGFP+APC+ cells, and plated into progenitor assays. Colonies were scored for colony forming unit (CFU)-granulocyte-macrophage (GM), monocyte (M), granulocyte (G), and granulocyte-erythroid-monocyte-megakaryocyte (GEMM). **Summary of Results:** As predicted, cells co-transduced with *PTPN11* mutant D61Y and pCD4 produced significantly more CFU-M than cells co-transduced with WT Shp-2 and pCD4 (37.2+/-3.6 v. 14.4+/-3.4, n=6, p=0.0008). However, upon co-transduction with GATA2, the number of CFU-M generated from D61Y-expressing cells was significantly reduced and was similar to that observed in cells expressing WT Shp-2. Quantitative RT-PCR verified restoration of GATA2 expression in cells co-transduced with D61Y and pCD4-GATA2. **Conclusion:** These findings demonstrate that restoration of GATA2 expression normalizes the propensity toward monocytic differentiation induced by the activating *PTPN11* mutant D61Y.

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PROFILE OF NONCOMPLIANCE WITH ORAL CHEMOTHERAPY IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA.

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OBJECTIVE: To assess rates of adherence to oral chemotherapy during maintenance chemotherapy in children with acute lymphoblastic leukemia (ALL). **BACKGROUND:** 25-30% of children with ALL eventually relapse. Relapse can be explained by high risk biological features in a minority of ALL patients, but why the majority of children relapses, has not been determined. An important part of treatment for leukemia consists of a prolonged period of maintenance oral chemotherapy. Prolonged low dose medication is thought to kill newly dividing leukemic cells. Discontinuing medication early might therefore increase the risk of relapse. **METHODS:** Children with ALL diagnosed at Riley Hospital were identified. Pharmaceutical claims data from Indiana Medicaid for 1/1992 – 12/ 2004 were analyzed to investigate chemotherapy use patterns for Methotrexate and 6-Mercaptupurine during maintenance chemotherapy and then were compared to prescription orders from written medical records. Multigraph timeline visualizations were used to analyze prescription data, medication fill data and laboratory data on each patient. **RESULTS:** A total of 56 patients who took 6-Mercaptopurine and Methotrexate were identified in the Medicaid database. Two measures were used for medication adherence: the continuous measure of medication availability during maintenance therapy and the continuous measure of medication gaps. Two-thirds of the children were prescribed at least 75% of the recommended protocol dosage for 6-Mercaptopurine and Methotrexate. However, only 32% of the children filled between 75-100% of the prescribed medication while 21% filled 50-75%, 14% filled 25-50%, and 12% filled less than 25% of the prescribed medication. 21% over filled. Only 46% of the children had medication available for 75-100 % of the days during maintenance chemotherapy whereas 11% had medication available less 25% of the time. **CONCLUSIONS:** Reducing the amount of maintenance therapy in clinical trials has been shown to reduce the event free survival for ALL. However adherence to medication is not studied or monitored in any of the national clinical trials. Pharmacy refill data allows analysis of large numbers of patients over prolonged periods of time and can help determine the upper limit of possible medication adherence. Our study shows that only 46% of the children filled enough of their medication to be able to take it more than 75% of the time. Multigraph timeline visualization of the prescription data, refill data and laboratory data gives a more complete picture of actual medication adherence than currently exists and can allow real-time monitoring of patients during their maintenance chemotherapy.

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IN VITRO CULTURED ALLOGENEIC CYTOTOXIC T CELLS MEDIATE GVL WITHOUT SYSTEMIC GVHD DESPITE EXPRESSION OF FUNCTIONAL LPAM.

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Background: Previous studies have shown that *in vitro* cultured alloreactive T cells had comparable GVL activity but decreased GVHD as compared to naïve cells. It has been shown that expression of LPAM on CD8 T cells is important for gut homing specificity in GVHD. Our preliminary data showed that the expression of LPAM is decreased upon *in vitro* culture of CTLs. Recently, retinoic acid (RA) has been shown to up regulate LPAM expression on naive T cells. We hypothesize that *in vitro* cultured CTLs without retinoic acid lack the ability to cause GVHD in part due to deficient LPAM expression. **Methods:** B6PL spleen/lymph node cells were stimulated against DBA splenocytes with IL-2 & IL-7 ± 100 nm RA. Day 14 CTL & CTLRA were infused into allogeneic recipients &compared to naïve T cells. **Results:** CTL & CTLRA showed CD8 LPAM expression of 0.7% & 61% ($p<.01$). Both groups had comparable *in vitro* migration towards to SDF, but CTLRA had ↑ migration towards TECK; 17.3% vs. 4.6% ($p<.01$). Homing analysis revealed ↑ migration of CTLRA to PP & MLN [Homing index (CTLRA/CTL) 2.3 & 2.5 respectively]. 600 cGy irradiated B6D2F1 mice were given $0.5x10^6$ P815 murine mastocytoma cells on day 0 followed by B6 BM cells with either $5x10^6$ CTLs or $10x10^6$ naïve lymphocytes on day 1. CTLs and naïve lymphocytes mediated a potent GVL effect in spleen & BM. Recipients of naïve lymphocytes developed lethal GVHD with high clinical GVHD scores (median survival 17 days; n=14) where as, CTL group had significantly improved survival due to attenuated GVHD (median 60 days; n=10, $p<0.001$). In pure GVHD experiments, CTL, CTLRA & naive lymphocytes induced lethal BM aplasia (n= 10, mean Hb 3g% in all) in the absence of BM rescue at d12 as compared to rad controls (n= 8, mean Hb 7.5g%) without systemic GVHD in any group. With BM rescue, naïve cells resulted in lethal systemic GVHD at d20 (n=8; Serum ALT 188 IU/L, clinical & histological GVHD scores of 8 & 11.5 respectively, $p<.01$). Where as recipients of CTL or CTLRA were alive >d150 (n=8; ALT 60 IU/L, clinical GVHD score of 2) despite ↑ migration of CTLRA cells to gut in contrast to CTL without RA ($p<.01$). **Conclusions:** We demonstrate that *in vitro* cultured alloreactive CTLs mediate a rapid GVL effect that was equivalent to the infusion of naïve T cells but with attenuated sub clinical GVHD. We also showed that *in vitro* cultured CTLs have defective gut homing which could be corrected by up regulating LPAM expression with the addition of RA. Thus defective gut homing does not explain the lack of lethal GVHD seen with *in vitro* cultured CTL.