REG3x is a Biomarker of Graft Versus Host Disease of the Gastrointestinal Tract

<table>
<thead>
<tr>
<th>Maximum GVHD Grade</th>
<th>REG3x (%95% CI)</th>
<th>p-value</th>
<th>1 year NRM</th>
<th>p-value</th>
<th>1 year OS</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low REG3x (%95% CI)</td>
<td>45% (5%65%)</td>
<td>0.001</td>
<td>24% (15-35)</td>
<td>0.001</td>
<td>49% (38-60)</td>
<td>0.001</td>
</tr>
<tr>
<td>High REG3x (%95% CI)</td>
<td>17% (11-27%)</td>
<td>0.57</td>
<td>83% (73-95)</td>
<td>0.57</td>
<td>43% (35-50)</td>
<td>0.57</td>
</tr>
<tr>
<td>Low KRT18 (%95% CI)</td>
<td>104% (24-45%)</td>
<td>0.54</td>
<td>36% (29-51%)</td>
<td>0.54</td>
<td>51% (47-69)</td>
<td>0.54</td>
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<tr>
<td>High KRT18 (%95% CI)</td>
<td>29% (29-40%)</td>
<td>0.54</td>
<td>71% (60-80)</td>
<td>0.54</td>
<td>54% (44-66)</td>
<td>0.54</td>
</tr>
</tbody>
</table>

**REG3x** is a Biomarker of Graft Versus Host Disease of the Gastrointestinal Tract

**1**

**REG3x** is a Biomarker of Graft Versus Host Disease of the Gastrointestinal Tract


**ABSTRACT BODY:** There are no validated plasma biomarkers specific to graft versus host disease (GVHD) of the gastrointestinal (GI) tract. Previously, we identified and validated elafin as a plasma biomarker for skin GVHD using an unbiased proteomics discovery approach (Science Transl Med, 2010). Using this same proteomics approach to analyze plasma pooled from ten patients with and without the disease, we identified 76 plasma proteins that were increased at least two fold in patients with acute GI GVHD. REG3x, a C-type lectin antimicrobial protein secreted by Paneth cells, emerged as the lead candidate from among proteins that were preferentially expressed in the GI tract and measurable by ELISA. We analyzed plasma levels of REG3x in a validation set of 570 BMT patients: 113 GI GVHD; 223 no GVHD; 52 non-GVHD (e.g. infectious) enteritis; and 182 skin only GVHD. Unrelated and HLA-mismatched donors were over-represented in the GVHD groups; groups were otherwise balanced for age, myeloablative conditioning and date of sample collection. REG3x levels were more than twice as high in the GI GVHD group compared to all others (mean SEM in ng/ml: 245±40, 64±8, 74±18, 112±22, respectively, p=0.004). REG3x could distinguish GVHD from other causes of enteritis with an area under the curve (AUC) of the receiver operating characteristic (ROC) curve of 0.75 (95%CI, 0.66-0.82). Importantly, high REG3x levels at onset of GI GVHD predicted lack of response to therapy (p=0.002). When we divided all patients with diarrhea (n=165) into two equal groups according to REG3x: low (<65 ng/ml, n=83) and high (>65 ng/ml, n=82), the high REG3x group developed more severe GVHD, experienced higher 1 year non-relapse mortality (NRM), and had significantly lower 1 year overall survival (OS) (Table 1). Because KRT18 has previously been reported as a marker for liver/GI GVHD (Blood, 2007); we compared REG3x and KRT18 levels in the same patient samples. KRT18 levels differentiated GI GVHD from non-GVHD enteritis (p=0.01), but were similarly elevated in patients with isolated skin GVHD and did not correlate with GVHD severity, NRM or OS (Table 1). In conclusion, we have identified and validated REG3x as a new diagnostic biomarker specific for GI GVHD with significant prognostic value.

**2**

**Ghrelin Suppresses Insulin Secretion and Compromises Beta-Cell Function in Healthy Humans**

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**ABSTRACT BODY:** Background and aims: The orexigenic gut hormone ghrelin and its receptor, the growth hormone secretagogue receptor 1a, are present in pancreatic islets. While ghrelin reduces insulin secretion in rodents, its effect on insulin secretion in humans has not been established. Our objective was to test the hypothesis that circulating ghrelin suppresses glucose-stimulated insulin secretion in healthy subjects.

**Materials and methods:** Acyl ghrelin (0.5 and 2.0 μg/kg/h) or saline was infused in 16 healthy subjects (8M/8F; age 28.3 ± 6.0 y; BMI 27.0 ± 2.7 kg/m2, fasting plasma glucose 94 ± 1.8 mg/dl, mean ± SEM) on 3 separate occasions in a counterbalanced fashion. The ghrelin was infused for 45 minutes to achieve steady-state levels and continued through a 180-minute frequently sampled intravenous (IV) glucose tolerance test (FSIGT). The acute (first phase) insulin response to IV glucose (AIRg) was calculated from plasma insulin concentrations between 2 and 10 min after the glucose bolus. Insulin sensitivity index (SI) was quantified using the minimal model of glucose kinetics. Disposition index (DI), a measure of β-cell function, was a product of AIRg and SI. IV glucose tolerance was measured by the glucose disappearance constant (Kg) from 10 to 20 min.

**Results:** Ghrelin infusion did not alter fasting plasma insulin or glucose, but both the 0.5 and 2 μg/kg/h ghrelin infusions reduced AIRg (622.3 ± 196.1, 569.6 ± 187.0 vs. 861.2 ± 288.5 min mL/1, respectively, p < 0.05 and < 0.01 ghrelin vs. control) and DI (1940.3 ± 569.7, 1668.7 ± 628.0 vs. 3824.8 ± 1282.9, respectively, p < 0.01 for both doses vs. control) significantly compared to the saline control. Furthermore, the 2 μg/kg/h ghrelin infusion decreased Kg significantly (0.016 ± 0.002 vs. 0.021± 0.003, p < 0.05, ghrelin vs. saline). Ghrelin administration also showed a trend towards lower SI during both ghrelin infusions (p = 0.076 for the 2 μg/kg/h dose).

**Conclusion:** Exogenous ghrelin reduces the first-phase insulin response to IV glucose and lowers β-cell function in healthy humans. These findings raise the possibility that endogenous ghrelin has a role in control of regulation of insulin secretion, and that ghrelin antagonists could improve β-cell function.

**3**

**Two Functional Promoter Variants of Sphingosine-1-Phosphate Receptor 3 Gene Decrease Human Susceptibility to Severe Sepsis-Associated Acute Lung Injury**

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**ABSTRACT BODY:** The genetic mechanisms underlying acute lung injury (ALI) is poorly understood. We have previously demonstrated that sphingosine-1-phosphate (SIP) and its receptor SIPR3, are intimately involved in lung inflammatory responses particularly in vascular barrier regulation. A combination of SIPR3 gene resequencing for SNP discovery, case-control association, promoter functional and transcription factor binding study was utilized. Initial studies identified common SIPR3 gene variants (80 SIPR3 variants, 51 novel) by direct DNA sequencing of a multietnic panel of 27 samples, and 9 cosmopolitan tagging SNPs were selected for subsequent genotyping (iPLEX GOLD). Initial studies identified common SIPR3 gene variants (80 SIPR3 variants, 51 novel) by direct DNA sequencing of a multietnic panel of 27 samples, and 9 cosmopolitan tagging SNPs were selected for subsequent genotyping (iPLEX GOLD).
Gold platform and TiaqMan allelic discrimination assays). Next, we performed association studies in case-control samples of unrelated individuals from both African and European American populations in Chicago (218 cases and 378 controls). In European Americans, the promoter SNPs m702279T (1899 T/G) and rs11137480 (1785 G/C) conferred decreased susceptibility of both severe sepsis and sepsis-induced acute lung injury (p<0.05). To address the effects of these two SNPs on SIRC3 transcription efficacy the SIRC3 promoter with SNPs -1899 T/G and -1785 G/C was cloned into a luciferase reporter vector and assessed for functionality in transfected human lung endothelium. Compared to promoter with -1899T and -1785G, promoter with either SNP-1899G, -1785C or both significantly decreased luciferase promoter activity in endothelial cells upon TNFa stimulation (60%, 50% or 80% decrease, respectively (p<0.05)). Finally, binding to the SIRC3 promoter by the transcription factor Cds1 detected by electrophoretic mobility shift assay was significantly interrupted by SNP-1899 T/G. These data indicate that functional SIRC3 promoter variants influence risks of sepsis and sepsis-associated ALL.

4 THE METABOLIC SYNDROME AND ADVANCED SUBCLINICAL ATHEROSCLEROSIS AS ASSESSED BY CORONARY CALCIUM
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CURRENT CATEGORY: Cardiology/Cardiovascular Disease
ABSTRACT BODY: Background: The Metabolic syndrome (Mets) is a risk factor for the development of cardiovascular disease. However, the relationship between metabolic syndrome and advanced subclinical atherosclerosis as assessed by upper quartile (≥ 75th percentile) coronary artery calcium score (CACS) is not well described.
Methods: We evaluated the predictors of upper quartile CACS in a cross sectional study of 233 subjects without known cardiovascular disease undergoing screening by CAC. The metabolic syndrome was defined with ≥ 3 of the following: waist circumference for men >40 inches and for women >35 inches; triglycerides ≥150 mg/dL; HDL cholesterol <40 mg/dL in men and <50 mg/dL in women; systolic blood pressure ≥130 and/or diastolic ≥ 85 mmHg or drug treatment for elevated blood pressure; fasting plasma glucose ≥100 mg/dL or drug treatment for elevated blood glucose.
Results: The mean age was 53.9 years ± 10.6 years with a range 25 to 80 years; 55% were males. The prevalence of Mets and diabetes was 35.4% and 9.5%, respectively. In univariate analysis of 32 variables the metabolic syndrome (p<0.001 for all subjects and p=0.004 for those without diabetes), diabetes mellitus (p<0.001), fasting blood sugar (p=0.01), systolic blood pressure (p=0.021), cholesterol medicine use (p=0.033), and the Framingham Risk Score (p=0.049) were significantly related to upper quartile CACS. After adjusting for diabetes, fasting blood sugar, systolic blood pressure and cholesterol medicine use, the metabolic syndrome was an independent predictor of upper quartile CACS [odds ratio 2.13 (95% CI 1.02-4.43)]. In a second model adjusting for age, waist circumference, systolic blood pressure, pulse pressure, hypertension, fasting blood sugar, diabetes, family history for premature coronary artery disease, aspirin use, cholesterol medicine use, unemployment, high sensitivity C reactive protein, and Framingham and Reynolds risk scores, Mets remained independently predictive of upper quartile CACS [OR 2.49 (1.05-9.5)].
Conclusions: The metabolic syndrome as defined by ATP III criteria is an independent predictor of advanced subclinical atherosclerosis as assessed by ≥ 75th percentile CACS.

5 ENDOTHELIAL CELL BARRIER REGULATION BY SIMVASTATIN IS MEDIATED BY CLAUDIN-5
W. Chen, J. Jacobson, J.G. Garcia Medicine, University of Illinois at Chicago, Chicago, IL.
CURRENT CATEGORY: Cardiology/Cardiovascular Disease
ABSTRACT BODY: Rationale: We previously reported that simvastatin, an HMG-CoA reductase inhibitor, confers endothelial barrier protection via differential regulation of Rho GTPases. Subsequently, we investigated endothelial cell (EC) tight junctions, a barrier-regulatory aggregation of junctional proteins, and found significant upregulation of claudin-5 mRNA and protein in EC treated with simvastatin associated with rearrangement of tight junctions. Methods/Results: To investigate potential mechanisms involved in the upregulation of claudin-5 by simvastatin, EC were treated with siRNA specific for VE-cadherin (siVE-cad) prior to treatment with vehicle or simvastatin (5 μM, 16 h). Western blotting confirmed decreased claudin-5 expression in both control and simvastatin-treated EC transfected with siVE-cad (100 nm, 3 d) compared to cells transfected with a non-specific siRNA (nsRNA). Mtx in transfed simvastatin did not affect VE-cadherin expression levels, immunofluorescence of simvastatin-treated EC revealed a redistribution of VE-cadherin characterized by increased and more discrete localization at the cell membrane. We next identified effects of simvastatin on FoxO1 and β-catenin, transcription factors regulated by VE-cadherin and known to mediate claudin-5 expression, as EC treated with simvastatin (5 μM, 5-10 min) demonstrated increased levels of phosphorylated FoxO1 and phosphorylated β-catenin. We then studied the functional effects of claudin-5 upregulation on simvastatin-mediated EC barrier protection and found that silencing of claudin-5 (siCLN-5, 100 nm, 3 d) did not alter transendothelial electrical resistance or flux of a high molecular weight marker (FITC-dextran, 2000 kD) across either vehicle or simvastatin-treated (5 μM, 16 h) EC monolayers administered thrombin (1 U/mL). However, compared to controls transfected with nsRNA, EC transfected with siCLN-5 (100 nm, 3 d) and grown on transwell inserts prior to treatment with vehicle or simvastatin (5 μM, 16 h) demonstrated a significant increase in thrombin-induced (1 U/mL, 1 h) permeability to the low molecular weight marker, sodium fluorescein (376 D). Finally, we performed in vivo studies in which mice were administered siCLN-5 or nsRNA intratracheally (10 mg/kg, 3 d) prior to treatment with simvastatin (20 mg/kg, IP) or vehicle and then administered of intratracheal LPS (1.26 mg/kg, 24 h). Lungs from these animals were then perfused with Hoechst stain H33258 (562 D) and imaging revealed an attenuation of LPS-induced dye extravasation associated with simvastatin treatment that was partially reversed in animals administered siCLN-5 compared to controls. Conclusions: Our data confirm the upregulation of claudin-5 by simvastatin is mediated by VE-cadherin and FoxO1 and is a key mediator of the vascular protective properties of statins.

6 MITOCHONDRIA-TARGETED ANTIOXIDANT, MITO-TEMPO, PREVENTS ANGIOTENSIN II MEDIATED CONNEXIN 43 REMODELING AND SUDDEN CARDIAC DEATH
A.A. Sovari, L. Gu, D. Mitchell, E. Jeong, M.G. Bonini, S.C. Dudley Cardiology, UIC, Chicago, IL; S. Irvanian Cardiology, Emory University, Atlanta, GA.
CURRENT CATEGORY: Cardiology/Cardiovascular Disease
ABSTRACT BODY: Introduction: Angiotensin II activation and associated elevation in reactive oxygen species (ROS) have been implicated in pathogenesis of many cardiovascular disorders including arrhythmia. Nevertheless commonly used antioxidants have been shown to be ineffective in clinical trials. We created a transgenic mouse model of cardiac restricted overexpression of angiotensin converting enzyme (ACE8/8). These mice show spontaneous ventricular tachycardia and fibrillation (VT/VF), sudden cardiac death (SCD), and a reduction in Cx43 level, which impairs conduction and predisposes to arrhythmia. We sought to determine the role and the major source of oxidative stress by angiotensin II in VT/VF and Cx43 remodeling.
Method: Wild type and ACE8/8 mice with and without 2 weeks of treatment with a NOS inhibitor (L-NO, 25mg/Kg IP injections daily), a mitochondria-targeted antioxidant (Mito-TEMPO, 0.7mg/Kg IP injections daily), a NADPH oxidase inhibitor (Apocynin 80mg/L in drinking water), and ACE8/8 crossed with P67DN were studied. Western blotting (with derivatization to dini- trophenylhydrozone to detect oxidized protein levels), and immunohistochemistry staining for Cx43 were performed. Electrophysiology study was performed by a 1.1F octapolar catheter through internal jugular vein access and pacing the right ventricle using a burst pacing protocol.
Results: Proteins were more oxidized (increased protein-carbonyl detection), and Cx43 was reduced in ACE8/8 to 33% of control. Treatment with Mito-TEMPO prevented SCD and improved survival in ACE8/8 mice (p<0.0005, hazard ratio 4.76 with 95% CI of 1.96 to 11.53). Inducibility of VT/VF was higher in ACE8/8 mice compared to WT (87.5% vs. 2.3%) and VT
inducibility was reduced with Mito-TEMPO treatment (50% in treatment group). Cx43 level (immunohistochemistry) was increased by 1.7 fold with Mito-TEMPO treatment. Treatments with L-NIO, Apocynin and crossing with P67DN mice did not prevent VT/VF and SCD in ACE8/8 mice. This result suggests that mitochondria are the major source of ROS by angiotensin II and mitochondria-targeted antioxidants may be effective antiarrhythmic drugs.

7 REGULATION OF ISLET BETA CELL SERCA2 EXPRESSION AND CALCIUM HOMEOSTASIS IN TYPE 2 DIABETES MELLITUS

T. Kono, D. Moss, C. Evans-Molina Medicine, Indiana University, Indianapolis, IN; G. Ahn, L. Gann, P. Fueger, T. Ogilha Wells Center for Pediatric Research, Indiana University, Indianapolis, IN; A. Zarin-Herzberg Dept. de Bioquimica, Facultad de Medicina, Universidad Nacional Autonoma de Mexico, Mexico D.F. Mexico.

CURRENT CATEGORY: Endocrinology/Metabolism

ABSTRACT BODY: The maintenance of intracellular Ca2+ homeostasis in the pancreatic β cell plays a vital role in insulin secretion, maintenance of endoplasmic reticulum (ER) health, and cellular survival. Cytosolic and ER Ca2+ levels are closely regulated by activity of the sarco-endoplasmic reticulum Ca2+ ATPase (SERCA) pump, which hydrolyzes 1 ATP molecule in order to move 2 Ca2+ molecules across the sarco- or ER membrane. Dysfunction of the pancreatic β cell plays a prominent role in the pathogenesis of Type 2 diabetes mellitus (T2DM), and the major premise of our research program is to define the biochemical pathways that govern gene expression and cellular function in the β cell in order to identify key sites of dysregulation in diabetes mellitus. Our data demonstrate a progressive and significant loss of β cell SERCA2 mRNA and protein expression in several representative models of T2DM, including islets isolated from obese diabetic db/db mice, lean C57BL6 mice treated with streptozotocin to induce islet inflammation, diabetic cadaveric human islets, as well as human islets and rat insulinoma (INS-1) cells treated with high glucose (25 mM) and the pro-inflammatory cytokine IL-1β. Loss of SERCA2 was found to correlate with elevated β cell basal Ca2+ levels, impaired Ca2+ response to glucose, insulin secretory defects, and activation of ER stress and death pathways. Previous studies have suggested that pharmacologic PPAR-γ activation in T2DM has direct effects to improve β cell function, which are independent of PPAR-γ effects to lower blood glucose and free fatty acid levels. Interestingly, treatment with the PPAR-γ agonist, pioglitazone (pio), restored SERCA2 expression in diabetic human islets and human INS-1 cells. We have now shown that pio increases SERCA2 homeostasis and insulin secretion. As confirmation that these effects were attributable to restoration of SERCA2 levels, SERCA2 was overexpressed in INS-1 cells that had been treated with high glucose and IL-1β, and insulin secretion was similarly rescued. To determine if the observed transcriptional effects of PPAR-γ activation were direct, luciferase assays were performed in INS-1 cells using fragments of the SERCA2 promoter. Results show that a region 259 bp upstream of the transcriptional start site, containing 2 putative PPAR responsive elements, is sufficient to confer PPAR-γ transactivation. These results were confirmed by ChIP and EMSA assays, which demonstrated that PPAR-γ directly binds the proximal PPRE in the SERCA2 promoter. Deletion and mutagenesis assays further indicate that the PPRE, which harbors a key regulatory region necessary for efficient SERCA2 transcription both under basal and hyperglycemic stimulated conditions. We next sought to characterize the mechanisms by which SERCA2 was downregulated in the Type 2 diabetic β cell. PPAR-γ mRNA and protein expression were found to be decreased under conditions of stress and restored with pio, suggesting an autoregulatory loop. Further, inhibition of the kinase S6 kinase 5 (S6K5), which has been shown to phosphorylate and inactivate PPAR-γ in adipose tissue, led to a restoration of SERCA2 expression and improved β cell survival under diabetic conditions. Taken together, these data indicate that dysregulation of SERCA2 plays a prominent role in the progressive β cell death and dysfunction observed in T2DM. Restoration of SERCA2 expression, either through gene replacement strategies, pharmacologic activation of PPAR-γ, or through inhibition of CDK5 may be viable therapeutic approaches to improve β cell function and survival. Future studies will be performed to fully characterize the pathways through which SERCA expression is altered in diabetes and to identify other key transcriptional regulators.

8 RAC1 INTERACTION WITH CYTOCHROME C MEDIATES MITOCHONDRIAL H2O2 PRODUCTION IN ASBESTOS-EXPOSED MACROPHAGES

H.L. Osborn, A.J. Ryan, A. Racila, S. Murthy, B. Carter Pulmonary and Critical Care, University of Iowa Hospitals and Clinics, Iowa City, IA.

CURRENT CATEGORY: Pulmonary/Critical Care

ABSTRACT BODY: Asbestos exposure is a prototypical cause of pulmonary fibrosis which is a progressive disease characterized by lung injury and associated inflammation that induces aberrant repair. Alveolar macrophages obtained from patients with asbestosis spontaneously release high levels of ROS, specifically H2O2, H2O2 generation is known to mediate the development of pulmonary fibrosis and inhibition of H2O2 generation attenuates the development of fibrosis. Rac1 is a GTPase belonging to the Rho family of GTP binding proteins that is linked to H2O2 production. We have shown that over expression of Rac1 increases mitochondrial H2O2 generation in macrophages and Complex III is known to be site of the majority of reactive oxygen species production in the mitochondria. The mechanism by which Rac1 induces mitochondrial H2O2 generation after asbestos exposure is unknown.

We hypothesize that Rac1 is imported into the mitochondria where it is activated by asbestos and generates H2O2 by interacting with Complex III.

We found that Rac1 is present in the mitochondria, the intermembrane space (IMS), and it is activated in the intermembrane space by asbestos.

The translocation of small proteins into the mitochondria requires the presence of conserved cysteine motifs to allow movement to and accumulation in the IMS. We performed site directed mutagenesis of the cysteine motifs in Rac1 and found that the C189S Rac1 mutant was not imported into the mitochondrial IMS, suggesting that this cysteine motif is required for translocation into the mitochondria. Cells expressing Rac1 C189S had significantly less mitochondrial H2O2 generation compared to cells expressing wild type Rac1.

To determine whether Rac1 interacted with Complex III, we overexpressed wild type Rac1 in macrophages cultured in the presence or absence of asbestos. Immunoprecipitation of Flag Rac1 from isolated mitochondria revealed that Rac1 interacted directly with Cytochrome C in cells exposed to asbestos. As expected, Rac1 C189S did not interact with Cytochrome C since it does not translocate to the mitochondria.

Rac1 plays an important role in the generation of ROS in response to a known mediator of pulmonary fibrosis, asbestos. Rac1 activity is increased in the mitochondria and interacts with Complex III (Cytochrome C) and increases H2O2 production. Cells expressing Rac1 C189S were not as responsive to asbestos treatment compared to Rac1 expressing cells.

These observations demonstrate a novel mechanism by which Rac1 induces mitochondrial H2O2 production and mediates asbestos-induced pulmonary fibrosis.

9 TAZ AS A REGULATOR OF MESENCHYMAL TRANSFORMATION AND CLINICAL AGGRESSIVENESS IN GLIOMAS

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CURRENT CATEGORY: Hematology and Oncology

ABSTRACT BODY: Glioblastoma (GBM) is a highly malignant, infiltrative brain tumor. Our lab has performed extensive microarray studies on GBM and has shown that a subset of these tumors have a gene expression signature characterized by those associated with the mesenchyme. Tumors which overexpress these genes are associated with poor outcome and resistance to treatment. In an effort to identify the molecular mechanisms by which this mesenchymal shift occurs, we have identified TAZ (transcriptional co-activator with PDZ-binding motif) as a transcriptional co-activator whose expression level is tightly associated with the mesenchymal change (elevated
TAZ is positively associated with higher expression of key mesenchymal genes. High expression of TAZ also correlated with higher grade glioma as well as poorer patient outcome. These data lead to our main hypothesis, that TAZ activation is critically important in the mesenchymal transition and aggressive clinical behavior in GBM.

To further discern TAZ’s role in GBM, we stably silenced or overexpressed TAZ in primary cell lines. Loss of TAZ did not affect proliferation, but did decrease invasion and dramatically decreased tumor formation when injected intra-cranially into severe combined immunodeficiency (SCID) mice. Five of 5 mice injected with control cell cultures developed tumors, while only 2 out of 10 TAZ-knockdown cultures resulted in tumor formation. Furthermore, the 2 tumors that formed were of lower histologic grade compared to controls. Cells expressing a constitutively active form of TAZ were injected into SCID mice and 5 of the 5 mice injected with the overexpression clone died less than 60 days post-injection, while the mice injected with the vector control (low TAZ levels) survived an average of 120 days post-injection. Overexpression of TAZ increased invasion, and induced osteogenesis, but did not alter proliferation. Further TAZ activation regulates the expression of signature mesenchymal genes, as shown by Affymetrix profiling of TAZ-transfected and TAZ-knockdown constructs. We also used the RCAS/N-tva system to overexpress TAZ in nestin positive brain cells. We found that when TAZ is co-expressed with PDGF-B, high grade gliomas form and result in a 50% survival probability of less than 5 weeks; however, as previously published, when PDGF-B is overexpressed alone, low grade gliomas form and result in a 50% survival probability of 11 weeks.

These data support the hypothesis that TAZ is a central regulator of mesenchymal gene expression in glioma. To further characterize TAZ function in human glioma, we examined the expression of its binding partners (TEAD1-4), as well as FN1, a marker of mesenchymal phenotype, in human tumors. Western analyses showed that TEAD4 was specifically upregulated in grade IV/GBM compared to lower grade gliomas, and TAZ to TEAD4 expression tightly paralleled FN1 expression. Inspection of the TAZ promoter region revealed a Cpg island in the promoter region that was methylated in most lower grade tumors, but not in grade IV GBMs implying that methylation may be one means by which TAZ is regulated and appears to be epigenetically silenced in tumors with a more favorable outcome. To confirm this, we found that treatment of TAZ-methylated primary cell lines with a demethylating agent resulted in an increase in TAZ expression in both the mRNA and protein levels.

We have identified TAZ as a critical regulator in the mesenchymal transition in gliomas. Since mesenchymal differentiation is associated with gliomasogenesis and tumor aggressiveness, strategies to target TAZ and its downstream targets may be warranted in alternative treatment options for patients. This study may also provide a rationale for developing inhibitors which directly target TAZ.

10 LOSS OF ACTIVATOR OF G PROTEIN SIGNALING 3 IMPAIRS RENAL TUBULAR REGENERATION FOLLOWING ACUTE KIDNEY INJURY IN RODENTS

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CURRENT CATEGORY: Nephrology

ABSTRACT BODY: Recovery of renal function following acute kidney injury (AKI) is often incomplete and subsequently increases the risk of chronic kidney disease, end-stage renal disease, and death. Therefore, the discovery of pathways that promote tubular repair is vital to improve outcomes in AKI. Renal ischemia precipitates tubular epithelial cell injury which is exacerbated upon reperfusion. Subsequently, renal tubular regeneration proceeds in a coordinated fashion, primarily by de-differentiation and proliferation of sublethally injured tubular epithelial cells. The intracellular mechanisms that promote this reparative response remain poorly understood. G protein-coupled receptors activate heterotrimeric G proteins to initiate and integrate a variety of critical intracellular signals, including those that influence cell proliferation. Heterotrimeric G protein signaling can be regulated independent of cell surface receptors through the action of activator of G protein signaling 3 (AGS3) which controls signaling intensity and accessibility to specific signaling molecules within cellular microdomains. In the present study, we demonstrate that AGS3 influences renal tubular regeneration following ischemia-reperfusion injury (IRI) in rodents. In normal rat kidneys, AGS3 protein expression was undetectable by Western blot analysis. Following IRI, there was a temporal induction of renal AGS3 protein expression that peaked with a 60-fold increase between 72 and 96 hours after reperfusion and corresponded to the repair and recovery phase following ischemic injury. Renal AGS3 expression was lost predominantly to the recovering outer medullary proximal tubular cells and was highly co-expressed with Ki-67, a marker of cell proliferation. Kidneys from AGS3 deficient mice exhibited impaired renal tubular regeneration following IRI compared to wild-type AGS3 expressing mice. This was characterized by significant increases in renal tubular dilatation and cast formation and a significant decrease in renal tubular epithelial cell density 7 days following ischemic injury. Mechanistically, genetic knock-down of endogenous AGS3 mRNA and protein in renal tubular epithelial cells reduced cell proliferation in vitro. Similar reductions in renal tubular epithelial cell proliferation were observed following incubation with gallic acid, a selective inhibitor of Gβγ subunit activity, and lentiviral over-expression of the carboxyl terminus of G protein coupled receptor kinase 2 (GRK2e2), a scavenger of Gβγ subunits. In conclusion, these data suggest that AGS3 facilitates renal tubular epithelial cell proliferation and renal tubular regeneration by influencing heterotrimeric G protein function through a G protein-coupled receptor-independent mechanism.

11 CD30/CD30L SIGNALING IN TREG MEDIATED SUPPRESSION OF T CELL FUNCTION

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CURRENT CATEGORY: Hematology and Oncology

ABSTRACT BODY: CD30 is a member of the TNF superfamily of proteins widely known to be responsible for regulating T cell function. Our previous work demonstrated the critical role of CD30 in regulatory T cells (Treg) function in a graft-versus-host disease (GVHD) model. Here, we demonstrated that CD30 and its ligand (CD30L or CD153) are differentially expressed on T cell subsets. With minimal expression at rest, CD30 is predominantly restricted to Treg while CD30L expression is upregulated predominately on CD4 and CD8 T cells upon TCR activation. We observe the same pattern of differential expression in vivo using an allogeneic cardiac transplant model. Effector function of conventional T cells which comprise of CD4+ and CD8+ T cells (Tcon) from CD30L KO or wild type (WT) mice are equally efficient in inducing GVHD upon allogeneic hematopoietic cell transplantation into a lethally irradiated recipients. However, suppression of GVHD by co-administration of Treg is only observed for WT Tcon and not CD30L KO Tcon, suggesting a significant role of CD30/30L in Treg-Tcon interaction. In cytotoxicity assays and syngeneic mouse tumor models, we further showed that mouse lymphoma cells (EL4) that express CD30 are relatively resistant to CD8 cell mediated cytotoxicity and this immunity is decreased with CD8 cells from CD30L KO mice. In sum, these findings support a model whereby CD30L-induced reverse signaling in Tcon may suppress their function, and this is mediated by the CD30 expression on stimulated Tregs and cancer antigen-specific T cells to evade cytotoxicity from tissue infiltrating effector lymphocytes.
13 NOVEL CANDIDATE GENES ASSOCIATED WITH THE PROTECTIVE EFFECTS OF SEW-2871 ON BRAIN DEATH-INDUCED ACUTE LUNG INJURY

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CURRENT CATEGORY: Pulmonary/Critical Care

ABSTRACT BODY: Lung transplantation, the only viable therapy for patients with end-stage lung disease, is severely compromised by the lack of available donor lungs. Currently, the vast majority of lung donors are from brain death (BD) individuals, however, the autonomic storm accompanying BD often results in neurogenic pulmonary edema (NPE) and induces irreversible lung injury. We previously demonstrated that sphingosine 1-phosphate (S1P), a phospholipid angiogenic factor and major barrier-enhancing agent, reduces vascular permeability and ischemia/ reperfusion (IR) lung injury in rodents via ligation of the S1P1 receptor, S1PR1. As primary lung graft dysfunction is induced by vascular endothelial cell barrier dysfunction, we hypothesized that SEW-2871, a S1PR1 agonist, may attenuate NPE when administered to the donor shortly after BD. In a rat brain death model with hemodynamic monitoring, 4h after BD rats exhibited significant lung injury with ~60% increases in bronchoalveolar lavage (BAL) total protein, BAL cell counts, and lung tissue W/D weight ratios. In contrast, rats receiving SEW-2871 (0.1 mg/kg) 15 minutes after the induction of BD and assessed at 4h later exhibited significant lung protection (~50% reduction, p<0.01) reflected by reduced BAL total protein, BAL cytokines concentration, BAL albumin, BAL total cell count and lung tissue wet/dry (W/D) weights ratio. Lung tissue microarray analysis at 4h after BD revealed significant roles in KS and PEL pathogenesis. In the first part of the study, we examined the role of COX-2 in PEL using nimesulide, a well-known COX-2 specific NSAID that is prescribed to approximately 500 million people in 50 different countries. Our data demonstrates that nimesulide is efficacious in inducing proliferation arrest in PEL (KSHV+/EBV+; BCBL-1 and BC-3, KSHV+/EBV+; JSC-1), EBV-infected (KSHV+/EBV+; Raji), and non-infected (KSHV+/EBV-; Akata, Loukes, Ramos, B/L) high malignancy human Burkitt’s lymphoma (BL) and KSHV+/EBV+ lymphoblastoid (LCL) cells. Tukey’s post hoc comparison analysis demonstrates that the anti-proliferative effects of nimesulide on KSHV+/EBV+ cells were more potent indicating the increased vulnerability of KSHV infected NHL cells to nimesulide mediated COX-2 blockade. Nimesulide induced sustained cell death and G1 arrest in BCBL-1 and down-regulated KSHV latent genes LANA-1 and vFLIP. Consequently, nimesulide treatment activated p53/p21 tumor-suppressor pathway by blocking LANA-1/p53 interaction, down-regulated cell survival kinases p-Akt and p-GSK-3β, VEGF-C, and PEL de-fining genes syndecan-1, aquaporin-3, and vitamin-D3 receptor, and cell cycle proteins cyclins E/A and cdC25. Overall, our data for the first time provides a comprehensive molecular framework linking COX-2 with PEL pathogenesis. In the second part of the study, we investigated the role of COX-2/PGE2/EP receptors in KS pathogenesis. COX-2, PGE2 and its receptors (EP1-4) were detected in KS lesions with the strong staining of COX-2/EP2/EP4. In latent KSHV infected endothelial cells, expression of COX-2/EP receptors and PGE2 secretion were up-regulated and EP receptor antagonists down-regulated LANA-1 expression as well as Ca2+, p-Src, p-PDK3, p-PTK3, p-PTK1, and p-NF-xB. Exogenous PGE2 and EP receptor agonists induced the LANA-1 promoter potentially through YY1, Sp1, Oct-1, Oct-6, CEBP and c-Jun transcription factors. PGE2/EP receptor induced LANA-1 promoter activity was down-regulated significantly by the inhibition of Ca2+, p-Src, p-PDK3, p-PTK3/4, and p-NF-xB. Thus, our data for the first time implicates the inflammatory EP receptors in any form of herpes virus latency and uncover a novel paradigm that demonstrates the evolution of KSHV genome plasticity to utilize inflammatory response for its survival advantage of maintaining viral gene expression. Collectively, our data strongly suggest that the potential use of anti-COX-2 and anti-EP receptor therapy may not only ameliorate the chronic inflammation associated with KS and PEL but could also lead to elimination of the KSHV latent infection. Our study also uncovered the chemotherapeutic potential of nimesulide in treating PEL. Additionally, correlation between COX-2 and poor NHL prognosis suggests its potential as a therapeutic model for similar NHLs.
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CD8+ T-CELL RESPONSE AGAINST HUMAN PAPILLOMAVIRUS TYPE 16 E6 PROTEIN IS ASSOCIATED WITH A FAVORABLE CLINICAL TREND


CURRENT CATEGORY: Infectious Disease

ABSTRACT BODY:

Background: The association between the CD8+ T-cell responses to human papillomavirus (HPV) type 16 E6 protein and a favorable clinical trend in women with cervical disease has been demonstrated previously. The roles of HPV-specific CD4+ T-cell responses and of regulatory T-cells (Tregs) were examined.

Methods: Subjects with abnormal Papanicolaou smear results were enrolled. Interferon-γ enzyme-linked immunospot assay and fluorescent activated cell sorter analysis to measure the frequencies of Tregs were performed.

Results: Subjects with histological diagnosis of cervical intraepithelial neoplasia 1, 2, or 3 were considered to have short-term persistence of cervical abnormality and were called “regressors” (n=51) while those of normal histology were designated “persistors” (n=51). A significantly higher percentage CD8+ T-cell response was detected in the regressors (15/33 or 45.5%) compared to the persistors (10/51 or 19.6%) (p = 0.015) for the E6 peptides. The CD4+ responses to certain E6 regions (E6[16–40], E6[91–115], E6[106–113], and E6[138–158]) were also significantly higher in the regressors compared to the persistors. Although there was no difference in the frequencies of Tregs between the two groups, low frequencies of Tregs were significantly associated with positive CD4+ T-cell responses within certain E6 regions (E6[16–40], E6[31–55], E6[76–100], E6[91–115], and E6[106–130]).

Conclusions: CD4+ T-cell responses to the HPV-16 E6 protein are associated with a favorable clinical trend. In addition, CD4+ T-cell responses to specific regions within the HPV-16 E6 protein are associated with regression and lower percentage of circulating Tregs. Collectively, a broad CD4+ T-cell immune response to E6 regions overall, and E6 regions (E6[16–40] and E6[106–130]), and E6[136–158] independently, may be important in the regression of cervical lesions and may be related to lower Treg frequency.

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MYOCARDIAL LATE GADOLINIUM ENHANCEMENT IS A NOVEL FINDING IN SICKLE CELL DISEASE: A CARDIAC MAGNETIC RESONANCE AND GENOMIC STUDY

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CURRENT CATEGORY: Cardiology/Cardiovascular Disease

ABSTRACT BODY: Rational: Cardiopulmonary complications are a major cause of death in patients with sickle cell disease (SCD). Our aim was to characterize myocardial structure and function in SCD patients using Cardiac Magnetic Resonance (CMR) imaging. Based on the imaging data, we then performed microarray analyses of isolated peripheral blood mononuclear cells (PBMC) on a subset of patients to identify novel genetic pathways potentially associated with cardiopulmonary subphenotypes in SCD and enhance our understanding of the development of these sequelae.

Methods: A total of thirty-one outpatients with SCD in steady-state (mean age 33 +/- 7yrs) prospectively underwent CMR. Using CMR, cardiac chamber size, ventricular function, presence of myocardial late gadolinium enhancement (LGE), and myocardial and hepatic iron deposition (T2*), abnormal myocardial T2*<20ms and hepatic T2*>13ms were determined. Microarray analyses of PBMC RNA utilized FDR<10% and fold-change>1.25.

Results: Patients with SCD exhibited preserved left ventricular (LV) ejection fraction (58.3 +/- 7%) with dilated LV (225 +/- 61mL, normal-150 +/- 31mL) and right ventricular (RV, 227 +/- 59mL, normal-173 +/- 39mL) and diastolic volumes (EDV) and LA volumes (117 +/- 34mL, normal-97 +/- 27mL). LGE was noted in 7/31 (23%), myocardial iron overload in only 2/31 (7%) and hepatic iron overload in 16/31 (52%) subjects. Subjects with LGE had lower hepatic T2* (5 +/- 3ms vs 22 +/- 13ms, p=0.01). Women with LGE (vs women without LGE) had significantly larger LVEDV index (153 +/- 9mL/m2 vs 108 +/- 25mL/m2, p=0.01), RVEDV index (230 +/- 13mL/m2 vs 194 +/- 41mL/m2, p=0.02), and LA volume (147 +/- 15mL vs 94 +/- 20mL, p=0.01). Microarray analyses revealed that patients with LGE (n=5) exhibited 159 differentially regulated transcripts versus those without LGE (n=11) with the regulation of apoptosis as the most significantly represented ontology followed by cell proliferation (p<0.01).

Conclusion: We report, for the first time, the presence of significant LGE in SCD. Patients with LGE demonstrated greater hepatic iron overload suggesting increased disease severity and women with LGE exhibited more adverse remodeling. Patients with LGE exhibited a unique gene expression profile representing significant apoptosis. Finally, we speculate that LGE may represent a new finding in cardiac pathology in SCD.

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REGULATION OF TUMOR IMMUNE MICROENVIRONMENTS BY EPHRINB2

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CURRENT CATEGORY: Hematology and Oncology

ABSTRACT BODY: Background: Tumor microenvironment consists of both inflammatory and vascular cells. Inflammatory cells in particular play important roles in tumor growth and resistance to therapy. Blood vessels, which supply oxygen and nutrients to growing tumors, may positively and negatively regulate the entry of inflammatory cells into tumor tissues. Interestingly, proteins known to play roles in vascular development during embryogenesis have been implicated in the control of leukocyte migration. For example, ephrinB2, which is a regulator of vascular patterning in developing embryos, is expressed in tumors and influences the activity of chemotactic signaling pathways in other models. A previous study demonstrated that ephrinB2 activation impaired the in vitro chemotaxis of T-cells towards chemokines such as CXCL12/SDF-1. We therefore hypothesized that deletion of microenvironmental ephrinB2 would enhance T-cell accumulation in tumor tissues.

Methods: EphrinB2+/-/lacZ mice were implanted with B16 tumor cells sc in flanks and hindlimbs. Macroscopic tumors were harvested and ephrinB2 expression assayed using x-gal staining. Vascular expression of ephrinB2 in B16 tumors was compared to KR158 gliomas, LLC lung carcinomas, and EL4 lymphomas in ectopic and in metastatic sites after iv tail vein injection. Mx1-cre/efnb2fl/fl mice were generated which allow inducible deletion of ephrinB2. Mx1-cre/efnb2+/- mice were generated which allow for the post-natal deletion of ephrinB2. Mx1-cre/efnb2+/- mice served as controls. Both experimental groups (n=7) were treated with poly(I:polyC) 3x per week for 2 weeks. 4 weeks after treatment, successful deletion of ephrinB2 was confirmed using a PCR based assay to detect deleted bands in bone cells. 1 million B16 tumor cells were implanted in hindlimbs and tumors were measured at least twice weekly. At the conclusion of the study, all tumors were harvested and stained with anti-CD3 antibodies to enumerate T-cell populations. In addition, tumors were stained with anti-CD34 for microvessel density analyses and anti-NG2 to quantify pericyte coverage as a surrogate marker of functional vasculature.

Results: EphrinB2 was expressed in vascular structures in B16, KR58, LLC, and EL4 tumors. Unexpectedly, ephrinB2 was upregulated in microvascular structures in adjacent muscle during tumor invasion, suggesting roles for ephrinB2 in vascular biology both within tumors and at the tumor/host interface. Inducible deletion of host ephrinB2 significantly reduced B16 tumor growth (control tumor volume 2846 mm3 versus deleted tumor volume 869 mm3, p = 0.002). EphrinB2 deletion did not affect CD34+ microvessel density or NG2+ pericyte coverage of vessels. Rather, tumors grown in ephrinB2 deficient microenvironments demonstrated a significant increase in infiltrating CD3+ T-cells (58.4 +/- 14.3 cells/field, p = 0.03). Increasing numbers of CD3+ T-cells inversely correlated with tumor volume.

Conclusion: EphrinB2 is upregulated in tumor vasculature when tumor mass increases. Using a novel imaging protocol, we have demonstrated that host expression of ephrinB2 is necessary for maximal tumor growth. Although ephrinB2 is critical for embryonic angiogenesis, post-natal deletion did not affect tumor angiogenesis. Instead, ephrinB2 deletion led to increased accumulation of CD3+ T-cells in tumor tissues, which inversely correlated with tumor growth. These findings demonstrate a novel role for ephrinB2 specifically and vascular cells generally in regulating immune microenvironments. Validation of these findings will therefore identify...
a novel method to increase the delivery of T-cells to tumor tissues with relevance to combination immunotherapeutic strategies.

19 ARTERIAL CALCIFICATION AND VASCULAR REMODELING IN CHRONIC KIDNEY DISEASE IS DIRECTLY MEDIATED BY S100A12 (EN-RAGE)
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CURRENT CATEGORY: Cardiology/Cardiovascular Disease
ABSTRACT BODY: Background: Increased serum levels of the pro-inflammatory cytokine S100A12 (also known as EN-RAGE), extracellular newly identified RAGE binding protein is associated with cardiovascular morbidity and mortality in hemodialysis patients, even after correction of other inflammatory markers such as IL-6 and CRP. We previously showed that transgenic expression of human S100A12 in vascular smooth muscle cells (VSMC) caused development of thoracic aortic aneurysms in the C57BL/6J mouse strain, and increased atherosclerosis and enhanced vascular calcification in the atherosclerosis prone ApoE null mice. In the current study, we tested the hypothesis that vascular expressed S100A12 predispose non-atherosclerosis prone C57BL/6J mice on normal rodent chow diet but exposed to the metabolic changes of chronic kidney disease (CKD) to develop vascular disease resembling that observed in patients with chronic kidney disease.
Methods: CKD was induced in S100A12 transgenic mice and wild type littermate mice not expressing human S100A12 by surgical ligation of the ureters. The aorta was analyzed after 7 weeks of moderately elevated BUN, and cultured aortic smooth muscle cells were studied.
Results: We found enhanced vascular medial calcification in S100A12tg mice subjected to CKD, while WT-CKD, S100A12qshg and WT-qshg aortas showed no or only scant calcification. Vascular calcification was mediated, at least in part, by activation of the receptor for S100A12, RAGE and by enhanced oxidative stress, since inhibition of NADPH-oxidase Nox1 (using shRNA) and limited access of S100A12 to RAGE (using soluble RAGE) attenuated calcification and gene expression of osteoblastic genes in cultured vascular smooth muscle cells.
Conclusion: S100A12 augments chronic kidney disease-triggered osteogenesis in murine vasculature, reminiscent of features associated of enhanced vascular calcification in patients with chronic and endstage kidney disease.

20 HOSPITAL-ACQUIRED CONDITIONS AND PAY-FOR-PERFORMANCE: THE IMPACT OF RISK ADJUSTMENT
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CURRENT CATEGORY: Health Care Research/Clinical Epidemiology/Quality Improvement
ABSTRACT BODY: Background: Medicare no longer pays for specific hospital-acquired conditions (HACs) as a form of pay-for-performance, regardless of a patient’s risk to develop HACs. The rules are also complex to detect and deny payment for HACs. Hospital rates of HACs without risk-adjustment will soon be reported by Medicare’s Hospital Compare website. Risk adjustment for all admissions will occur in 2015 for hospitals in the top quartile of risk-adjusted HAC rates. The underlying premise is that hospital performance rather than missions will occur in 2015 for hospitals in the top quartile of risk-adjusted HAC rates. The impact of Medicare’s pay-for-performance reform in hospitals will be measured using observed-to-expected ratios.
Methods: We evaluated claims data for 462,176 adult Medicare discharges from 125 acute care Michigan hospitals using the 2007 Healthcare Cost and Utilization Project State Inpatient Dataset. Using the example of catheter-associated urinary tract infections (CAUTIs), cases were identified using ICD-9-CM codes assuming all secondary diagnoses could be HACs. We assessed how hospital comparisons using observed (unadjusted) HAC rates would be modified by simple risk-adjustment using observed/expected ratios based on each hospital’s number of discharges per Diagnosis-Related Group (DRG) and each DRG’s statewide mean rate of urinary tract infections (UTIs) as secondary diagnoses.
Results: Compared to all adult Medicare discharges, the 14% of discharges with secondary diagnosis UTIs (including CAUTIs) were more likely female (57% vs. 70%), older (mean age 73 vs. 77) and more likely to have comorbid diabetes (32 vs. 35%), renal failure (17 vs. 21%), heart failure (17 vs. 24%), paraplegia (4 vs. 8%) and decubitus ulcers (3% vs. 9%). Hospital rates of UTIs as secondary diagnoses ranged from 7 to 24%. Yet only 1% of UTIs were identified as CAUTIs using the catheter code 996.64, thus, because medical record reviews indicate many UTIs in claims data are in fact catheter-associated UTIs, hospitals were compared by UTI rates. Hospitals’ UTI rates varied greatly by DRG, with high mean rates for sepsisemia (>50%), renal failure (30%), rehabilitation (30%), and intracranial hemorrhage/cerebral infarction (19%), and lower rates among other common DRGs such as heart failure/shock (14%), simple pneumonia (14%), major joint replacement of lower extremity (8%), and chronic obstructive pulmonary disease (7%). Observed-to-expected ratios of UTIs using hospitals’ DRGs ranged from 0.6 to 1.8. Of 31 hospitals identified in the top quartile as poor performers using unadjusted UTI rates, 8 were reassigned to better quartiles after risk adjustment using observed-to-expected ratios.
Conclusions: New pay-for-performance strategies such as non-payment and public reporting for HAC’s have important complexities that will affect how and which hospitals and patients are impacted, including: 1) inaccuracy of claims data to identify HACs (such as not using a catheter-code to identify UTIs as CAUTIs) and 2) unequal risks to develop HACs related to patient characteristics and reason for admission (DRG). Even with simple risk adjustment by discharge DRGs, 1 in 4 hospitals identified as poor performers would be reassigned as better performers; thus, risk adjustment should be developed carefully prior to public reporting and hospital pay modifications by HAC rates.

21 FUSION OF AGONIST-INDUCED ENDOSONES WITH INTRACELLULAR VESICLES TO CREATE NOX1 REDOX-ENDOSOMES
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CURRENT CATEGORY: Mechanisms of Disease
ABSTRACT BODY: Over the past decade, Nox1, a catalytic subunit of NADPH oxidase, has emerged as an important mediator of physiologic and pathologic conditions. Nox1-derived reactive oxygen species (ROS) are responsible for redox-dependent signaling that contributes to the development of cancer, cardiovascular disease and other inflammatory diseases. Localization appears to be a regulatory mechanism for Nox1 activation. However, little is known regarding the relative membrane distribution of Nox1 and whether it traffics in response to agonist stimulation. Previous studies of Nox1 localization are limited by the use of heterogeneous overexpression systems. The goal of this study was to characterize endogenous Nox1 localization and trafficking under basal and stimulated conditions. Studies utilized cultured murine and rat aortic smooth muscle cells (SMCs). Biotinylation of plasma membrane (PM) proteins reveal that at basal conditions ~5% of total cellular Nox1 resides at the PM. Having previously shown that tumor necrosis factor (TNF)-α and thrombin activate Nox1 in SMCs, we found that treatment of SMCs with TNF-α protects biotinylated Nox1 from surface biotin cleavage, indicating internalization of PM Nox1 in response to TNF-α. In contrast, treatment of cells with thrombin does not protect Nox1 from surface biotin cleavage, signifying the absence of Nox1 internalization in response to thrombin. Stimulation of SMCs with TNF-α or thrombin in the presence of biotin increased the amount of biotinylated Nox1, suggesting possible recruitment of intracellular Nox1 to the PM. However, inhibiting endocytosis with a dominant-negative dynamin or 4°C prevents this increase in biotinylated Nox1 in response to TNF-α or thrombin. We interpret this finding to indicate that Nox1 is not recruited to the PM after agonist stimulation, but instead, agonist-induced endosomes fuse with intracellular Nox1-containing vesicles to create Nox1 redox-endosomes. These novel findings confirm differential activation of Nox1 in response to different agonists and provide the first evidence for Nox1 trafficking in response to stimulation. Further characterization of Nox1 trafficking is likely to identify additional therapeutic targets for the treatment of cancer, cardiovascular disease and multiple inflammatory diseases.
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NOD2 EXPRESSION UPREGULATED BY ORGANIC DUST VIA NF-κB NEGATIVELY REGULATES ORGANIC DUST-INDUCED INFLAMMATORY RESPONSES

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CURRENT CATEGORY: Mechanisms of Disease

ABSTRACT BODY: Rationale: Chronic organic dust exposure in the agricultural industry results in significant airway disease. Since we have recently demonstrated high concentration of bacterial peptidoglycan in large animal farming environments, we examined the role of the intracellular peptidoglycan breakdown product sensor, nucleotide oligomerization domain 2 (NOD2), in regulating organic dust-induced inflammation.

Methods: Human THP-1 monocytes were exposed to swine facility organic dust extract (ODE) and NOD2 expression by real-time PCR and Western blot was evaluated. NF-κB binding following ODE stimulation was analyzed by EMSA. Cells were also pretreated with NF-κB pathway inhibitors, caffeic acid phenethyl ester (CAPE) and BAY 11-708, and subsequently stimulated with ODE whereupon NOD2 expression was evaluated. Next, isolated primary lung macrophages from NOD2 knock-out (NOD2−/−) and wild-type (WT) mice were ex vivo stimulated with ODE and cytokine/chemokines levels were quantitated by ELISA. Utilizing an established in vivo model, bronchoalveolar lavage (BAL) and lung tissues were collected from WT and NOD2−/− mice challenged once or repetitively for 3wks to ODE or saline.

Results: Relative mRNA and protein expression of NOD2 increased following ODE stimulation at 24 and 48 hours, respectively. NF-κB translocation increased rapidly (30-90 mins) following ODE stimulation, and inhibitors of the NF-κB pathway significantly reduced ODE-induced NOD2 expression. Murine NOD2−/− lung macrophages demonstrated significantly enhanced CXCL1, CXCL2, and IL-6, but not TNF-α production, following ODE stimulation as compared to ODE-stimulated WT macrophages. Next, airway neutrophils and BAL CXCL1 levels were significantly enhanced in NOD2−/− mice as compared to WT following a one-time ODE exposure. After 3 weeks of repetitive, daily exposure to ODE, there was a significant increase in lung parenchymal inflammation indices in NOD2−/− mice as compared to WT mice.

Conclusions: The peptidoglycan breakdown product intracellular sensor, NOD2, is enhanced following organic dust exposure by an NF-κB-dependent pathway, and NOD2 appears to selectively negatively regulate organic dust-induced pro-inflammatory mediator production in macrophages and also airway inflammatory outcomes.

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CURRENT CATEGORY: Disease Modulation/Intervention

ABSTRACT BODY: Objective: We assessed relationships between pediatric mean age 12) risk factors for cardiovascular disease (CVD), type 2 diabetes (T2DM), and high blood pressure (HBP) and the development of CVD, T2DM, and HBP by age 40 years.

Design: 592 schoolchildren were first studied at mean ± SD age 12.4 ± 3.4 and then 27 years later at age 39.8 ± 5.0 years. Relationships between pediatric triglycerides (TG), HDL cholesterol (HDLc), LDL cholesterol (LDLc), blood pressure (BP), glucose, and BMI and the adult development of CVD, T2DM, and HBP were assessed by stepwise logistic regression. Pediatric TG, BP, LDLc, BMI, and glucose above and HDLc below established pediatric cutoffs, along with race, were explanatory variables.

Setting: Public and parochial suburban schools, 27 year prospective follow-up.

Participants: 592 schoolchildren first studied at mean age 12.4 and then 27 years later at mean age 39.8 years.

Outcome Measures: CVD, T2DM, and HBP in young adulthood, at mean age 39.8 years.

Results: By stepwise logistic regression, adult CVD (16 yes, 576 no) was associated with pediatric high TG, odds ratio (OR) 4.9, 95% confidence interval (CI) 1.8-13.5, p = 0.002. Adult T2DM (3 yes, 549 no) was associated with pediatric high BP (OR 2.7, 95% CI 1.2-6.1, p=0.016), black race (OR 2.8, 95% CI 1.4-5.7, p=0.005), pediatric low HDLc (OR 2.5, 95% CI 1.2-5.1, p =.015), and with pediatric glucose (OR 3.2, 95% CI 1.1-9.3, p=0.032). Adult HBP (81 yes, 496 no) was associated with pediatric high BMI (OR 4.1, 95% CI 2.5-6.7, p <0.0001), and with pediatric high BP (OR=2.1, 95% CI 1.2-4.0, p =.015).

Conclusions: Pediatric risk factors at age 12 were significantly, independently related to adult (age 40) CVD, T2DM, and HBP. Pediatric TG was associated with adult CVD. Pediatric BP, low HDLc, high glucose, and race were associated with adult T2DM, and pediatric BMI and BP were associated with adult HBP. Identification of pediatric risk factors for CVD, T2DM, and HBP facilitates initiation of primary prevention programs to reduce development of adult CVD, T2DM, and HBP.

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RACE-BASED DISPARITIES IN POST PERCUTANEOUS CORONARY INTERVENTION (PCI) OUTCOMES PERSIST INDEPENDENT OF SOCIOECONOMIC STATUS (SES) AND ACCESS TO HEALTHCARE

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CURRENT CATEGORY: Cardiology/Cardiovascular Disease

ABSTRACT BODY: Background: Previously, we reported that both African-Americans (AA) patients and lower socioeconomic status (SES) patients had poorer outcomes after percutaneous coronary intervention (PCI). However, these two variables evidenced significant collinearity. We now extend these observations with additional follow-up and investigate the interactions between these factors.

Methods: 1,432 patients undergoing PCI at a large public health system (PHS) hospital were analyzed as an open cohort. Patients were included if complete data were available and uniform access to healthcare was provided through the same PHS. Patients were analyzed by race, SES and other key variables and followed for MACE (death, MI, urgent TVR). Multivariate models were constructed and survival data analyzed.

Results: 1,432 patients (57 ± 10 yrs, 32% female, 47% AA, 21% White) underwent PCI for STEMI (17.1%), NSTEMI (27.9%), unstable angina (26%) or stable CAD (29.1%) over 4.5 years. Clinical follow-up was obtained in 99% of patients (n=1,415, mean 2.2 yrs ± 1.9 yrs). Overall mortality was 8.6% at 2 years (6.8% as the initial MACE event). AA patients evidenced lower MACE-free survival than non-AA (78% vs. 86%; p=0.001, Fig. 1) at 2 years. Mortality for the AA cohort was significantly higher than the non-AA group in absolute terms (5.7% vs. 2.8%, p=0.005) as well as relative to overall MACE. Lower SES patients (dichotomized by median income) evidenced significantly greater MACE rates than higher SES patients as reported previously (78.8% vs. 85.1%, p=0.006) however this observation was now independent of race.

Conclusions: PHS AA patients remain a high-risk post-PCI cohort despite similar presumed access to healthcare as non-AA patients. Death appears to be the first post-PCI MACE event in a significant proportion of PHS patients. The observed race-based disparity seems to be independent of SES. MACE events remain unacceptably high and underscore the need for additional research and intervention.

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NORMALIZATION OF SERUM VITAMIN D IN HYPERCHOLESTEROLEMIC, VITAMIN D DEFICIENT PATIENTS, PREVIOUSLY STATIN INTOLERANT BECAUSE OF MYOSITIS-MYALGIA, RESTORES STATIN TOLERANCE WITHOUT MYOSITIS-MYALGIA

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CURRENT CATEGORY: Cardiology/Cardiovascular Disease

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ABSTRACT BODY: Introduction: Vitamin D deficiency is common in diverse clinical populations in variegated settings and environments. Low serum 25 (OH)D levels have been associated with various health issues and it has been hypothesized that vitamin D deficiency is involved in the pathogenesis of the disease. In this study, we evaluated the role of vitamin D supplementation in the treatment of hypercholesterolemic patients who are previously statin intolerant.

Methods: In 68 hypercholesterolemic patients, unable to tolerate one or more statins because of myositis-myalgia, vitamin D deficiency was found to be having low serum vitamin D with their symptoms improving with Vitamin D supplementation.

Conclusion: In hypercholesterolemic patients, unable to tolerate one or more statins because of myositis-myalgia, vitamin D deficiency was found to be having low serum vitamin D with their symptoms improving with Vitamin D supplementation.

Results: At mean 3 months follow-up, on vitamin D supplementation and statins, 62/68 (91%) previously statin-intolerant patients tolerated statins well and were asymptomatic without myositis-myalgia. In these 62 patients, on vitamin D supplementation, mean ± SD vitamin D rose from 22 ± 7 to 43 ± 13 ng/ml (p<.0001), and LDL cholesterol fell from 162 ± 57 to 34 ± 34 mg/dl (p<.0001). Despite vitamin D supplementation, 6 of 68 patients (9%), had myositis-myalgia on re-instituted statins at 3 months follow-up. In treatment on these 6 patients, mean ± SD vitamin D rose from 22 ± 3 to 42 ± 7 ng/ml (p=.03), and LDL cholesterol fell from 177 ± 33 to 114 ± 40 mg/dl (p=.03). In 91% of hypercholesterolemic, vitamin D deficient patients, previously statin intolerant because of myositis-myalgia, normalization of serum vitamin D restored statin tolerance without myositis-myalgia.

Objective: To evaluate whether S1PR1 may serve as a potentially novel biomarker in hypercholesterolemia.

Methods: We used a novel transgenic mouse model of hypercholesterolemia which we developed in our laboratory. This model mimics human hypercholesterolemia and statin intolerance. We measured serum vitamin D levels and evaluated the effect of vitamin D supplementation on statin intolerance.

Conclusion: Our study demonstrated that vitamin D supplementation improved statin tolerance in hypercholesterolemic patients who are previously statin intolerant.

27 ADOLESCENT OLGOMENORRHEA IN A HEALTHY, BI-RACIAL SCHOOLGIRL COHORT: A SIMPLE CLINICAL PARAMETER PREDICTING YOUNG ADULT INSULIN, GLUCOSE, AND HOMA INSULIN RESISTANCE FROM AGE 19 TO 25

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CURRENT CATEGORY: Endocrinology/Metabolism

ABSTRACT BODY: Objective: Olgomenorrhea is associated with increased type 2 diabetes mellitus (T2DM) in adult women. We hypothesized that age 14 to 19 oligomenorrhea would predict insulin, glucose, and HOMA insulin resistance (IR) from age 19 to 25, and impaired fasting blood glucose ≥100 mg/dl (IFG) plus T2DM from age 10 to 24.

Methods: The study design was a prospective 14 year follow-up involving healthy, bi-racial girls (n=370), in urban and suburban schools, starting at age 10 years in the Cincinnati clinic of the National Growth and Health Study. There were no interventions and outcome measures included insulin, glucose, HOMA IR, impaired fasting glucose (IFG), and type 2 diabetes mellitus (T2DM). Girls with type 1 DM and glucose ≥126 mg/dl at age 10 were excluded from the analysis cohort.

Results: Age 14 waist circumference was the most important explanatory variable for average insulin ages 19 to 25 (partial R2=29.7%), for average glucose ages 19-24 (6.6%), and for HOMA IR (30.1%) from ages 19 to 24 (fall p<.0001). At age 14 androgens were associated with age 19-25 insulin (free testosterone [FT], partial R2 = 1.1%), glucose (dehydroepiandrosterone sulfate [DHEAS] 2.1%), and HOMA IR (DHEAS 1.6%, FT 0.9%), all p<.025. Age 14–19 oligomenorrhea was associated with age 19-25 insulin (partial R2=1.9%), glucose (2.9%), and HOMA IR (2.8%), all p≤.004. Correlates of IFG + T2DM from age 10 to 24 included childhood insulin (OR 1.05, 95% CI 1.03-1.06), age 14 DHEAS (OR 1.04, 95% CI 1.01-1.07), and oligomenorrhea category, age 14-19 (OR 1.53, 95% CI 1.04-2.23).

Conclusions: Olgomenorrhea in healthy bi-racial schoolgirls during ages 14–19 predicts insulin, glucose, and HOMA IR from ages 19–25, and IFG + T2DM from ages 10–24, facilitating primary prevention of hyperinsulinemia, IFG, and T2DM.
Results: S1PR3 was identified by mass spectroscopy analysis among 10 nitrated IP4 plasma proteins in ALI mice, results confirmed in other murine models. Furthermore, serum from humans with severe sepsis-induced ALI (n=15) exhibited elevated S1PR3 levels whereas normal and ICU controls had minimal S1PR3 detection (p < 0.01). EC exposure to barrier-disrupting agents (LPS, thrombin) induced S1PR3 nitration and shedding from the EC plasma membrane within S1PR3-containing microparticles. Shed microparticles produced significant declines in normalized EC electrical resistance consistent with increased permeability which was attenuated by silencing of EC S1PR3 expression.

Conclusions: These results suggest that S1PR3 nitration and subsequent shedding into circulation as microparticles represents a potentially viable and novel biomarker in ALI.

29
NORMALIZATION OF SERUM VITAMIN D IN 125 HYPERCHOLESTEROLEMIC, VITAMIN D DEFICIENT PATIENTS, PREVIOUSLY STATIN INTOLERANT BECAUSE OF MYOSITIS-MYALGIA, RESTORES STATIN TOLERANCE WITHOUT MYOSITIS-MYALGIA
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CURRENT CATEGORY: Endocrinology/Metabolism
ABSTRACT BODY: In 125 hypercholesterolemic patients, unable to tolerate ≥ 1 statin because of myositis-myalgia, selected by low (<32 ng/ml) serum vitamin D, we prospectively assessed whether resolution of vitamin D deficiency would result in statin tolerance, free of myositis-myalgia. We studied 58 men, 67 women, median age 60, 107 white, 18 black. On no statins, 50,000 units of vitamin D was given twice/week for 3 weeks, and then continued once/week.

After 3 weeks on vitamin D, statins were restarted. Patients were re-assessed every 3–4 months on statins and vitamin D supplementation. On vitamin D supplementation and re-instituted statins for a median of 7.5 months, serum vitamin D normalized in 96 patients (77%), and 107 patients (86%) were asymptomatic.

Symptomatic myositis-myalgia in hypercholesterolemic statin-treated patients with concurrent vitamin D deficiency may reflect a reversible interaction between vitamin D deficiency and statins on skeletal muscle.

Table 1. Vitamin D supplementation in 125 hypercholesterolemic, vitamin D deficient patients, previously statin-intolerant because of myositis-myalgia.

<table>
<thead>
<tr>
<th>Duration of treatment</th>
<th>Vitamin D (ng/ml)</th>
<th>1,25D (ng/ml)</th>
<th>VD normalized (≥32 ng/ml) on follow-up (%)</th>
<th>Myalgia-free on follow-up (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>months</td>
<td>Study entry</td>
<td>Follow-up</td>
<td>Study entry</td>
<td>Follow-up</td>
</tr>
<tr>
<td>&lt;3 months</td>
<td>19.9 ± 20</td>
<td>40 ± 12.41 ♦</td>
<td>161 ± 60</td>
<td>155</td>
</tr>
<tr>
<td>3–5 months</td>
<td>23 ± 5</td>
<td>44 ± 15.42 ♦</td>
<td>156 ± 47</td>
<td>166</td>
</tr>
<tr>
<td>5–8 months</td>
<td>40 ± 6.4</td>
<td>70 (1.7)</td>
<td>178 ± 52</td>
<td>195</td>
</tr>
<tr>
<td>≥9 months</td>
<td>18 ± 27</td>
<td>28 ± 14.28</td>
<td>148 ± 55</td>
<td>169</td>
</tr>
<tr>
<td>K=14 months</td>
<td>11 ± 3.5</td>
<td>14 ± 6.9</td>
<td>119 ± 46</td>
<td>105</td>
</tr>
<tr>
<td>16–20 months</td>
<td>21 ± 17</td>
<td>23 ± 31.42</td>
<td>148 ± 34</td>
<td>105</td>
</tr>
<tr>
<td>21–25 months</td>
<td>15 ± 21</td>
<td>19 ± 38.39</td>
<td>142 ± 101</td>
<td>103</td>
</tr>
<tr>
<td>26–30 months</td>
<td>29 ± 5.5</td>
<td>22 ± 38.5</td>
<td>150 ± 57</td>
<td>146</td>
</tr>
<tr>
<td>31–35 months</td>
<td>35 ± 10.9</td>
<td>22 ± 40.3</td>
<td>156 ± 97</td>
<td>146</td>
</tr>
<tr>
<td>Total cohort</td>
<td>39.9 ± 7.5</td>
<td>35.9 ± 7.5</td>
<td>125</td>
<td>125</td>
</tr>
</tbody>
</table>

*p <.05, § p <.01, # p <.001 by paired Wilcoxon test

30
SOLUBLE CD14 IN SYNOVIAL FLUID FROM PATIENTS WITH OSTEOARTHRITIS (OA) AND MENISCAL INJURY MODULATES LPS ACTIVATION OF FIBROBLAST-LIKE SYNOVIOCYTES
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CURRENT CATEGORY: Rheumatology/Immunology/Allergy
ABSTRACT BODY: Background and Objectives: Low-grade, chronic synovial inflammation is a frequent manifestation of OA and joint injury, and has been associated with severity of symptoms and progression of cartilage degeneration. It has been hypothesized that inflammation is triggered in OA via stimulation of pattern-recognition receptors such as the Toll-like Receptors (TLRs) by endogenous products of tissue degradation. We tested whether a TLR-4 stimulating factor was present in synovial fluid (SF) from patients with meniscal injury with or without OA.

Materials and Methods: SF was obtained from patients undergoing arthroscopic surgery for meniscal tears, with or without concomitant OA, and tested for LPS contamination using the LAL assay (Pierce Chemicals). HEK293 cells transfected with either TLR-4, or TLR-4 + MD2, were used to screen SF for the ability to induce IL-8 production. Fibroblast-like synoviocytes (FLS) cell lines were established from patients and post-mortem tissue donors, and used between passages 4 and 8. FLS were stimulated with a TLR-4 stimulus (ultrapure LPS 100 ng/ml, Invivogen Inc.), SF alone, or SF + LPS for 6 or 18 hours. IL-8 in stimulated culture supernatants was measured by ELISA. In blocking experiments, SF was pre-incubated with anti-CD14 (clone MEM-18), or immunoadsorbed with anti-CD14 bound to protein-G coupled beads, prior to using as stimuli. scD14 levels in SFs were measured by ELISA.

Results: Only 2 of 17 SFs stimulated IL-8 production from HEK transfec-tants. Moreover, SF inhibited LPS production in response to a TLR-4 ligand (LPS). Instead, the addition of SF (0.09-25%) to LPS prior to stimulation resulted in significant augmentation (>10 fold) of IL-8 production by FLS. CD14, a cofactor for TLR-4 responses to LPS, is expressed on cell-surfaces and has been associated with severity of symptoms and progression of cartilage degeneration. It is currently being explored.

Conclusions: In vitro, SF augments the response of Fibroblast-like Synoviocytes to LPS. This effect appeared to be largely due to scD14. As FLS are expected to be in contact with SF in vivo, these results suggest that SF scD14 in the setting of OA and meniscal injury can sensitize FLS to respond to inflammatory stimuli such as TLR-4 ligands. scD14 levels in various anatomic states, and the effect of SF on other TLRs, is currently being explored.

31
ADOLESCENT OLGOMENORRHEA IN A BIRACIAL SCHOOLGIRL COHORT: A SIMPLE CLINICAL PARAMETER PREDICTING IMPAIRED FASTING GLUCOSE PLUS TYPE 2 DIABETES, INSULIN, GLUCOSE, INSULIN RESISTANCE, AND CENTRIPETAL OBESITY FROM AGE 19 TO 25
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CURRENT CATEGORY: Endocrinology/Metabolism
ABSTRACT BODY: Objective: We hypothesized that childhood oligomenorrhea (age 14 to 19) would Independently predict impaired fasting glucose (≥ 110 mg/dl, IFG) plus type 2 diabetes (≥126 mg/dl, T2DM), insulin, glucose, and homoeostatic model assessment (HOMA) insulin resistance (IR) from age 19 to 25 years.

Materials/Methods: Prospective 15 year follow-up of a biracial cohort of 370 girls in urban- suburban schools starting at age 10 years.
Results: Age 14 waist circumference (cm) was the most important significant explanatory variable for IFG + T2DM from ages 19-24 (p=.002), odds ratio (OR) 1.06, 95% confidence interval (CI) 1.02-1.10, along with oligomenorrhea category from age 14 to 19 (0, 1, 2 ≥ 3 reports over 6 years, p=.032), OR 1.82, 95% CI (1.05-3.14). IFG plus T2DM at ages 19 to 24, were more common in girls having 1 (6%), 2 (11%), and ≥ 3 (38%) oligomenorrhea reports from ages 14 to 19 than in girls without oligomenorrhea (3%). Explanatory variables (all p<.025) for HOMA insulin resistance, ages 19-24, included waist circumference at age 14 (partial β=-30.1%), black race (4.0%), oligomenorrhea frequency during ages 14-19 (3.0%), age 14 DHEAS (1.7%), and free testosterone (0.9%).

Conclusions: In 15-year prospective follow-up of a healthy, bi-racial schoolgirl cohort, there were significant, independent associations between age 14 waist circumference and adolescent oligomenorrhea (ages 14-19) and IFG + T2DM, ages 19-24. Age 14 waist circumference, oligomenorrhea frequency ages 14-19, and age 14 DHEAS and free testosterone were significantly, independently associated with IR, ages 19-24, potentially facilitating primary prevention of IFG, T2DM, and hyperinsulinemia.

32 CLINICAL PREDICTORS OF TREATMENT RESPONSE TO DIVALPOEX AND RISPERIDONE AMONG YOUTH WITH PEDIATRIC BIPOLAR DISORDER (PBD)

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CURRENT CATEGORY: Disease Modulation/Intervention

ABSTRACT BODY: Pharmacotherapy is the front-line approach for reducing symptoms of PBD; however, findings in the literature frequently indicate nonresponse rates of more than 40% (Emslie et al., 2003). Understanding factors that differentiate bipolar youth who respond to treatment versus those who do not will inform effective interventions. The current study examined three relevant clinical risk factors at baseline that may influence response to pharmacotherapy among children and adolescents with PBD: (1) high levels of aggression, (2) high levels of irritability, and (3) comorbid disruptive behavior disorders. In addition, we examined the interactions between clinical predictors and medication type (risperidone versus divalproex) to begin to address the question of what works under which conditions. Data for this study were collected as part of a six-week double-blind, placebo-controlled, randomized outpatient medication treatment trial of risperidone versus divalproex for mania. Sixty-six children and adolescents (mean age = 10.9 years, SD = 3.3) with PBD were followed prospectively throughout pharmacotherapy treatment. Aggression and irritability were assessed at baseline via the Overt Aggression Scale-Aggression and Irritability subscales (OAS); mean splits were used to categorize high versus low levels of aggression and irritability. Presence of comorbid disruptive behavior disorders at baseline was assessed using a structured diagnostic interview (WASH-U-K-SADS). Outcome measures of PBD symptoms included the Young Mania Rating Scale (YMRs) and the Child Depression Rating Scale-Revised (CDRS-R). All outcome measures were administered weekly for 8 weeks of treatment to examine both the magnitude and trajectory of symptom change over time.

Mixed-effects regression models were examined separately for each clinical predictor (Aggression, Irritability, and Comorbidity) and each outcome measure (YMRs, CDRS-R, and CAFAS). Models included interactions between time and the clinical predictor in addition, Time x Clinical Predictor x Active Drug (risperidone vs. divalproex) effects were included in each model to examine differential medication responses among the clinical predictors.

Findings indicated that the clinical predictors significantly influenced treatment response. PBD youth with high levels of baseline aggression experienced a greater rate of change in depressive symptoms over time than PBD youth with low levels of aggression, across medication groups (Estimate = -1.95; SE = .39, t (339) = -1.98, p = .05). Youth with high irritability at baseline experienced faster improvement in manic symptoms for those receiving risperidone as compared to low-irritable youth (Estimate = 0.96, SE = .46, t(329) = 2.10, p = .037); high-irritability youth also showed a greater rate of change in depressive symptoms across treatment than low-irritability youth, across medication groups (Estimate = -5.81, SE = 2.53, t(329) = 2.30, p = .022). Last, youth with comorbid disruptive behavior disorders experienced a greater response to risperidone versus divalproex (Estimate = 7.17, SE = 2.78, t (356) = 2.58, p = .01).

33 THE DEVELOPMENT OF G4 POLYMAMDOAMINE (PAMAM) DENDRIMER AS AN IDEAL NANOSCALE DELIVERY SYSTEM FOR ISLET TRANSPLANT

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CURRENT CATEGORY: Endocrinology/Metabolism

ABSTRACT BODY: Purpose: Islet transplantation is a promising therapy for type 1 diabetes mellitus (T1DM), but shows variable success. A longstanding goal is to develop an efficient system for the delivery of molecular cargos to islets. Islets are clusters of 1000 cells. Common viral and non-viral systems only can deliver molecular cargos to the periphery cells of an islet. Furthermore, most of these systems compromise islet function, pose potential oncogenic risks, and increase immunogenicity. Dendrimers are nanostructures with high biocompatibility containing void spaces in their interior regions and modifiable surface groups that allow conjugating therapeutic drugs and proteins. G4 dendrimers have been demonstrated to have superior efficacy to enter cells without auxiliary agents showing minimal cytoxicity. We hypothesize that G4 dendrimers could provide a unique vehicle to deliver molecular cargos uniformly to islets. Methods: G4 dendrimers were conjugated with FITC and transfection efficacy was tested in rodent and human islets using confocal microscopy and optimal concentration was determined. Impact of G4 dendrimers on islet function was evaluated by measuring the calcium influx and insulin secretion in response to glucose. Results: (i) Confocal microscopy images showed high uptake of G4 dendrimers including the islet cores. (ii) 100 aM of G4 dendrimer and 48 hours of incubation was determined as the optimal condition for the dendrimer to penetrate the islet cells. (iii) The functionality of G4 dendrimer-treated islets was well preserved. Conclusion: This is the first time that G4 dendrimers have been applied as a delivery system in islets with high transfection efficacy. The development of G4 dendrimers as a functional in vivo and ex vivo system is under investigation.

34 TRANSCATIONATION OF THE EP4A2 RECEPTOR IS REQUIRED FOR HYALURONAN/CD44-MEDIATED HUMAN PULMONARY ANGIOGENESIS

F. Lennon, T. Mirzapoizova, N. Mbambetsarie, B. Mbambetsarie, G. Cervantes, S. Ravi, S.A. Patrick University of Chicago, Chicago, IL.

CURRENT CATEGORY: Mechanisms of Disease

ABSTRACT BODY: Rationale: Angiogenesis or the formation of new blood vessels is important in the growth and metastatic potential of various cancers including lung cancer. Therefore, understanding the mechanism(s) by which pulmonary angiogenesis occurs has important therapeutic implications in lung cancer growth and metastasis. We have demonstrated that the glycosaminoglycan low molecular weight hyaluronan (LHM-HA, ~ 2,500 Da), produced in pathological states by degradation of endogenous high molecular weight hyaluronan (HMW-HA, ~ 1 million Da), promotes human pulmonary endothelial cell (EC) barrier disruption and angiogenesis. However, the mechanism(s) by which this occurs are poorly defined.

Methods: Human pulmonary microvascular EC were used for in vitro examination of LWM-HA-mediated angiogenesis utilizing proliferation assays, migration assays and tubule formation on Matrigel. Examination of EC signaling was evaluated using siRNA, immunoblotting and RhoA activation assays.

Results: Our data indicate that treatment of human EC with LWM-HA induces CD44v10 (HA receptor variant) association with the receptor tyrosine kinase, EphA2, transactivation (tyrosine phosphorylation) of EphA2, and recruitment of the PDZ domain scaffolding protein, Pat1, to the cell periphery. Silencing (siRNA) CD44, EphA2 or PAT1 blocked LWM-HA-mediated...
angiogenesis (EC proliferation, migration and tubule formation). In addition, silencing EphA2, Patj, Src or Dbx expression blocked LMW-HA RhoA activation. Finally, silencing Dbx blocked LMW-HA-mediated angiogenesis.

**Conclusions:** Our results indicate LMW-HA-mediated transactivation of EphA2 is required for Patj and Dbx membrane recruitment and subsequent RhoA activation required for human pulmonary angiogenesis. These results suggest that targeting downstream effectors of LMW-HA can inhibit angiogenesis which can be a potential therapeutic intervention for lung cancer treatment.

**35 CALORIC RESTRICTION AND AUTOPHagy ACTIVATION SUPPRESS TAu TOXICITY IN A DROSOPHILA MODEL OF TAUOPATHY**

G.R. Jackson Neurology, University Of Texas Medical Branch, Galveston, TX; M. Bakhoun Neuroscience and Cell Biology, University Of Texas Medical Branch, Galveston, TX

**CURRENT CATEGORY:** Mechanisms of Disease

**ABSTRACT BODY:** Alzheimer’s Disease (AD) is the most common cause of dementia. It is characterized by the deposition of extracellular amyloid plaques as well as intracellular neurofibrillary tangles. Recent findings in a mouse model have shown that the microtubule-associated protein, Tau, potentiates the toxic effects of amyloid Aβ. Postmortem studies have also shown that neurofibrillar tangles deposition, predominantly made of tau, strongly correlates with the severity of the disease. Not a single tau mutation has yet been linked to AD, and the mechanism by which Tau aggregation, oligomerization, phosphorylation or truncation impairs neuronal function or survival is still unknown. In a previously developed Drosophila model of tauopathy, expression of human Tau under an eye-specific promoter produced a dosage-sensitive rough eye phenotype. Here we show that autophagy activation through either environmental, chemical or genetic stimuli results in the suppression of Tau-induced toxicity. Caloric restriction causes reversion of the rough eye phenotype by 50%. Moreover, induction of autophagy by feeding flies on rapanycin, an inhibitor of TOR (Target Of Rapamycin) also results in suppression of the tau-induced rough phenotype. Genetic upregulation of key autophagic genes such as Atg2 and Atg7 also suppress the rough phenotype, whereas downregulation of key autophagic genes, such as Atg1, Atg4, Atg6 and Atg18 results in the enhancement of the rough phenotype. We also show that expression of human Tau in the eye induces autophagy in a dosage-dependent manner. Formation of LC3-GFP punctae were observed when tau was expressed meaning that autophagic genes, such as Atg1, Atg4, Atg6 and Atg18 results in the enhancement of the rough phenotype. We further demonstrate that the activation of autophagy suppresses tau toxicity. These findings could inspire novel therapeutic approaches to modulate the burden of tauopathies and provides insight into the mechanism by which tau mediates cytotoxicity in neurodegenerative diseases.

**36 TULA-2, A NOVEL TYROSINE-PHOSPHATASE, REGULATES SYK PHOSPHORYLATION IN OSTEOCLAST SIGNALING PATHWAYS**

S. Back, N. Adapala, D. Holland, T.N. Newman, M.F. Barbe, A.Y. Tsygankov, A. Sanjay Temple University, Philadelphia, PA; N. Carpino Stony Brook University, Stony Brook, NY

**CURRENT CATEGORY:** Rheumatology/Immunology/Allergy

**ABSTRACT BODY:** Hematopoietic stem cells (HSC) are committed to the osteoclast lineage when the cell surface receptor, RANKL is activated by RANK. The osteoclasts contain the cytoplasmic domain of the receptor residues and activation of the signaling enzyme Syk which can then act on downstream targets leading to the transcription of osteoclast specific genes and enhanced bone resorption. Syk dephosphorylation is hence necessary to attenuate this signal and prevent continued differentiation of precursor cells into osteoclasts or hyperactivity of mature osteoclasts. Recently, a novel tyrosine phosphatase, T-cell ubiquitan ligand -2 (TULA-2) has been shown to dephosphorylate specific phosphotyrosine residues on Syk in various systems. The goal of our project is to determine how TULA-2 mediated dephosphorylation of Syk regulates osteoclast differentiation and function. TULA-2 is a member of the TULA family of proteins, TULA and TULA-2. In spite of a significant homology and similar domain organization between TULA and TULA-2, only TULA-2 has significant phosphatase activity. Furthermore, whereas TULA is expressed only in lymphocytes, TULA-2 is expressed in most tissues albeit a higher level of expression is seen in cells of hematopoietic origin. In vivo analysis including x-ray, histomorphometry and uCT indicated that mice that lack both TULA and TULA-2 (DKO) have decreased bone mass compared to wild-type (WT) counterparts. In vitro cell differentiation assay indicated that osteoclast-like cells cultured from DKO bone marrow were more numerous when compared to WT. However, there was no difference in the ability of DKO versus WT osteoclasts to survive longer in the presence of RANKL. At the molecular level, a probe for total tyrosine phosphorylation revealed increased phosphoryrosines at various molecular weights in DKO osteoclasts as well as single knockout TULA-2 (SKO2) osteoclasts when compared to WT osteoclasts. A probe for phosphorylation of Syk revealed increased phosphorylation at tyrosine 525/526 in DKO osteoclasts when compared to WT osteoclasts. Cumulatively, the above data indicates that the absence of TULA proteins, particularly TULA-2, results in hyperphosphorylation of Syk, leading to more numerous osteoclasts which contributes to decreased bone mass in mice suggesting that the phosphatase activity of TULA 2 is required for regulated bone resorption.
CARDIAC TROPOinin ELEVATION IN CRITICALLY ILL PATIENTS WITH NO CORONARY DISEASE: SIGNIFICANCE AND TREATMENT

F. Rothenberg University of Cincinnati, Cincinnati, OH; N. Ibrahim, A. Ejar, R. Vandivier Cardiology, Veterans Administration Medical Center, Cincinnati, OH.

ABSTRACT BODY: The presence of elevated serum cardiac troponins (cTn) in patients experiencing an acute coronary syndrome or heart failure has a robust association with increased mortality and subsequent cardiovascular events. Investigations have shown a similar relationship between elevated serum cTn and high 30-day or in-hospital mortality in patients who have been diagnosed with sepsis, however the mechanism underlying this relationship is not known but presumed to be related to cardiovascular causes. Many of these patients may have concurrent coronary artery disease which may provide the main driving force for increased mortality. Treatment of patients with elevated cTn elevations and critical illness is further complicated by the fact that the mainstay of therapy in patients with an acute coronary syndrome or heart failure is medical and/or surgical intervention rather than early revascularization. We hypothesized that beta-blockers given to critically ill patients with elevated serum cTn and with no known coronary disease would reveal no benefit in 30-day and 1-year mortality. We performed a retrospective chart review of all male patients with no prior history of coronary artery disease admitted to any ICU in the VAMC Cincinnati from January 1, 2005 to December 31, 2008 to determine whether the degree of cTn elevation was associated with increased risk, and whether treatment with beta-blockade provided benefit or harm.

Between 1/1/05 and 12/31/08, there were 560 male patients admitted to all VAMC Cincinnati ICUs for whom troponin measurements were elevated. Peak cTnT or cTnI was used for this investigation. Of these patients, 266 had no prior or current evidence of coronary artery disease and no associated surgical procedure preceding the elevated cTn. The mean age of this group was 68.3 years, 65% had the primary diagnosis of sepsis. Serum cTnT was elevated in 49% of these patients, cTnI in the remaining 51%. At this institution, cTnT is intermediate when between 0.03–10 ng/ml whereas cTnI is intermediate between 0.05–0.78 ng/ml; cTnT is “high” above 0.10 ng/ml and cTnI is “high” above 0.78 ng/ml. There was no difference in 30-day or 60-day mortality when comparing intermediate and high cTnT elevations; however there was a statistically significant increase in 1-year mortality between these groups. Critically ill patients with high cTnT had a 65% 1-year mortality, patients with an intermediate cTnT had a 48% 1-year mortality (p=0.006), and patients with a negative cTnT had a 25% 1-year mortality. In those patients placed on or already on beta-blockers, the 1-year mortality in those with elevated cTnT or cTnI was 35% as compared to 48% 1-year mortality in patients who were not on beta-blockers (p=0.03). In patients who were critically ill but had no elevation of serum cTn, beta-blockers had no effect on 1-year mortality in patients who were not on beta-blockers (p=0.03). In patients who were critically ill and had no elevation of serum cTn, beta-blockers had no effect on 1-year mortality (25% in both groups). In summary, elevated serum troponin is a marker for increased 1-year mortality in patients who are critically ill. Administration of beta-blockers is associated with a statistically significant 13% absolute reduction in 1-year mortality in patients with serum cTn elevation, but has no apparent effect in critically ill patients for whom there is no elevation in serum cTn. Measurement of serum cTn in all critically ill patients may identify a subset for whom aggressive therapy including beta-blockers may provide significant survival advantage.

We are using this retrospective analysis to develop a parallel human and mouse translational program to identify the mechanism of cardioprotection associated with critical illness, and to develop a diagnostic and therapeutic regimen to effectively reduce the high cardiovascular morbidity and mortality associated with critical illness.

IFHI RS1990760 SNP IS ASSOCIATED WITH DIFFERENTIAL INTERFERON ALPHA RESPONSE AND DS DNA AUTOANTIBODIES IN LUPUS PATIENTS

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ABSTRACT BODY: Objective: Interferon alpha (IFN-α) is a heritable risk factor for systemic lupus erythematosus (SLE). Interferon induced with helicase C domain 1 (IFIH1) is a cytoplasmic dsDNA sensor which activates interferon-regulatory factors, stimulating IFN-α production. The rs1990760 (A946T) single nucleotide polymorphism (SNP) in IFIH1 has been associated with susceptibility to SLE and type 1 diabetes in European ancestry populations. We hypothesized that the SLE-risk variant of IFIH1 may be associated with IFN-α pathway activation and autoantibodies directed at nucleic acid in SLE patients.

Methods: 541 SLE patients were studied, including 275 African-American, 164 European-American, and 102 Hispanic-American patients. Serum IFN-α was measured using a reporter cell assay. The rs1990760 SNP in the IFIH1 locus was genotyped using Taqman primer-probe sets. Logistic regression models were used to detect genetic associations with autoantibody traits. Differences in serum IFN-α were tested using the Mann-Whitney U statistic, and multiple linear regression was used to analyze PBMC gene expression in the context of serum IFN-α.

Results: The rs1990760 T allele was associated with anti-dsDNA antibodies across all studied ancestral backgrounds (meta-analysis OR=1.29, p=0.026). In African- and European-American subjects, there was a strong enrichment of the T allele in subjects with anti-dsDNA antibodies who lacked anti-Ro antibodies (OR=1.93, p=1.2×10^-3). The rs1990760 T allele was associated with lower serum IFN-α in subjects who had anti-dsDNA antibodies (p=0.0047). When we studied simultaneous serum and peripheral blood mononuclear cell (PBMC) samples from SLE patients, we found that the IFIH1 rs1990760 T allele was associated with increased IFN-α induced expression in PBMC in response to a given amount of serum IFN-α in those patients with anti-dsDNA antibodies. This effect was independent of STAT4 genotype, which we have previously shown to modulate sensitivity to IFN-α in a similar way.

Conclusions: These data suggest that rs1990760 SNP in the IFIH1 locus is associated with dsDNA antibodies, and in the subset of patients with anti-dsDNA antibodies IFIH1 genotype influences responses to IFN-α. These studies suggest a role for the IFIH1 risk allele in SLE in vivo.

MORTALITY AMONG PATIENTS WITH COMMUNICATION DISABILITIES: POST-HOSPITALIZATION

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ABSTRACT BODY: Background: Provider-patient communication is one of the key components of patient care. Communication disabilities affect patient treatment, medication adherence, and health outcomes. We previously found a greater risk for 30 day rehospitalization among a cohort of hospitalized adult patients with communication disabilities. The purpose of this study was to assess the mortality of these formerly hospitalized patients with communication disabilities.

Methods: We followed a sample of 121 patients up to 18 years after discharge from the University of Illinois at Chicago Medical Center. Mean patient age was 50.0 years (sd=12.3). Thirty six patients (30%) had at least one or more serious hearing, vision, or speech disabilities. Classification of patients according to barrier status was made by agreement between the attending physician, a second- or third-year resident, and one or two medical students when available. In cases in which a consensus could not be reached, the patient was classified as not having the barrier in question. We used the name and birthday information from the original sample to verify mortality status in the Social Security Death Index (SSDI), a comprehensive online database of names, birthdays, places of residence, social security numbers, and mortality information. We constructed a multivariate Cox regression model of the data to analyze the significance of factors potentially related to mortality including: age, comorbidity (Charlson Score), presence of a communication disability, insurance, hospital charges, and duration of hospitalization.

Results: Of the 121 patients we found evidence of mortality in 58 (48%). Mortality rates were 22/36 (61%) for patients with communication disabilities and 36/85 (42%) for patients without such disabilities. Communication disabilities did not increase the risk for mortality (Hazard Ratio [HR]
While our previous findings showed that there was an increased risk for 30 day hospital readmission in patients with communication disabilities, this did not translate to an increased risk of mortality after adjusting for age and comorbidity up to 18 years later. Communication problems between healthcare providers and patients at the time of hospital discharge can potentially impede provider-patient communication and lead to inadequate patient understanding of medications, instructions for chronic disease self management, outpatient follow-up schedules, and contingency planning for unexpected medical events. Larger studies evaluating health outcomes of diverse patient populations with communication disabilities are needed to better understand long-term risks associated with these conditions.

43 FAMILIAL AGGREGATION OF HIGH TUMOR NECROSIS FACTOR ALPHA LEVELS IN SYSTEMIC LUPUS ERYTHEMATOSUS

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CURRENT CATEGORY: Rheumatology/Immunology/Allergy

ABSTRACT BODY: Introduction: Systemic lupus erythematosus (SLE) is an autoimmune disease that is highly heterogeneous and attacks multiple organ systems, and tumor necrosis factor alpha (TNF-alpha) is an inflammatory cytokine which is actively involved in the immune dysregulation observed in SLE patients. It is not currently known whether TNF-alpha is a primary or secondary pathogenic factor in SLE. To explore this question, we studied TNF-alpha levels in SLE families to determine whether high levels of TNF-alpha were heritable and aggregated in SLE families.

Methods: We studied samples from more than 200 SLE patients and their families from varying ancestral backgrounds. 23 unaffected spouses of SLE patients and 62 unrelated healthy controls were also obtained from similar registries. TNF-alpha was measured using a commercial ELISA. Familial correlations, concordance, and relative recurrence risk rates for the high TNF-alpha trait were assessed statistically using standard methods.

Results: The SLE affecteds had the highest TNF-alpha, followed by their healthy first degree relatives, and the lowest values were observed in the healthy unrelated controls. TNF-alpha levels were significantly higher in unaffected first degree relatives as compared to healthy unrelated subjects (p<0.0025). No Mendelian patterns of inheritance were observed with the high TNF-alpha trait, but familial aggregation was strong. Having a high TNF-alpha SLE patient in the family was predictive of having at least one first degree relative with high TNF-alpha (OR=3.33, p<1.2 x 10^-4). 28.4% of first degree relatives of SLE patients had high TNF-alpha levels, as compared to 6.9% of healthy unrelateds, resulting in a first degree relative recurrence risk rate of 4.11. Interestingly, spouses of SLE patients frequently had high TNF-alpha as well, and the median TNF-alpha value in spouses was similar to the first degree relatives. Concordance of the TNF-alpha trait (high vs. low) in SLE patients and their spouses was strikingly high at 78.2%.

Conclusions: These data suggest that high TNF-alpha is heritable to some degree, and would support the idea that high TNF-alpha is involved in SLE pathogenesis in a primary way. The high degree of concordance in SLE patients and their spouses suggests that environmental factors may also play a role in the familial aggregation we have observed.

45 DEK ONCOGENE EXPRESSION IS IMPORTANT FOR HEAD AND NECK SQUAMOUS CELL CARCINOMA DEVELOPMENT

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CURRENT CATEGORY: Hematology and Oncology

ABSTRACT BODY: The human DEK gene was initially discovered as a fusion with the CAN/NUP214 nucleoprotein in leukemia, providing the first indication that DEK may be an oncogene. Subsequent in vitro models demonstrated that DEK promotes cellular proliferation, survival and resistance to chemotherapies via p53-dependent and -independent mechanisms. Our data have established for the first time that DEK is a bona fide oncogene in squamous cell carcinoma (SCC) wherein DEK1 overexpression in human keratinocytes caused hyperplasia in organotypic epithelial rafts, 2) cooperation with defined oncogenes increased squamous cell carcinoma (SCC) formation in immunodeficient mice, and 3) knockout mice were resistant to chemically induced skin papillomas. Herein we focus on the role of DEK in malignant head and neck (HN) SCC. Head and Neck Cancer (HNC) is the sixth most common malignancy worldwide and has traditionally been linked to nicotine and alcohol use. In recent years, Human Papillomavirus (HPV) was identified as an additional risk factor that is detected in as many as 50% of oropharyngeal cancers. Using human head and neck cancer (HNC) tissue microarrays, we show that the human DEK proto-oncogene is upregulated in HN SCC. Previous work from our laboratory has demonstrated that DEK is...
induced by the HPV E7 oncogene in cultured cells and in the epithelium of HPV16 E7 transgenic mice. In these mice, E7 oncogene expression is targeted to squamous epithelium using the Keratin14 (K14) promoter. K14 E7 transgenic mice are uniquely susceptible to HNC formation when exposed to the chemical carcinogen 4-nitroquinoline-1-oxide (4-NQO) in the drinking water. Here we have crossed K14E7 with DEK knockout mice, and report that DEK expression is at least in part required for HNC formation in vivo. A comparison of DEK deficient with DEK proficient K14E7 epidermis reveals defects in cellular proliferation, particularly in the basal cell compartment, accompanied by reduced tumor formation and increased survival. Late stage cancers, including HNC, are notoriously difficult to treat. We are currently developing novel mechanisms to target DEK depletion specifically in tumor cells in collaboration with other investigators for potential human use. Targeting cellular proteins such as DEK which are important for cellular growth and chemoresistance hold promise for novel therapies and head and neck cancers in the future.

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A TISSUE STEM CELL NICHE REGULATES OXIDATIVE-MECHANICAL LRP5 AORTIC VALVE OSTEOSTABOGENESIS

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CURRENT CATEGORY: Cardiology/Cardiovascular Disease

ABSTRACT BODY: Introduction: Calcific aortic valve disease (CADV) is the most common indication for valve surgery in the USA. Cellular mechanisms are under investigation. This study hypothesizes that CADV develops secondary to Wnt3a/Lrp5 activation via oxidative-mechanical stress a tissue stem cell niche resident in the aortic valve. Methods: eNOS-/-, Lrp5-/-, and ApoE-/- mice were tested with experimental diets including a control (n=20), cholesterol (n=20), cholesterol + Atorvastatin (n=20). Different genotypes were utilized for examining oxidative stress aortic valve tissue. In vitro studies were performed to measure Wnt3a secretion from aortic valve endothelial cells and to determine oxidative stress by measuring eNOS activity in these cells. Anion exchange chromatography was performed to isolate the mitogenic protein. Myofibroblast cells were tested to induce bone formation. Results: Cholesterol treated mice developed severe stenosis with increased Wnt3a and Lrp5 levels. We next performed immunohistochemistry in the aortic valves as compared to the tricuspid aortic valves. Tricuspid Lrp5-/- mice developed no CADV. Tricuspid ApoE-/- developed mild stenosis by echo histology. MicroCT and RT-PCR for bone markers. In vitro studies were performed to measure Wnt3a secretion from aortic valve endothelial cells and to determine oxidative stress by measuring eNOS activity in these cells. Anion exchange chromatography was performed to isolate the mitogenic protein. Myofibroblast cells were tested to induce bone formation. Conclusion: Targeting the Wnt3a/Lrp5 pathway in valve calcification and activation of osteogenesis is via oxidative-mechanical stress in CADV. These findings provide a foundation for targeting the cross talk mechanism in the aortic valve.

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T CELL CO-STIMULATORY MOLECULE OX40 ENHANCES INTRAVASCULAR LEUKOCYTE TRAFFICKING AND ADHESION

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CURRENT CATEGORY: Rheumatology/Immunology/Allergy

ABSTRACT BODY: The role of T lymphocytes is increasingly appreciated in the pathogenesis of vasculitis, atherosclerosis, and other inflammatory vascular diseases. OX40 is an inducible co-stimulatory molecule expressed by activated T cells. In addition to its essential role in lymphocyte activation and proliferation, emerging evidence suggests that OX40 is also implicated in cell trafficking and adhesion. However, the mechanism by which OX40 mediates inflammatory infiltration remains to be fully elucidated. The iris vasculature is an expedient platform for real-time imaging of leukocyte migration. Thus, we aimed to study the impact of OX40 on T cell-mediated leukocyte-endothelial interaction during the process of ocular inflammation. In this study, intravitreal microscopy demonstrated that intravitreal administration of ovalbumin (OVA) elicited significant leukocyte rolling and adhesion in the iris vessels of DO11.10 mice, whereas depletion of CD4+ cells by GK1.5 antibody abolished the local inflammation. Compared to the control group without cognate antigen challenge, quantitative PCR showed a marked increase of ocular OX40 and ICAM-1 transcripts. To directly assess the role of OX40 in leukocyte-vascular interaction, activated CD4+ DO11.10 T cells with and without additional in vitro OX40 agonistic antibody stimulation were intravenously transferred into BALB/c mice, and a local immune response in the recipient animals was induced by intravitreal injection of OVA. Adoptive transfer of OX40-stimulated lymphocytes augmented leukocyte rolling and adhesion in the ocular vasculature, whereas ICAM-1 blocking antibody significantly attenuated OX40-enhanced leukocyte adhesion. This study suggests that the T cell co-stimulatory molecule OX40 promotes leukocyte migration and adhesion in part by an ICAM-1-mediated mechanism.

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THE SPINDLE ASSEMBLY CHECKPOINT IS COMPOSED OF TWO INDEPENDENT AND CONSERVED BRANCHES: ONE THAT REQUIRES AURORA B KINASE ACTIVITY AND ANOTHER THAT REQUIRES CENP-H AND CENP-I

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CURRENT CATEGORY: Molecular Medicine and Bioinformatics

ABSTRACT BODY: Kinetochores generate a spindle assembly checkpoint (SAC) signal which prevents anaphase by inhibiting activation of the anaphase promoting complex (APC) until each kinetochore is attached to spindle microtubules (Hardwick et al, 2002). The SAC is strongly induced by spindle poisons, a powerful class of chemotherapeutics that interfere with microtubule function (Pines et al, 1999). Despite this clinical importance, it remains unclear exactly what interaction is monitored at the kinetochore and how the SAC is generated. We previously identified a point mutant in yeast Mad3 (an Ipl1 kinase substrate) that does not arrest in the presence of unpaired chromosomes (which lack tension) but does arrest in the microtubule destabilizing drug benomyl. This result led us to hypothesize that the SAC is composed of two independent branches, one that senses kinetochore-microtubule attachment and another that senses poleward tension. Seeking to separate these branches, we used our Mad3 mutant strain and screened the budding yeast genome for genes required for our Mad3 mutant to arrest in benomyl, reasoning that these genes would be necessary to sense microtubule attachment. The screen yielded Ctf3 and Ctf19. In both mutants, cells arrested in mitosis generally, but the arrest requires Mad3. We next sought out human proteins with sequence homology to Ctf3 and Ctf19 and identified the centromere proteins CENP-H and CENP-I respectively. Since generating unreplicated mitotic chromosomes in human cells is difficult, we instead modified a previously reported assay in which inhibition of Aurora B (the human Ipl1 kinase homolog) caused taxol-arrested cells to exit mitosis, while nocodazole treated cells remained arrested, suggesting that unattached kinetochore arrest in an Aurora B independent manner (Hauf et al, 2003). To determine if HeLa cells in nocodazole and Aurora B inhibitors arrest due to CENP-H and CENP-I activity, HeLa cells were treated with siRNA and placed in nocodazole, then exposed to an Aurora B inhibitor. Under these conditions, control cells remained arrested while CENP-H and CENP-I knock-down (KD) cells exited mitosis in a dose-dependent manner. This indicates that nocodazole triggers two pathways that can arrest cells, one requiring Aurora B activity and a second requiring CENP-H and CENP-I. Consistent with this model for checkpoint arrest, CENP-H and CENP-I are not required for taxol arrest.

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CENP-I KD cells arrest in an Aurora B dependent manner and indicate that the role of CENP-H and CENP-I in the SAC may be to help recruit Mad2.

Our data suggest a mechanism whereby cells with unattached kinetochores generate two independent signals. Yeast harboring point mutants in the spindle checkpoint protein Mad3 do not arrest in the presence of unpaired chromosomes, but arrest in benomyl with the help of Ctf3 and Ctf19, and this role is conserved. Human cells knocked down in either CENP-H or CENP-I arrest at the same concentration of taxol as control cells but require Aurora B activity to arrest in nocodazole. Moreover, we show that Aurora B has a SAC role independent of its established role in releasing microtubules.

49 DIO2 IS A NOVEL CANDIDATE GENE LINKING THYROID METABOLISM TO PROTECTIVE RESPONSES TO ACUTE INFLAMMATORY LUNG INJURY


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CURRENT CATEGORY: Pulmonary/Critical Care

ABSTRACT BODY: Rationale: Critically ill patients including those with ALI express increased serum concentrations of the prohormone, thyroxine (T4) and the active hormone, 3, 3, 5-triiodothyronine (T3). While the exact role of thyroid hormones remains poorly understood. Microarray studies were applied in murine models of Ventilation-induced lung injury (VILI) and ALI to assess potential dysregulated genes which are involved in thyroid synthesis or metabolism.

Methods: Microarray assay was applied to identify dysregulated genes among pulmonary gene expression profiles from wild type mice and mice exposed to LPS (1ug/kg, 4 hrs) and VILI (40ml/kg, 4 hrs). Gene expression levels were evaluated by Real-time PCR and Western blotting. Protein expression and localization were detected with immunohistochemical staining. Liposome mediated gene silencing were applied in mice lung. Pulmonary vascular leak was determined by measuring bronchoalveolar lavage (BAL) fluid total protein concentrations and Thyroid function was evaluated via T4 and TSH assessments in plasma.

Results: Microarray data shows a significant increase in expression of Dio2 (9.5 fold change at 30 ml/kg tidal volume and 26.2 fold change at 40 ml/kg tidal volume), a dioiodinase which converts premature thyroxine (T4) to bioactive triiodothyronine (T3), in inflammatory lung tissues whereas the Dio1 and Dio3 remain unchanged. Findings were verified by RT-PCR and Western Blotting, immunohistochemical staining of Dio2 revealed markedly increased Dio2 expression in pulmonary endothelium and alveolar epithelium in VILI mice. Compared with control mice, reduced TSH and T4 were observed in the lung via liposome mediated siRNA and aggravated lung injury in Dio2 silenced mice suggests a protective role in lung inflammation.

Conclusion: Dio2 expression is increased in inflammatory lung tissues, aggravated lung injury in Dio2 silenced mice suggests a protective role in ALI, which strengthens the premise that thyroid hormone metabolism is intimately linked to the response to inflammatory injury in critically ill patients.

50 EZRIN/RADIXIN/MOESIN PROTEINS DIFFERENTIALLY REGULATE SIP-INDUCED PULMONARY ENDOTHELIAL CELL BARRIER ENHANCEMENT


CURRENT CATEGORY: Pulmonary/Critical Care

ABSTRACT BODY: Rationale: Endothelial cell (EC) barrier dysfunction induced by inflammatory agonists is the direct underlying cause of vascular leak and pulmonary edema in sepsis and an essential component of angiogenesis, tumor metastasis, and atherosclerosis. Preservation of vascular barrier integrity, therefore, has the potential for profound clinical impact. We have previously described potent EC barrier enhancement induced by the lipid growth factor, sphingosine-1 phosphate (SIP), to involve Rac GTPase-dependent cortical actin remodeling, focal adhesion and adherens junction rearrangements as an integral step. The ezrin, radixin, and moesin (ERM) family of actin-binding proteins link the actin cytoskeleton with plasma membrane proteins and serve to transduce signals from agonists to induce cytoskeletal remodeling. Here we further characterize the role of the ERM family in modulating SIP-induced cytoskeletal rearrangement and EC barrier function.

Methods: To study the involvement of ERM in EC barrier regulation in vitro, we used immunoblotting, immunocytochemistry, transendothelial monolayer resistance (TER) measurements (a sensitive indicator of EC barrier function), Rac GTPase activity assay, coimmunoprecipitation assays, and RNA interference in cultured human pulmonary artery EC.

Results: Our data demonstrate that SIP (1 μM) promotes ERM phosphorylation on critical threonine residues (Ezrin-567, Radixin-564, Moesin-558), which is a hallmark of ERM activation. This phosphorylation peaks at 10–20 min after SIP and is dependent upon activation of PKC isoforms and Rac1 as pharmacologic inhibitors of these signaling molecules attenuate ERM phosphorylation. Immunofluorescent studies reveal that SIP-mediated ERM phosphorylation occurs at the cell periphery. Immunoprecipitation of individual ERM proteins reveal that the majority of ERM phosphorylation on these critical threonine residues after SIP occurs in moesin and ezrin. Baseline radixin phosphorylation is higher than in the other two ERM proteins but does not increase after SIP. SIP-induced moesin and ezrin threonine phosphorylation is not mediated by the barrier enhancing receptor SIPR1 because siRNA downregulation of SIPR1 fails to inhibit these phosphorylation events, while stimulation of EC with the SIPR1-specific agonist S1P271 fails to induce these phosphorylation events. Interestingly, Rac1 activity, which is required for maximal barrier enhancement by SIP, appears to be both upstream and downstream of ERM in the SIP response because siRNA downregulation of all 3 ERM proteins or radixin alone (but not moesin alone) significantly inhibits Rac activation after SIP. Importantly, siRNA depletion of either radixin alone, or of all three ERM proteins, significantly attenuates SIP-induced increase in barrier function (TER), cortical ring formation, accumulation of peripheral di-phospho-MLC and paxillin, FAK, and VE-cadherin peripheral redistribution. In contrast, moesin depletion has the opposite effect on barrier function as demonstrated by enhanced SIP-induced cortical ring formation, MLC phosphorylation and increase in TER.

Conclusions: These data suggest that despite their structural similarities and reported functional redundancy, the ERM proteins differentially participate in SIP-induced EC barrier enhancement, with radixin promoting barrier function and moesin opposing it.

51 AGE 14 SEX HORMONE BINDING GLOBULIN, OLIGOMENORRHEA, AND CHILDHOOD INSULIN PREDICT METABOLIC SYNDROME AND CLASS III OBESITY AT AGE 24

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CURRENT CATEGORY: Endocrinology/Metabolism

ABSTRACT BODY: Objective: Oligomenorrhea, hyperandrogenemia, and low sex hormone binding globulin (SHBG) are associated with cardiovascular disease (CVD) in adult women. We hypothesized that age 14 oligomenorrhea, low SHBG, and metabolic syndrome plus childhood insulin would predict metabolic syndrome and Class III obesity at age 24.

Design: National Growth and Health Study (NGHS), Prospective 10 year schoolgirl study ages 14-24 (n=436).

Results: Free testosterone was associated with oligomenorrhea at age 14 (OR 1.94, 95% CI 1.3 - 2.9, p=0.0013). Childhood insulin (OR 1.03, 95% CI 1.01-1.06), and age 14 SHBG (OR 0.89, 95% CI 0.82-0.96) and oligomenorrhea (OR 4.95, 95% CI 1.45-16.8) were associated with metabolic syndrome at age 24, AUC=0.85. Age 14 bottom decile SHBG (OR 6.63 95% CI 2.2-20.0), oligomenorrhea (OR 5.24 95% CI 1.54-17.8), and top decile childhood insulin (OR 4.30 95% CI 1.46-12.6) predicted metabolic syndrome at age 24, AUC = 0.80. Black race (OR 14.3 95% CI 1.46-12.6), and top decile childhood insulin (OR 4.24, 95% CI 1.6-11.2) predicted Class III obesity at age 24, AUC=0.80.
Conclusions: Modifiable childhood insulin and age 14 low SHBG and oligomenorrhea predict metabolic syndrome and Class III obesity age 24, facilitating identification of adolescents in whom diet, exercise, and (possibly) metformin could be used in primary prevention of metabolic syndrome and class III obesity.

52 COR TacTACTIN MODULATES ALLERGIC AIRWAY RESPONSES IN A MURINE MODEL OF ASTHMA
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CURRENT CATEGORY: Pulmonary/Critical Care
ABSTRACT BODY: RATIONALE: The actin-binding protein cortactin is a key regulator of cytoskeletal dynamics and contractile cell machinery, and we have previously reported that polymorphisms in the cortactin gene are associated with severe asthma. However, the potential functional role of cortactin in asthma pathophysiology remains completely unknown.

METHODS: We therefore examined the levels of cortactin protein and phosphorylation in human lung samples from severe asthma patients, and studied the effects of cortactin gene silencing via small interfering RNAs (siRNAs) in a model of asthma induced by ovalbumin (OVA) challenge.

RESULTS: Immunohistochemical staining of lung biopsy sections revealed significant increase in human lung endothelium of severe asthma patients (n=5), with no obvious change in total cortactin protein expression level. Transfection of cortactin-specific siRNA (5 mg/kg, intratracheal instillation, 3 days) significantly reduced cortactin levels in delivery system significantly reduced expression of cortactin in murine lung tissue as measured by Western blotting (55% reduction, p<0.05). Cortactin siRNA significantly suppressed airway hyperresponsiveness (AHR, 18% suppression, p<0.05) and cosinophila (25% reduction of BAL eosinophil counts, p<0.05) after OVA challenge. Cortactin siRNA also attenuated OVA-induced BAL protein content increases (16% suppression, p<0.05).

CONCLUSIONS: These findings suggest that cortactin is a novel determinant of asthmatic inflammation and may serve as a therapeutic target in asthma.

Supported by NIH grant HL88144, HL 58094

53 IS REPEAT STOOL TESTING NEEDED FOR DIAGNOSIS OF CLOSTRIDIUM DIFFICILE INFECTION USING TOXIN (A AND B) ENZYME IMMUNO ASSAY?
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CURRENT CATEGORY: Infectious Disease
ABSTRACT BODY: Background and Aims: Clostridium difficile infection (CDI) is a hospital-acquired infection with increasing incidence and severity. Early diagnosis is critical so that treatment and containment strategies can be implemented expeditiously. The stool enzyme immunoassay (EIA) for toxins A and B is the most commonly used laboratory test to diagnose CDI. Frequently more than one stool sample is tested for C. difficile toxin on the assumption that the diagnostic yield increases. The aim of the study is to determine if repeat stool testing improves diagnosis of CDI.

METHODS: A retrospective cohort study was designed using a database of all stool toxin EIAs that were performed on hospitalized patients at the Cleveland Clinic, a tertiary care hospital between January 2005 and December 2008. The results of all tests were taken into consideration for each episode of diarrhea. Transition probabilities were calculated based on repeat testing results.

RESULTS: 29,373 stool samples from 17,971 patients over 3 year period were submitted for C. difficile toxin detection. A total of 2,692 (9.17%) were diagnosed with CDI. Of these 2,675 patients (99.36%) were detected based on the first stool test. The second test was positive in 90.7%; an additional 6.6% patients tested positive when the stool was tested a second time and an additional 2.0% when the stool was tested a third time. If the first test was negative, the probability that the second test was positive was 2.2%. Similarly, if the first 2 tests were both negative, the probability that the third test was positive was 2.3%.

Conclusions: 91% of the stool samples tested positive for C. difficile toxin on the first stool test. 8.6% had positive tests subsequently, when the first stool was negative. Repeat testing does not improve the diagnostic utility significantly since the probability that second and third stool test will yield a positive result is low. We do not recommend repeat testing routinely except in patients where there continues to be a high clinical suspicion.

54 HUMAN ALVEOLAR EPITHELIAL CELLS ARE A TARGET FOR BACILLUS ANTHRACIS LETHAL TOXIN
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CURRENT CATEGORY: Infectious Disease
ABSTRACT BODY: Rationale: The lung is the site of entry for Bacillus anthracis in inhalation anthrax, the most deadly form of the disease. B. anthracis does not, however, cause disease in the lung. Rather, spores are internalized by resident lung cells. Subsequently, vegetative bacterial forms disseminate to cause systemic disease. Of the cell types resident in the lung, three have been reported to take up B. anthracis spores: alveolar macrophages (AM), dendritic cells (DC) and alveolar epithelial cells (AEC); however, the major cell type responsible for dissemination of B. anthracis to the peripheral blood is unknown. Originally, AM were thought to be the most important cell for dissemination, although recent evidence suggests a more prominent role for DC and AEC. The ability of A. anthracis to use a cell for dissemination may be driven by the cell's sensitivity to Lethal Toxin (LT), a major virulence factor demonstrated to cleave mitogen-activated protein kinase kinases (MEKs) in human epithelial cells but not in AM. We hypothesize that B. anthracis spores will be engulfed by, and survive within, cells that disseminate it. Further, LT may promote epithelial barrier penetration by B. anthracis. AEC may be involved in two dissemination routes. The first is a transcellular route. When AEC engulf spores, the spores may survive and translocate from the apical to basolateral side, thus crossing the epithelial barrier without first being ingested by AM or DC. In the second route, AEC barrier function may be disrupted by LT, allowing spores, or AM or DC carrying spores, to penetrate the epithelium.

Methods: We investigated spore uptake and barrier function using a human lung organ culture model and cultured primary human AEC.

RESULTS: We found that spore ingestion was most significant in phagocytic cells, specifically AM and DC, but AEC also ingest spores. Also, we found that treatment of AEC with LT causes actin cytoskeleton rearrangement and MEK cleavage, but does not decrease viability. This cytoskeletal rearrangement may lead to increased epithelial permeability.

Conclusions: Our results provide evidence for multiple routes of dissemination. Spores may be internalized by AEC and cross an intact epithelium without initial uptake by AM or DC. Alternatively, spores may penetrate an epithelium damaged by LT, either before or after being ingested by AM or DC. Both mechanisms are consistent with a rapid clearance of the pathogen from the alveolar space and entry into the blood, as observed in natural infections of B. anthracis.

55 A LINK BETWEEN HIV PROTEASE INHIBITOR-INDUCED ER STRESS AND AUTOPHAGY IN ADIPOCYTES AND HEPATOCYTES
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CURRENT CATEGORY: Mechanisms of Disease
ABSTRACT BODY: HIV protease inhibitors (PI) have been directly linked to Highly Active Antiretroviral Therapy-induced lipodystrophy and dyslipidemia syndromes, which are major risk factors for atherosclerosis and cardiovascular disease. However, the underlying molecular mechanism remains unclear. We have previously shown that in key cells involved in lipid metabolism, HIV PIs induce ER stress and downstream cellular lipid metabolism dysregulation. Recently, it has been reported that autophagy is closely related to ER stress and plays an important role in lipid metabolism. The aim of this
study was to determine whether HIV PI-induced ER stress is associated with autophagy induction in adipocytes and hepatocytes, which would give a clearer mechanism behind this effect and will attempt to translate these findings to a live mouse model of ischemic heart disease. We found that HIV PIs induce ER stress, which leads to induction of autophagy causing lipid metabolism dysregulation in key cells. This must be further investigated to fully understand the molecular significance of HIV PI side effects, and how to inhibit these effects in order to improve the lives of patients treated with these therapies.

## 56
HDAC1 AND HDAC2 ACT REDUNDANTLY TO CONTROL P63 AND P53 FUNCTIONS IN EPIDERMAL PROGENITOR CELLS

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**CURRENT CATEGORY:** Dermatology

**ABSTRACT BODY:** Epidermal and hair follicle development from surface ectoderm progenitor cells require coordinated changes in gene expression. Histone deacetylases alter gene expression programs through modification of chromatin and transcription factors. We find that deletion of ectodermal Hda1 and Hda2 results in dramatic failure of hair follicle specification and epidermal proliferation and stratification, phenocopying loss of the key ectodermal transcription factor p63. While expression of p63 and its positively regulated basal cell targets is maintained in Hda1/2 deficient ectoderm, targets of p63-mediated repression, including p21, 14-3-3z and p16/INK4a, are ectopically expressed and HDAC1/2 bind and are active at their promoter regions in normal undifferentiated keratinocytes. Mutant embryos display increased levels of acetylated p53, which opposes p63 functions, and p53 is required for HDAC inhibitor-mediated p21 expression in keratinocytes. Our data identify critical requirements for HDAC1/2 in epidermal development, and indicate that HDAC1/2 directly mediate repressive functions of p63, and suppress p53 activity.

## 57
LYSOCARDIOLIPIN ACYLTRANSFERASE (LYCAT) IS A NOVEL CANDIDATE GENE IN RADIATION-INDUCED PULMONARY FIBROSIS

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**CURRENT CATEGORY:** Pulmonary Medicine

**ABSTRACT BODY:** Radiation-induced pulmonary fibrosis (Lycat), which codes for a protein involved in the remodeling of cardiolipin, a mitochondrial structure and function protein. We hypothesized that Lycat expression plays a critical role in minimizing radiation-induced fibrosis and lung injury.

**METHODS:** C57BL6 mice were subjected to a single dose of thoracic radiation (20 Gy) and then administered (intratracheally) either control or Lycat siRNA once weekly beginning at 10 weeks post irradiation through week 18. Fibrosis was evaluated by H & E staining, and Mason’s trichrome blue staining for collagen in lung tissue. Pulmonary leak was assessed by increases in BAL fluid protein.

**RESULTS:** Loss of function studies (siRNA knock down of Lycat) demonstrated significant weight loss, increased vascular permeability and infiltration of inflammatory cells in BAL fluid compared to irradiated control mice. Immunohistochemical staining for collagen deposition and measurements of acid soluble collagen demonstrated increased lung collagen content in irradiated Lycat-silenced mice compared to irradiated controls. In addition, compared to irradiated control mice, histology of lungs from irradiated Lycat-silenced mice revealed augmented edema formation, airway inflammation and fibrotic foci while computed tomography showed increased areas of consolidation and ground-glass opacification. Further, irradiated lung tissue exhibited increased Lycat protein expression and activity compared to control mice.

**Conclusions:** These data firmly support Lycat as a novel candidate gene in radiation pulmonary fibrosis and suggest that over-expression of Lycat or increased Lycat activity may be beneficial in patients with radiation-induced pulmonary fibrosis and injury.

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60 VASODILATOR-STIMULATED PHOSPHOPROTEIN DEFICIENCY POTENTIATES PAR-1-INDUCED INCREASE IN ENDOTHELIAL PERMEABILITY IN MOUSE LUNGS
J. Profricovic, J. Han, A.V. Andreeva, R.F. Neamu, T.A. Veyno-Vasenetskaya
University of Illinois, Chicago, IL; U. Walter University of Würzburg, Würzburg, GERMANY
CURRENT CATEGORY: Cardiology/Cardiovascular Disease
ABSTRACT BODY: Vasodilator-stimulated phosphoprotein (VASP) is implicated in the protection of the endothelial barrier in vitro and in vivo. VASP function in thrombin signaling in the endothelial cells (ECs) is not known. For the first time we studied the effects of VASP deficiency on EC permeability and pulmonary vascular permeability in response to thrombin receptor stimulation. We provided the evidence that VASP deficiency potentiates the increase in endothelial permeability induced by activation of thrombin receptor in cultured human umbilical vein endothelial cells (HUVECs) and isolated mouse lungs. Using transendothelial resistance measurement, we showed that siRNA-mediated VASP downregulation in HUVECs leads to a potentiation of thrombin- and protease-activated receptor 1 (PAR-1) agonist-induced increase in endothelial permeability. Compared to control cells, VASP-deficient HUVECs had delayed endothelial junctional reassembly and abrogated VE-cadherin cytoskeletal anchoring in the recovery phase after thrombin stimulation, as demonstrated by immunofluorescence studies and cell fractionation analysis, respectively. Measurement of the capillary filtration coefficient in isolated mouse lungs demonstrated that VASP(-/-) mice have increased microvascular permeability in response to infusion with PAR-1 agonist compared to wild type mice. Lack of VASP led to decreased Rac1 activation both in VASP-deficient HUVECs after thrombin stimulation and VASP(-/-) mouse lungs after PAR-1 agonist infusion, indicating that VASP effects on thrombin signaling may be correlated with changes in Rac1 activity. This study demonstrates that VASP may play critical and complex role in the regulation of thrombin-dependent disruption of the endothelial barrier function.

62 IMPACT OF MEDICARE-SPECIALIZED NURSE CASE MANAGEMENT: FAVORABLE HOSPITAL AND EMERGENCY UTILIZATION OUTCOMES FOR A MEDICARE ADVANTAGE POPULATION COMPARED TO UNMANAGED TRADITIONAL MEDICARE EXPERIENCE
CURRENT CATEGORY: Quality Improvement
ABSTRACT BODY: 73% of Medicare spending is associated with a population with 5 or more chronic conditions, and frequently, psychosocial barriers. (1) Aetna’s case management program for Medicare Advantage members was designed to improve health outcomes as and thereby reduce the need for hospital inpatient stays and emergency department visits, and to accomplish this through member-friendly telephonic assistance designed to meet the frequently complex needs of elderly and/or disabled. We intervene by reaching out to assist 18% of our membership identified with multiple conditions and care barriers, targeting those with the highest combination of risk and opportunity based upon predictive modeling from multiple data sources including claims, utilization data, and self-reported data from Health Risk Assessment surveys of new members. Case managers are not directly responsible for patient care; however work closely with the members, physicians, subspecialty providers, and others to facilitate care to manage the complex cardiac, oncologic, psychiatric, social, other medical, and/or end-of-life needs of the participants. Identified needs are addressed on scheduled calls with the member (typically 2-3 times per week initially, with frequency decreasing as issues resolve). Common issues covered include health education, safety and emergency measures, medication regimen and care coordination needs, barriers such as dementia, depression and access issues, and issues associated with Terminal illness including available options and support. Member satisfaction with case management is strong, with 94% of those participating in case management reporting high satisfaction with the program. In April 2010, we sought to validate the success of this ongoing case management program by studying the outcomes for our Medicare Advantage population compared to a benchmark loosely managed, risk-adjusted, Medicare Fee-for-service comparison population provided by Milliman, Inc (2). Results for 152, 053 Aetna Medicare Advantage HMO and PPO members (120,703 HMO and 31,350 PPO) were compared across six regions in the continental United States for the most recent one year interval 7/1/2008-6/30/2009 with completed data available. After adjusting for medical risk and for geographic variation, and excluding any component of differences attributable to utilization management programs, our population experienced significantly better outcomes than the comparison group in each region for acute hospital inpatient days as well as for emergency visits. The HMO population served by our Medicare-specialized case management program required 34% fewer acute hospital inpatient days (p<0.05) and, 14% fewer emergency department visits (p<0.05) per thousand members per year than did the risk-adjusted comparable population of Medicare beneficiaries, while the PPO population required 31% fewer hospital days and 24% fewer emergency visits (p<0.05). The results were uniformly positive for all regions where the healthplan had sufficient membership (over five thousand members). These results demonstrate that a member-centered quality-oriented case management program targeting improved care coordination and reduced barriers to care for high risk seniors improved health outcomes in a way that added true value to the healthcare system, reducing illness that required inpatient hospital care and also reducing emergency department visits and the fragmented care that may entail.


63 EFFICACY OF CANAKINUMAB (ACZ885), A FULLY HUMAN ANTI-INTERLEUKIN (IL)-1BETA MONOCLONAL ANTIBODY, IN THE PREVENTION OF FLARES IN GOUT PATIENTS INITIATING ALLOPURINOL THERAPY
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CURRENT CATEGORY: Rheumatology/Immunology/Allergy
ABSTRACT BODY: Background: Gout patients initiating urate lowering therapy have increased an risk of flares. Inflammation in gouty arthritis is induced by IL-1beta. Canakinumab targets and inhibits IL-1beta effectively in clinical studies. This study compared different doses of canakinumab vs colchicine in preventing flares in gout patients initiating allopurinol therapy.
Methods: In this 24 week double blind study, gout patients (20–79 years) initiating allopurinol were randomized (1:1:1:1:1:1:2) to canakinumab s.c. single doses of 25, 50, 100, 200, 300 mg, or 150 mg divided in doses every 4 weeks (50+50+25+25 mg [q4wk]) or colchicine 0.5 mg p.o. daily for 16 weeks. Primary outcome was to determine the canakinumab dose giving comparable efficacy to colchicine with respect to the number of gout flares occurring during first 16 weeks. Secondary outcomes included number of patients with gout flares and C-reactive protein (CRP) levels during the first 16 weeks.
Results: 432 patients were randomized and 391 (91%) completed the study. All canakinumab doses were better than colchicine in preventing flares and therefore, a canakinumab dose comparable to colchicine could not be determined. Based on a negative binomial model, all canakinumab groups, except 25 mg, reduced the flare rate ratio per patient significantly compared to colchicine group (rate ratio estimates 25 mg 0.60, 50 mg 0.34, 100 mg 0.28, 200 mg 0.37, 300 mg 0.29, q4wk 0.38, p<0.05). The percentage of patients with flares was lower for all canakinumab groups (25 mg 27.3%, 50 mg 16.7%, 100 mg 14.8%, 200 mg 18.5%, 300 mg 15.1%, q4wk 16.7%) compared to colchicine group (44.4%). All patients taking canakinumab were significantly less likely to experience at least one gout flare than patients taking colchicine (odds ratio range [0.22–0.47]; p<0.05 for all). The median baseline CRP levels were 2.86 mg/L for 25 mg, 3.42 mg/L for 50 mg, 1.76 mg/L for 100 mg, 3.66 mg/L for 200 mg, 3.21 mg/L for 300 mg, 2.23 mg/L for q4wk canakinumab groups and 2.69 mg/L for colchicine.
group. In all canakinumab groups with median CRP levels above the normal range at baseline, median levels declined within 15 days of treatment and were maintained at normal levels (ULN=3 mg/L) throughout the 16 week period. Adverse events (AEs) occurred in 52.7% (25 mg), 55.6% (50 mg), 51.9% (100 mg), 51.9% (200 mg), 54.7% (300 mg), and 58.5% (q4wk) of patients on canakinumab vs 53.7% of patients on colchicine. Serious AEs (SAEs) were reported in 2 (3.6%; 25 mg), 2 (3.7%; 50 mg), 3 (5.6%; 100 mg), 3 (5.6%, 200 mg), 3 (5.7%, 300 mg) and 1 (1.9%, q4wk) patients on canakinumab and in 5 (4.6%) patients on colchicine. One fatal SAE (myocardial infarction, not related to study drug) occurred in colchicine group.

Conclusion: In this large randomized, double-blind active controlled study of flare prevention in gout patients initiating allopurinol therapy, treatment with canakinumab led to a statistically significant reduction in flares compared with colchicine (standard of care), and was well tolerated.

64 GRANULATION TISSUE DERIVED STEM CELLS – A NOVEL SOURCE OF ADULT STEM CELLS FOR REPAIR OF ACUTE KIDNEY INJURY

J. Patel, N. Pancholi, G. Dunea Hektoen Institute of Medicine, Chicago, IL.; K.P. Gudehithlu, A.K. Singh John H. Stroger, Jr. Hospital of Cook County, Chicago, IL.; J.A. Arruda University of Illinois at Chicago and the Chicago VAMC, Chicago, IL.

CURRENT CATEGORY: Nephrology

ABSTRACT BODY: Earlier we showed that a foreign body induced subcutaneous granulation tissue is a regenerating tissue that is a rich source of stem cells. These cells, called granulation tissue derived stem cells (GTSC), were characterized and found to be of mesenchymal origin (Singh AK et al., 2007, Patel J et al., 2010). Here we test the efficacy of GTSC to ameliorate acute kidney injury (AKI) induced in Fischer (F344) in-bred rats (baseline plasma creatinine 0.7 ± 0.06 mg/dl and blood urea nitrogen (BUN) 23 ± 1.6 mg/dl). Acute kidney injury (ischemia/reperfusion injury of the kidney) was induced by unilateral (right) nephrectomy, occlusion of the left renal pedicle for 45 minutes followed by de-occlusion and reperfusion of the kidney. The injury was characterized by a rapid increase in plasma creatinine and BUN with tubular dilatation, necrosis, congestion and casts. After inducing AKI injury, the rats were divided into two groups. Three hours after injury, group 1 (treated, n=8) rats received one intravenous injection of GTSC (3-4 million cells in 0.7 ml volume) and group 2 (control, n=8) rats received 0.7 ml of vehicle. Within 24 hours after injury, compared to control rats, treated rats had significantly lower plasma creatinine (1.0 ± 0.04 mg/dl vs. 1.6 ± 0.12 mg/dl) and BUN (33 ± 2.7 mg/dl vs. 65 ± 3.3 mg/dl) (p<0.0001). The plasma creatinine and BUN levels in the treated group remained low and reached near baseline levels by day 4 whereas it did not reach baseline levels in controls until day 7. Consistent with the accelerated recovery the treated rats showed significantly higher tubular cell proliferation in the cortico-medullary region of the kidney (by PCNA staining) and significantly lesser tubular cell apoptosis (by TUNEL staining) than in controls. Histological analysis of the kidney at day 1 for tubular dilatation, necrosis, congestion and casts was not significantly different in the two groups of rats. To understand the mechanism of the GTSC effect, mRNA levels of several growth, inflammatory and anti-inflammatory factors were quantified in cultured GTSC. The GTSC were found to have 2-8 fold higher expression of FGF2, and compared with those in cultured glomerular epithelial cell (GEC; a non-inflammatory and anti-inflammatory factors were quantified in cultured GTSC. The mechanism of the GTSC effect, mRNA levels of several growth, inflammatory and anti-inflammatory factors were quantified in cultured GTSC. One fatal SAE (myocardial infarction, not related to study drug) occurred in colchicine group.

Conclusion: In this large randomized, double-blind active controlled study of flare prevention in gout patients initiating allopurinol therapy, treatment with canakinumab led to a statistically significant reduction in flares compared with colchicine (standard of care), and was well tolerated.

65 ACTIVATED OMENTUM ATTENUATES PROGRESSION OF CHRONIC KIDNEY DISEASE IN A RAT REMNANT KIDNEY MODEL

N. Pancholi, J. Patel, G. Dunea Hektoen Institute of Medicine, Chicago, IL.; K.P. Gudehithlu, A.K. Singh John H. Stroger, Jr. Hospital of Cook County, Chicago, IL.; J.A. Arruda University of Illinois at Chicago and the Chicago VAMC, Chicago, IL.

CURRENT CATEGORY: Nephrology

ABSTRACT BODY: In previous work we showed that fusion of the activated omentum, a tissue rich in stem cells and growth factors, to an injured organ facilitates repair of injured tissue (Singh AK et al, 2009, Pancholi N et al. 2010.). Here we tested whether activated omentum could ameliorate chronic kidney disease (CKD) in rats. CKD induced in rats by renal mass reduction (5/6 nephrectomy by removal of right kidney and excision of the two poles of the left kidney) is characterized by a gradual increase in plasma creatinine and blood urea nitrogen (BUN) accompanied by glomerulosclerosis and tubulointerstitial fibrosis. After inducing CKD, rats were divided into two groups. Group 1 rats (treated; n = 20) received intraperitoneal injection of polydextan gel particles to activate the omentum and promote its fusion to the injured kidney, while in Group 2 rats (control; n = 20) omentum was prevented from fusing to the kidney by performing omentectomy. At weeks 6 and 12 after inducing CKD, omentum treated rats had significantly lower plasma creatinine and BUN and significantly higher creatinine clearance as compared to control rats (Table 1). Histologically, compared to controls, omentum treated rats showed significantly less glomerulosclerosis and tubulointerstitial fibrosis as assessed by kidney injury scores. Immune staining of remnant kidney tissue for α-SMA showed fewer deposits of extracellular matrix in the tubulointerstitial area of treated rats as compared to control rats. Also, collagen type IV immune staining showed lesser thickening of basement membrane in the glomeruli and tubules of treated rats than controls. To understand the mechanism of omentum mediated amelioration of CKD we measured and found mRNA levels of growth (FGF2, HGF, IGF-1, VEGF) and immune-suppressive (IL-4, IL-10) factors to be upregulated in the omentum attached to the remnant kidney. Consistent with upregulation of growth factors in the omentum we found cellular proliferation, as assessed by Brdu incorporation, to be highest in the region of the kidney in immediate contact with the fused omentum. These data suggest that fusion of activated omentum to the kidney results in attenuation of progression of CKD, and that this effect could be due to diffusion of angiogenic, growth and immuno-modulatory factors from the omentum to the injured kidney.

Table 1.

<table>
<thead>
<tr>
<th>Week 6</th>
<th>Week 12</th>
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<tbody>
<tr>
<td></td>
<td>TREATED</td>
</tr>
<tr>
<td>Plasma Creatinine (mg/dl)</td>
<td>1.5 ± 0.0*</td>
</tr>
<tr>
<td>Blood Urea Nitrogen (mg/dl)</td>
<td>48.1 ± 0.2*</td>
</tr>
<tr>
<td>Creatinine Clearance (mL/min/100g)</td>
<td>0.22 ± 0.0*</td>
</tr>
</tbody>
</table>

*p<0.05 versus control
opportunities for social interaction (mean ES = −0.062, p = 0.67). These results are consistent with the theory that abnormal social cognition plays an important role in the etiology of persistent loneliness. Future loneliness reduction interventions should therefore emphasize social cognitive training over other approaches.

67 ACUTE ALDOSTERONE TREATMENT ENHANCES SPAK AND NCC PHOSPHORYLATION
B. Ko, L. Moddes Medicine, University of Chicago, Chicago, IL; R. Hoover Medicine, Emory University, Atlanta, GA.
CURRENT CATEGORY: Nephrology
ABSTRACT BODY: In animal studies, aldosterone (aldo) increases sodium chloride cotransporter (NCC) function and total abundance at 3-5 days. Recent work shows that NCC and STE20/SPS-1-related proline/alanine rich kinase (SPAK) phosphorylation increases at greater than 72 hrs of aldo stimulation. The acute effects of aldo on NCC have not been studied. We previously demonstrated that in a cell model with native NCC activity (mDCT15), aldo stimulated NCC activity by 26% at 6 hrs and 52% at 24 hrs with a hyperdynamic surface expression profile. To determine the mechanism for this, mDCT15 cells were incubated for 24 hrs with 100μM aldo or vehicle. Cells were lysed and immunoblotting using phospho-specific antibodies was performed. NCC Thr60 phosphorylation increased significantly with aldo administration compared to control (33%, p<0.05, n=4). SPAK 373 phosphorylation also increased (34%, p<0.05, n=4). This indicates that aldo enhances both SPAK and NCC phosphorylation, suggesting a potential signaling cascade whereby aldo activates SPAK, which phosphorylates NCC, which then triggers an increase in individual transporter activity. Further study is necessary to characterize this mechanism.

68 SUPERNORMAL EJECTION FRACTION AND LEFT VENTRICULAR DIASTOLIC DYSFUNCTION
M. Gargas Guthrie Clinic, Sayre, PA; I.A. Goeker, A.N. Magalski Mid America Heart Institute, University of Missouri Kansas City, Kansas City, MO.
CURRENT CATEGORY: Cardiology/Cardiovascular Disease
ABSTRACT BODY: Background: Left Ventricular Diastolic Function (LVDF) is an important entity in determining cardiac function. The impact of a hyperdynamic left ventricle(LV) with supernormal ejection fraction (EF) on LVDF has not been well studied. We evaluated the relationship of a hyperdynamic LV (EF >65%) with LVDF.
Methods: Stable outpatients who were not on a positive or negative inotropic agent and with no significant valvular, wall motion, pericardial, right heart, intracardiac shunt or intracardiac mass abnormality were included in the study. Baseline characteristics, 2D, m-mode and Doppler echocardiographic features including LVDF of 112 consecutive patients with EF >65% were compared with those of 112 consecutive patients with normal EF (55-65%).
Results: Patients with supernormal EF were more likely to be obese (52 vs 34 p= 0.02) and more likely to have left ventricular hypertrophy (LVH) (41 vs 26 p = 0.04). Age, gender distribution, prevalence of coronary artery disease, hypertension, diabetes, COPD and smoking was similar in both groups. More patients with supernormal EF had evidence of Grade I left ventricular diastolic dysfunction (36 vs 21 p=0.03). Prevalence of higher grade left ventricular diastolic dysfunction was low and similar in both groups.
Conclusion: This study suggests that to be more prevalent in obese patients and more likely to be present in patients with LVH. Higher EF was also seen to be associated with LV relaxation abnormality (Grade I left ventricular diastolic dysfunction). The reason and significance of these findings need to be further evaluated in larger studies.

69 MICROBIOLOGICAL EVALUATION OF IMMUNOTHERAPY AGENTS IN CLINICAL PRACTICE
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CURRENT CATEGORY: Infectious Disease
ABSTRACT BODY: Purpose of Study: Immunotherapy is being increasingly used in treatment of allergies that exacerbate many otolaryngology-related illnesses such as otitis media, chronic rhino-sinusitis. Upon literature review, in-office compounding of immunotherapy vials seems to be a safe practice with low incidence of bacterial infections. There are stringent protocols for proper preparation of vials and administering injections. Moreover, substances with bacteriostatic properties, such as glycerin, phenol or a combination of both can be added to the vials. The concentrations of these substances used in practice vary based on desired shelf life, physician preference or manufacturer. There is no in vitro data studying the optimal concentration for bacteriostatic or bactericidal effect of these substances used in subcutaneous injectable immunotherapy. This is the purpose of our study.
Methods Used: We used increasing concentrations of 2 substances used as preservatives, phenol and glycerin and their combination, to which we added 1X10^5 bacteria per vial. Staphylococcus aureus ATCC strain number 25923 was used since it is a common skin associated pathogen. The experiment was performed in triplicate and read at 24 and 48 hours of incubation. We followed this with log dilutions of bacteria in microtitre plates, creating a checkerboard with the single highest concentration of different preservatives, to assess turbidity and bacterial growth by colony counts.
Summary of Results: When large bacterial inocula of 1X10^5 were incubated with graded concentrations of the three substances; the visible turbidity was inversely proportional to their concentration. With the checkerboard technique in microtitre plates, there was no visible turbidity. Upon plating on Mueller-Hinton agar, bactericidal effect was seen with concentration of 10^-3/ml or less of bacteria, which simulate bacterial burden in clinical settings. Glycerin at concentration of 25% was as active as a combination of glycerin/phenol 25/0.4%, whereas phenol alone at 0.4% was less active.
Conclusions: Given the results, we recommend the highest concentration in use or highest tolerated concentration of glycerin or glycerin/phenol combination in immunotherapy vials for optimal bacteriostatic and likely bactericidal effect.

70 HYALURONIDASE-I IS A NOVEL REGULATOR OF HUMAN PULMONARY ENDOTHELIAL BARRIER DISRUPTION
T. Mirzapoi佐ova, N. Mambetsariév, B. Mambetsariév, F.E. Lennon, P.A. Singleton Medicine, University of Chicago, Chicago, IL.
CURRENT CATEGORY: Pulmonary/Critical Care
ABSTRACT BODY: Rationale: Endothelial cell (EC) barrier dysfunc- tion results in increased vascular permeability, a feature of several disease states including acute lung injury (ALI). Therefore, understanding the mechanisms of EC barrier disruption can have important therapeutic implications. We have previously demonstrated that the glycosaminoglycan, high molecular weight hyaluronan (HMW-HA, ~1 million Da), promotes human pulmonary endothelial cell (EC) barrier enhancement while low molecular weight hyaluronan (LMW-HA), produced in various lung disease states including acute lung injury (ALI). Therefore, understanding the mechanisms of EC barrier disruption can have important therapeutic implications. Total hyaluronan concentration and hyaluronan degradation were evaluated using HA enzyme-linked immunosorbent assays, fractionation based on molecular weight and Alcian blue gel staining. In vivo, male C57BL/6j mice (~8-10 weeks, Jackson Laboratories, Bar Harbor, ME) were treated with intra- tracheal administration of lipopolysaccharide (LPS, 2.5 mg/kg) for 24 hours to induce ALI with pulmonary vascular hyper-permeability as determined by murine lung immunohistochemistry and bronchoalveolar lavage total protein concentration. Immunoblot analyses of HYAL-1 were performed on treated EC lysates, mouse lung homogenates, mouse bronchoalveolar lavage (BAL) fluid and mouse plasma.
Results: Our data indicate that treatment of C57BL/6j mice (~8-10 weeks, Jackson Laboratories, Bar Harbor, ME) with intratracheal administration of lipopolysaccharide (LPS, 2.5 mg/kg) for 24 hours to induce ALI with
Our results indicate that HYAL-1 is upregulated in ALI and psychological stress increases the CROD infection had no effect on the levels of lymphangiogenesis; V. M. Reddy, S. Rawal, P. Wang, C. J. Glueck & Midwestern Regional Program Abstracts

E2 was 40.3 pg/ml, correlated with E2

Mice were subjected to water avoidance stress for 1 hour

Volume 59, Number 4, April 2011

These data demonstrate an unprecedented and unique role of Hypogonadal men given conventional doses of T as a gel (50 mg/day) develop high E2, with attendant increased risk of cardiovacular events, and thrombosis. Given the high population frequency of familial thrombophilias (Factor V Leiden [5%]), Prothrombin gene mutation [6%]), therapy with exogenous T often increases E2 to above normal levels and superimposes high E2-driven pharmacologic thrombophilia on heritable thrombophilias, and, thus, may increase the risk of thromboembolic morbidity and mortality.

There was a significant positive correlation between change in free testosterone (FT) and change in E2 on T therapy, as shown in the table

<table>
<thead>
<tr>
<th>Hormone level</th>
<th>Before treatment</th>
<th>After treatment</th>
<th>Change</th>
<th>nPaired Wilcoxon</th>
<th>Spearman correlation, change in T, FT and change in E2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total T</td>
<td>(normal: 280-400 ng/ml)</td>
<td>241±213</td>
<td>375±188</td>
<td>133±295</td>
<td>0.16</td>
</tr>
<tr>
<td>Free T</td>
<td>(normal: 7.2±2 pg/ml)</td>
<td>5.2±3.6</td>
<td>9.6±5.5</td>
<td>4.3±7.9</td>
<td>0.04</td>
</tr>
<tr>
<td>Estradiol</td>
<td>(normal: 5±4 pg/ml)</td>
<td>24.3±0.1</td>
<td>18.6±1.7</td>
<td>4.4±1.4</td>
<td>0.04</td>
</tr>
<tr>
<td>No of men with E2 &gt;42.0 pg/ml</td>
<td>(0%)</td>
<td>3(3%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

71 IDENTIFYING GENETIC VARIANTS CONTRIBUTING TO CELLULAR SENSITIVITY TO TAMOXIFEN USING A GENOME-WIDE CELL-BASED MODEL

D. Ziliak, E. Gamazon, H. Im, N. Cox, M. Dolan, R. Huang University of Chicago, Chicago, IL; Z. Desta, D. Flockhart Indiana University, Indianapolis, IN.

CURRENT CATEGORY: Hematology and Oncology

ABSTRACT BODY: Tamoxifen is one of the most commonly used agents in the management of estrogen receptor positive breast cancers. Currently the few known predictors are inadequate in predicting tamoxifen efficacy and toxicity in many breast cancer patients. Therefore, we utilized a genome-wide cell-based model to comprehensively evaluate genetic variants for their contribution to cellular sensitivity to tamoxifen. Our model incorporated genotype, gene expression, and cellular growth inhibition following tamoxifen treatment in lymphoblastoid cell lines from the International HapMap project. Growth inhibition was measured using AlamarBlue Assay in 60 unrelated CEU (individuals of northern and western European descent) samples. All cell lines were treated with increasing concentrations of endoxifen, an active metabolite of tamoxifen, for 72 hours. Log2 transformed percent survival at each concentration and IC50 were used as cellular sensitivity to drug phenotypes. A step-wise genome-wide association studies were performed among genotype, gene expression and endoxifen sensitivity phenotypes. We identified 10 SNPs associate with endoxifen sensitivity through the expression of 13 genes in the CEU population. Interestingly 2 of the candidate genes (TES and SMARCA2) identified in CEU both play a role in hormone biosynthesizes pathway. This genome-wide approach allows us to identify genetic variants.Genes that have not been previously discovered to relate to tamoxifen. Further validation of these cell-based model findings may significantly improve our ability to predict tamoxifen treatment efficacy and toxicity in breast cancer patients.

72 TESTOSTERONE TREATMENT FOR HYPOGONADISM LEADING TO CLINICALLY SIGNIFICANT HIGH ESTRADIOL

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CURRENT CATEGORY: Endocrinology/Metabolism

ABSTRACT BODY: Introduction: High estradiol (E2) levels in men have been associated with myocardial infarction, ischemic stroke, and pulmonary embolism. When E2 is given to men, the overall rate of cardiovascular disease deaths increases significantly. Endogenous and exogenous testosterone (T) is converted to E2 by aromatization. Our specific aim was to assess the development of high E2 when hypogonadal men were given T gel 50mg/day.

Methods and results: In 9 hypogonadal men, we measured E2 levels before and after T gel 50 mg/day. Of the 9 men, 3 developed high E2. There was a significant positive correlation between change in free testosterone (FT) and change in E2 on T therapy.

Twenty two hypogonadal men were studied on 50 mg/day T gel only, without measurement of antecedent T or E2. On treatment mean ± SD T was 459±247 ng/ml, and the correlation between total T and E2 was r=−0.50, p<0.017 (Spearman). On treatment, free T was 13.5±14.4 pg/ml, correlated with E2 (r=−0.54, p<0.01). On treatment, mean ± E2 was 40.3±24.1 pg/ml, and 8 of the 22 (36%) men had high E2 (≥42.6 pg/ml).

Discussion: One third of hypogonadal men given conventional doses of T as a gel (50 mg/day) develop high E2, with attendant increased risk of cardiovascular events, and thrombosis. Given the high population frequency of familial thrombophilias (Factor V Leiden [5%]), Prothrombin gene mutation [6%]), therapy with exogenous T often increases E2 to above normal levels and superimposes high E2-driven pharmacologic thrombophilia on heritable thrombophilias, and, thus, may increase the risk of thromboembolic morbidity and mortality.

73 LYMPHANGIOGENESIS IN WATER AVOIDANCE INDUCED STRESS AND CITROBACTER RODENTIUM INDUCED GUT INFLAMMATION

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CURRENT CATEGORY: Gastroenterology/Clinical Nutrition

ABSTRACT BODY: Background: Psychological stress increases the risk for developing, maintaining or re-activating human and experimental colitis. The present study was conducted to understand the role of psychological stress (water avoidance stress, WAS) and its regulation of inflammation and lymphangiogenesis in a mouse model of colitis. To produce a model targeted gut inflammation water avoidance stress (psychological stress) was combined with Citrobacter rodentium infection (CROD) to provoke gut inflammation.

Methodology: Mice were subjected to water avoidance stress for 1 hour daily for 10 days and 30 days respectively. One group is inoculated with LB broth + Citrobacter rodentium (CROD) and other group with LB broth only (Sham). Colonies were sampled and evaluated for lymphangiogenesis by immunohistochecistry (VEGFR3). Quantification and Statistical analysis was done with one way ANOVA and two way student’s t-test.

Results: CROD infection had no effect on the levels of lymphangiogenesis in the infected colon. However, WAS in both sham and CROD infected colons significantly reduced the level of lymphangiogenesis. There was a significant decrease in the number of lymphatic vessels between WAS groups at both 10 days and 30 days post infection, compared to sham and CROD treated mice not subjected to WAS (P<0.001).

Conclusion: These data demonstrate an unprecedented and unique role of psychological stress in negatively remodeling colon lymphatics. This is in contrast to the role that has been described for stress in both colon and gastric tumor angiogenesis and lymphangiogenesis. Mechanisms responsible for this altered regulation are currently under investigation. However this unique regulation may help explain the link between stress and colitis with fewer vessels leading to reduced tissue perfusion increasing ischemic damage leading to barrier dysfunction and reduced immunological surveillance and regulation of the intestinal tissues (lymphatic vessels) providing several possible links between stress and it’s observed effects on colon health and colitis.
74 ATEROTHROMBOSIS: Atherosclerosis, Thrombophilia, Hypofibrinolysis and Progressive Coronary Artery Disease

A. Gal, C. Abuchaibe, C. Glueck, P. Wang, I. M. Jewish Hospital Cincinnati, Blue Ash, OH.

**CURRENT CATEGORY:** Cardiology/Cardiovascular Disease

**ABSTRACT BODY:** Our specific aim was to examine interactions between atherosclerosis, thrombophilia-hypofibrinolysis, and atherothrombotic coronary artery disease (CAD) and ischemic stroke (CVA) in 28 patients (mean ± SD age 50 ± 11 years). Our second specific aim was to assess efficacy and safety of anticoagulant therapy with Coumadin in 12 patients (age 50 ± 11) with concurrent atherosclerosis-thrombophilia-hypofibrinolysis and progressively worsening CAD despite aspirin-plavix, repetitive stenting-bypass surgery and maximal lipid lowering therapy with resultant mean ± SD LDL cholesterol 83 ±35 and triglyceride 124 ± 66 mg/dl. Measures of thrombophilia included the Factor V Leiden, Prothrombin, MTHFR mutations, ACLA IgG and IgM, lupus anticoagulant, proteins C, S (total and free), Antithrombin III, Homocysteine, Factors VIII and XI. Measures of hypofibrinolysis included the plasminogen activator inhibitor-1 (PAI) 4G4G mutation, plasminogen activator inhibitor activity (PAI-fx), and Lp (a). Of the 28 patients, 24 had CAD, 3 CVA, 1 mesenteric artery thrombosis, with events < age 35 in 8 patients, 35 to <45 in 3, and 45 to <171. The most common coagulation abnormality was high Factor VIII in 7 patients, PAI-1 in 5 (PAI 4G4G4G, PAI-Fx-1), lupus anticoagulant in 3, anticoagulin antibody in 3, Factor V Leiden heterozygosis in 3, MTHFR C677T homozygosis in 3, prothrombin gene mutation in 2, low Protein C in 2, high Lp (a) in 2, low antithrombin III in 2, homocysteinein in 2, and low Protein S in 1. Twelve patients had progressive CAD despite stents-bypasses, lipid lowering, and antplatelet therapy; 5 had high Factor VIII, 6 lupus anticoagulant-high ACLA, 1 homocysteineim and 2 the prothrombin gene mutation. To try to arrest the progressively worsening course of CAD, Coumadin was given to these 12 patients (INR targeted to 2.5–3.5), for 70 ± 28 months (minimum 21, maximum 120 months). In these 12 patients, on Coumadin, there was no further progression of CAD, and they were able to function normally, without major bleeding episodes. In patients with premature CAD and CVA, and in patients with rapidly progressive CAD despite medical-surgical therapy, familial and acquired thrombophilias and hypofibrinolysis appear to interact with atherosclerosis, an interaction which can often be safely stopped and reversed with anticoagulation. We suggest that interactions between thrombophilia-hypofibrinolysis and hyperlipidemia-atherosclerosis be systematically assessed in patients with premature CAD and CVA, and in patients with CAD progression, to optimize short- and long-term secondary prevention with anticoagulation.

75 NORMALIZATION OF SERUM VITAMIN D IN HYPERCHOLESTEROLEMIC, VITAMIN D DEFICIENT PATIENTS, PREVIOUSLY STATIN INTOLERANT BECAUSE OF MYOSITIS-MYALGIA, RESTORES STATIN TOLERANCE WITHOUT MYOSITIS-MYALGIA

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**CURRENT CATEGORY:** Endocrinology/Metabolism

**ABSTRACT BODY:** Background: Myalgia-myositis is the most common cause of statin intolerance.

**Methods:** In 68 hypercholesterolemic patients, unable to tolerate ≥ 1 statins because of myositis-myalgia, selected by low (<32 ng/ml) serum vitamin D, we prospectively assessed whether resolution of vitamin D deficiency would result in statin tolerance, free of myositis-myalgia. On no statins, 50,000 units of vitamin D was given twice/week for 3 weeks, and was then continued once/week. After 3 weeks on vitamin D, statins were restarted, and patients were re-assessed after 3 months on statins and vitamin D supplementation.

**Results:** At mean 3 months follow-up, on vitamin D supplementation and re-instituted statins, 62/68 (91%) previously statin-intolerant patients now tolerated statins well and were asymptomatic without myositis-myalgia. In these 62 patients, on vitamin D supplementation and statins, mean ± SD vitamin D rose from 22 ± 7 to 43 ± 13 ng/ml (p<0.0001), and LDL cholesterol fell from 162 ± 57 to 99 ± 34 mg/dl (p<0.0001).

**Conclusions:** Symptomatic myositis-myalgia in hypercholesterolemic statin-treated patients with concurrent vitamin D deficiency may reflect a reversible interaction between vitamin D deficiency and statins on skeletal muscle.

76 PRIMARY LANGUAGE IN HISPANICS WITH CHRONIC KIDNEY DISEASE AND MEDICAL MANAGEMENT OF DIABETES, HYPERTENSION, AND HYPERLIPIDEMIA

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**CURRENT CATEGORY:** Nephrology

**ABSTRACT BODY:** Introduction and Purpose: Hispanics are greatly burdened by chronic kidney disease (CKD). Primary language has been found to influence health outcomes in Hispanics; however, this factor has not been studied in the context of CKD. The purpose of this study is to examine the cross-sectional association of primary language with clinical management of diabetes, hypertension, and hyperlipidemia in a cohort of Hispanics with mild to moderate CKD.

**Methodology:** We performed a cross sectional analysis of Hispanics enrolled in a prospective observational cohort study, the Hispanic Chronic Renal Insufficiency Cohort (HCRIC) Study. At study entry participants were between the ages of 21 to 74 with mild to moderate CKD as assessed by estimated glomerular filtration (eGFR) rate. Primary language was self-reported. Based on treatment guidelines, the parameters chosen as defining adequate medical management were as follows: Blood Pressure (BP) <130/80, glycosylated hemoglobin <7%, and total cholesterol <200 mg/dl.

**Results:** A total of 327 participants were included in the analysis. Approximately 69% of participants are Mexican American, 16% are Puerto Rican, and 25% have another Latin American ancestry. Approximately 80% of study participants were primary Spanish speakers. Compared to primary English speakers, Spanish speakers were older (57.9 vs. 53.6 years, p<0.008), more likely to have an income <$20,000 (82% vs. 62%, p<0.002), more likely to have less than six grade educational attainment (55% vs. 9%, p<0.001), and were less likely to have health insurance (41% vs. 26%, p=0.03). Primary Spanish speakers were also more likely to report a diagnosis of congestive heart failure (p=0.04). There was no difference in self-reported diabetes, hypertension, or other cardiovascular disease. Primary Spanish speakers had a higher systolic BP (138.8 vs. 131.4 mmHg, p=0.03), were less likely to be current smokers (5.6% vs. 13.8%, p=0.03), and were less likely to be on an angiotensin converting enzyme (ACE)-inhibitor or angiotensin receptor blocker (ARB) (66.8% vs. 81.0%, p=0.03). In multivariable analysis, there was no association between primary language and medical management of diabetes, hypertension, or hyperlipidemia. However, primary Spanish language speakers were 58% less likely to be on ACE-inhibitor or ARB compared to English speakers OR 0.42 (95% CI 0.19, 0.93).

**Conclusion:** In the Hispanic CKD population we examined, those with primary Spanish language appear to be particularly vulnerable and burdened with a lower socioeconomic status. We found an association between Spanish as a primary language and higher systolic BP and lower use of ACE-inhibitor or ARB. Follow-up of the HCRIC participants is ongoing and will permit an evaluation of the relationship between primary language and progression of CKD.

77 SLEEP DURATION MODIFIES THE HERITABILITY OF BODY MASS INDEX

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**CURRENT CATEGORY:** Genetic Medicine

**ABSTRACT BODY:** Objective: Previous research has found that short sleep duration (< 7 hrs/night) is associated with elevated body mass index (BMI). The current study examined whether sleep duration modified genetic and environmental influences on BMI. Participants were drawn from the University of Washington Twin Registry, a population-based sample of U.S. twins (N=1811 pairs, 933 MZ, 878 DZ; 63% female; M age = 36.6 years, SD=15.8 years). Participants provided self-report survey information on height and weight (used to calculate BMI, M=25.4 kg/m², SD=5.43) and...
habitual sleep duration (M=7.18 hrs/night; SD=1.24). Data were analyzed using behavioral genetic interaction models. The heritability of sleep duration was 32%; shared environmental influences on sleep duration were negligible (eMZ = 0.34; rDZ = 0.12). As previously reported, longer sleep duration was associated with decreased BMI (b = 0.24, SE = 0.09, P < 0.01). Behavioral genetic modeling indicated that there were significant interactions between sleep duration and both genetic [a0 = 9.35, ax = -0.71, both P < 0.05, where total genetic variance = a0 + ax* (sleep duration)] and shared environmental influences [c0 = -0.09, c1 = 0.04, both P < 0.05] on BMI. The heritability of BMI when sleep duration equaled 7 hours (h2 = 70%) was more than twice as large as the heritability of BMI when sleep duration equaled 9 hours (h2 = 33%). In conclusion, shorter sleep duration is associated with increased BMI and increased genetic influences on BMI, suggesting that shorter sleep duration may increase expression of genetic risks for high body weight. At the same time, longer sleep duration may suppress genetic influences on body weight. Future research aiming to identify specific genotypes for BMI may benefit from considering the moderating role of sleep duration.

78 SERUM CYTOKINES IN SARCOIDOSIS

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CURRENT CATEGORY: Rheumatology/Immunology/Allergy

ABSTRACT BODY: Rationale: Sarcoidosis is a systemic, granulomatosus condition of unknown etiology that predominantly affects the lungs but also commonly involves extra-pulmonary organs. The immunopathogenesis of sarcoidosis is largely unknown; immune cells are recruited and activated via poorly understood cell signaling mechanisms. Levels of cytokines in the lung are frequently altered. There may be important alterations in circulating cytokines as well. To explore this hypothesis, we measured circulating cytokines in patients with sarcoidosis and in controls.

Methods: We used a bead-based multiplex cytokine assay to measure the levels of 17 cytokines in 56 patients with biopsy-established, chronic sarcoidosis, and in 20 controls. IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17, G-CSF, GM-CSF, IFN-γ, MCP-1, MIP-1β, and TNF-α were measured simultaneously in serum samples. Non-parametric tests were performed to compare cytokine levels in controls to cytokine levels in patients with sarcoidosis. Significance was defined as a p value < 0.0029, which reflects a Bonferroni correction for multiple comparisons (17 cytokines).

Results: Serum IL-7 was elevated in patients with sarcoidosis compared to controls (p=0.0027). In contrast, MIP-1β and IL-5 were decreased in patients with sarcoidosis compared to controls (p=0.0001 and p=0.0012, respectively).

Conclusions: In a comprehensive evaluation of serum cytokines in sarcoidosis, we found serum IL-7, MIP-1β, and IL-5 to be significantly altered in sarcoidosis. These data provide a window into the immunopathogenesis of sarcoidosis, and support the concept that there are important and detectable alterations in the systemic immune system in sarcoidosis. These data also provide attractive targets for follow-up studies.

79 HUMAN UMBILICAL CORD BLOOD-DERIVED MESENCHYAL STEM CELLS IN THE CULTURED RABBIT INTERVERTEBRAL DISC - A CELL THERAPY APPROACH

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CURRENT CATEGORY: Mechanisms of Disease

ABSTRACT BODY: Back pain associated with intervertebral disc (IVD) degeneration is a common clinical condition. Human umbilical cord blood-derived mesenchymal stem cells (hUCB-MSCs) differentiate into cells of the adipogenic, osteogenic, and chondrogenic lineages. Furthermore, injection of mesenchymal stem cells into the IVD is an attractive approach for disc regeneration. In this pilot study, we determined the effectiveness of mesenchymal stem cell therapy for repair of the intervertebral disc in vitro.

First, hUCB-MSCs, labeled with a lipophilic dye (DiI), were injected into cultured rabbit IVD explants. 80,000 cells were injected into each explant with a 26G micro-syringe. The explants were maintained in culture for one month. Cell survival was then assessed by staining with CellTracker Green, which stains live cells. Survival of the injected cells was confirmed with fluorescence microscopy. Subsequently, to examine the function of transplanted stem cells, expression of the human collagen type II by the stem cells was assessed by RT-PCR. Finally, to examine the effects of stem cells on the host rabbit IVD, rabbit collagen type II and matrix metallopeptidase-13 (MMP-13) mRNA levels were quantified by real-time PCR using Taqman assays specific for the rabbit genes.

Our data show that the hUCB-MSCs injected into the cultured disc explants survived for at least one month and expressed the human collagen type II gene. Host rabbit type II collagen mRNA level increased by two-fold, host MMP-13 mRNA level decreased by three-fold in IVDs injected with stem cells, when compared to non-injected IVDs.

In conclusion, we have shown that hUCB-MSCs have survived for at least one month, and expressed the human collagen type II gene when injected into rabbit IVD. Furthermore, the host rabbit IVD expressed a higher level of type II collagen mRNA (an anabolic marker), and a lower level of MMP-13 mRNA (a catabolic marker). This is indicative that stem cells injected into the rabbit IVDs may delay the degenerative process that occurs when cultured in vitro, thus supporting the potential of a cell therapy approach for disc repair. Future studies in animal models in vivo should be performed before clinical trials in humans.
AGEs were higher in diabetic Balb/cJ mice compared to other groups. These findings suggest that different populations of sensory neurons in the DRG are susceptible to different types and degrees of damage in diabetic neuropathy. Underlying genetic differences in the expression of GLO1 may also alter the ability of DRG neurons to detoxify reactive dicarbonyls and limit the formation of AGEs with reduced expression leading to neuronal damage and symptoms of diabetic neuropathy. Therefore, AGEs and the glyoxalase system may play a role in the development and modulation of diabetic peripheral neuropathy.

81 A MOLECULAR NETWORK REVEALS THE EXQUISITE SENSITIVITY OF DIVIDING CANCER CELLS TO MICROTUBULE PERTURBATION
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CURRENT CATEGORY: Hematology and Oncology
ABSTRACT BODY: Microtubules have long been an important target in cancer therapy. Some of the oldest anti-cancer drugs include vinca alkaloids and paclitaxel, which affect microtubule stability by perturbing the balance between the assembly and disassembly of microtubule polymers. Given the sensitivity of dividing cells to microtubule poisons, these drugs are very effective at inducing mitotic cell death. However, they also perturb microtubules in neurons and the resulting peripheral neuropathy is a dose-limiting side-effect. This adverse toxicity invokes the need to identify cellular targets that influence microtubule dynamics in a selective manner during mitosis.

Here, we reveal a molecular network that acts at the attachment site of microtubules to chromosomes during mitosis. This network, which is comprised of the proteins astrin, Kif2b, and CLASP1, fine tunes microtubule dynamics in a temporal manner as cells progress through the division process. In early mitosis, a CLASP1/Kif2b complex promotes microtubule dynamics to allow chromosomes to establish proper attachment to the mitotic spindle. Furthermore the dynamic nature of microtubule polymers at this stage of division allows for the correction of attachment errors of chromosomes to microtubules. We previously showed these errors could lead to chromosome mis-segregation and chromosomal instability if left uncorrected. In late mitosis, however, the CLASP1/Kif2b complex is replaced by a CLASP1/astrin complex which in turns stabilizes microtubules and silences the mitotic cell cycle checkpoint allowing cells to proceed to anaphase. Failure to stabilize microtubules at this stage leads to a cell cycle arrest.

By perturbing components of this network, we show that dividing cancer cells are surprisingly much more sensitive to the magnitude and timing of disruption of microtubules than previously thought. This sensitivity stems from the cellular need to balance microtubule stability that ensures the timely progression through the cell cycle, with microtubule dynamics that safeguard from chromosome segregation errors. Importantly, we show that perturbing microtubule behavior by as little as two-fold has a drastic effect on cancer cell viability. In summary, our work identifies the astrin/Kif2b/CLASP1 network as an important therapeutic target that influences microtubules specifically in dividing cells. The effect on microtubule dynamics is much more subtle than that exhibited by traditional anti-microtubule cancer drugs and thus offers new avenues to exploit the sensitivity of cancer cell to microtubules poisons while minimizing adverse side-effects on neurons.

82 ACTIVATION OF CARBOHYDRATE RESPONSE ELEMENT BINDING PROTEIN (CHREBP) BY ETHANOL
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CURRENT CATEGORY: Mechanisms of Disease
ABSTRACT BODY: Carbohydrate response element binding protein (ChREBP) is a transcription factor involved in hepatic lipogenesis in the presence of high carbohydrate. Its function is under the control of AMP-activated protein kinase (AMPK) and protein phosphatase 2A (PP2A). Ethanol treatment of cultured hepatoma cells and of mice inhibited the activity of AMPK and the activation of PP2A by increasing ceramide content. Given the effect of ethanol on AMPK and PP2A, it is plausible that ethanol might enhance fatty acid synthesis by increasing the activity of ChREBP. We hypothesized that another potential pathway of ethanol-induced hepatic steatois is mediated by activation of ChREBP. Methods: The effects of ethanol on ChREBP were assessed in rat hepatoma cells and in C57BL/6J mice fed with the Lieber-DeCarli diet. Results: When the cells were exposed to ethanol (50 nM) for 24 hrs, the activity of a liver pyruvate kinase (LPK) promoter-luciferase reporter, which contains a carbohydrate response element (Chore), was increased by ~4-fold compared to controls, suggesting that ethanol increased ChREBP activity in vitro. Ethanol feeding in mice resulted in the translocation of ChREBP protein from cytosol to the nucleus and increased DNA binding ability of ChREBP when compared to pair-fed controls. These effects of ethanol on ChREBP were associated with an increase in the expression of genes known to be regulated by ChREBP. PP2A activity was increased in the liver of ethanol-fed mice by 22%. We found no difference in the levels of hepatic Xu-5-P (a known PP2A activator) between ethanol-fed mice and controls (1.2 ± 0.5 vs. 2.3 ± 1.4 mmol/g, p = 0.15), suggesting that the effect of ethanol on PP2A is mediated through an Xu-5-P independent pathway. Transfection of the constitutively active AMPK plasmid suppressed the basal activity of the LPK luciferase reporter by ~50% and significantly reduced the effect of ethanol on the reporter. On the other hand, transfection of rat hepatoma cells with a dominant negative AMPK plasmid induced basal LPK luciferase activity by ~20% and there was now additive effect of ethanol on activation of the reporter. The effect of ethanol on ChREBP was attenuated in the presence of okadaic acid, an inhibitor of PP2A. Conclusions: The effects of ethanol on AMPK and PP2A leads to the activation of ChREBP, providing another potential mechanism for ethanol-induced hepatic steatois.

83 EPIDEMIOLOGY OF INFECTIONS CAUSED BY STREPTOCOCCUS ANGINOSUS GROUP AT A TERTIARY CARE HOSPITAL IN SPRINGFIELD, ILLINOIS
O. Mansuri Internal Medicine, Southern Illinois University School of Medicine, Springfield, IL; V. Sundaresan, N.M. Khandori Internal Medicine, Infectious Diseases, Southern Illinois University, Springfield, IL.
CURRENT CATEGORY: Infectious Disease
ABSTRACT BODY: BACKGROUND: Streptococcus anginosus group (SAG), earlier referred to as Streptococcus milleri, can be further classified as Streptococcus anginosus, Streptococcus constellatus, and Streptococcus intermedius. While it is the normal flora of the oral, nasopharyngeal, gastrointestinal, and vaginal membranes, SAG can cause infections associated with abscess formation, commonly in the abdomen and brain. Although these infections carry high morbidity, the mortality rate from infections with SAG is low with the use of appropriate antibiotics. There exists a significant variation in reporting methodologies for this organism due to the old and new nomenclature. Often, it is reported as Alpha Streptococcus and not identified further. This may lead to a clinical decision of dismissing such results as contaminants.
AIM: The objective of this study was to determine the epidemiology of clearly identified SAG infections at a tertiary care referral hospital in Springfield, Illinois over the last 5 years.
METHOD: We retrospectively reviewed the charts of 54 patients (15 males, 39 females) with infections caused by SAG between 2004-2009. After receiving Institutional Review Board approval, the microbiology laboratory of Memorial Medical Center, Springfield, Illinois, was approached for a list of all patients with positive cultures (from sterile sites) with this group of organisms. We used the various nomenclatures of the organism to search the literature. Often, it is reported as Alpha Streptococcus and not identified further. This may lead to a clinical decision of dismissing such results as contaminants.
RESULTS: The mean age of the patients was 52. 15 patients had underlying medical conditions including diabetes mellitus, malignancy, and other causes of immunosuppression. Abscesses (brain and liver) accounted for the most common presentation of infection with SAG (14 cases), followed by empyema (9 cases), bacteremia (7 cases), endocarditis (2 cases) skin and soft tissue infections (4 cases), osteomyelitis (3 cases), and urinary tract infection.
84 GENDER DIFFERENCES IN CARDIAC REPOLARIZATION FOLLOWING INTRAVENOUS SOTALOL ADMINISTRATION

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CURRENT CATEGORY: Cardiology/Cardiovascular Disease

ABSTRACT BODY: Background: Females are more susceptible to drug-induced Torsade de Pointes (TdP), a potentially lethal polymorphic ventricular tachycardia, which is associated with excessive prolongation of the heart rate corrected QT interval (QTc). Sotalol prolongs the cardiac action potential that is seen as QTc prolongation on the surface electrocardiogram (ECG) and can induce TdP. The aim of this study was to assess gender differences in sotalol induced QTc prolongation.

Methods: 15 healthy volunteers, 9 female and 6 male (age: 32 ± 11.6 years) were studied. Following intravenous sotalol (75 mg intravenous sotalol over 2.5 hr at a constant infusion rate). A 12-lead ECG was recorded at baseline, 0.5, 1, 2, 3, 4, and 5 hrs following the start of the infusion. Blood samples were collected simultaneously and analyzed by LC-MS/MS technique. The QT and RR intervals were measured in each ECG lead and each QT was corrected for heart rate by the Fridericia and the Framingham formulas. For each 12-lead ECG, the average RR, QT and QTc intervals were calculated and used for data analysis. The data analysis included repeated measures of ANOVA, univariate analysis, and linear regression analysis.

Results: The QT intervals were similar in females and males at baseline (389±21 vs. 385±34 ms, NS) and the QT increased significantly (p<0.001) during the infusion in both females (from 389±21 to 436±28 ms) and males (from 385±34 to 413±28 ms). The Fridericia and Framingham formulas resulted in virtually identical QTc intervals. At 1 hr of infusion, QTc became significantly greater in females than it was at baseline (QTc 411±13 vs. 427±18 ms, p<0.001) while in males the increase was not significant (395±23 vs. 400±26 ms). The longest QTc intervals were observed at 2 hr in both genders. Compared to baseline, the increase was very significant in females (411±13 vs. 438±13 ms, p<0.001), while it was less significant in males (395±23 vs. 413±27 ms, p=0.05). The magnitudes of individual changes from baseline were significantly greater in females than in males (See Table).

QTc and serum sotalol concentration strongly correlated in both genders (r=0.93, p<0.001). Males had greater body weight (75.5±8.4 vs. 66.0±1.6 kg, p<0.05) and body surface area (1.91±0.11 vs.1.72±0.10m², p<0.001) than females, but neither correlated with QTc measurements or predicted the magnitude of QTc prolongation. The single predictor for the greater QTc prolongation was female gender.

Conclusion: Sotalol induced a greater QTc prolongation in females than males. This enhanced response to drug action may explain the higher incidence of drug induced TdP seen in females from QT prolonging drugs.

Maximum Individual Changes from Baseline Measurements by Gender

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<th>Female</th>
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<td>QTc (ms)</td>
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<td>NS</td>
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<td>QTc Framingham (ms)</td>
<td>54±8</td>
<td>21±12</td>
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85 EVALUATION OF THE AUTOIMMUNE DISEASE RISK ALLELE OF UBE2L3 IN AFRICAN-AMERICAN LUPUS PATIENTS

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CURRENT CATEGORY: Rheumatology/Immunology/Allergy

ABSTRACT BODY: Objective: UBE2L3 is associated with susceptibility to systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) in European ancestry populations, and this allele has largely not been investigated in non-European populations. Interferon alpha (IFN-α) and autoantibodies are pathogenic factors in SLE, and these factors likely play a role in RA pathogenesis as well. We studied the UBE2L3 risk allele for association with SLE, IFN-α, and autoantibodies in a predominantly African-American SLE cohort.

Methods: The rs5754217 single nucleotide polymorphism (SNP) in UBE2L3 was genotyped with Taqman probe-primer sets in 252 African-American and 99 European-American SLE patients, and 229 African-American and 105 European-American healthy controls. All patients had serum IFN-α and serology data available. Logistic regression models were used to detect genetic associations with autoantibody traits. Differences in serum IFN-α were tested using the Mann-Whitney U statistic. Results: The UBE2L3 rs5754217 T allele was strongly enriched in African-American patients with anti-La antibodies as compared to controls, and a recessive model was the best fit for this association (OR=2.56, p=0.0061). Serum IFN-α was higher in African-American patients with the TT genotype as compared to those with GT or GG genotypes (p=0.026), again fitting a recessive model. Stratifying the African-American cohort by anti-La antibodies, the TT/anti-La positive patients formed a significantly high IFN-α subgroup (p=0.0040) and this subgroup could account for all of the genetic effect of UBE2L3 genotype upon serum IFN-α. Similar non-statistically significant patterns of association were observed in the European-American SLE patients. Case-control analysis did not show large allele frequency differences, supporting the idea that this allele is most strongly associated with a particular subgroup of patients.

Conclusions: The UBE2L3 rs5754217 T allele associated with susceptibility to SLE and RA was associated in a recessive fashion with anti-La antibodies and serum IFN-α in SLE patients of African-American ancestry. This complex pattern of recessive influence upon subphenotypes will not produce a strong signal in standard case-control studies, and subphenotypes should be included in future studies of this locus.

86 VALIDATION OF VELOCITY VECTOR IMAGING ECHOCARDIOGRAPHY WITH MAGNETIC RESONANCE IMAGING IN MOUSE MODELS OF CARDIOMYOPATHY

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CURRENT CATEGORY: Cardiology/Cardiovascular Disease

ABSTRACT BODY: Background: Although deformation (strain) imaging has emerged as a valuable tool for echocardiographic assessment of LV function, its validity in small animal models is uncertain.

Methods: We compared circumferential (CS) and radial strain (RS) measured with echocardiography (vector velocity imaging [VVI]; Siemens) with tagged magnetic resonance imaging (MRI) in mouse models of cardiomyopathy. In 3 month old mice with gene targeted deficiency of cardiac myosin binding protein-C (cMyBP-C-/−; n=6 and cMyBP-C+/−; n=6) and muscle LIM protein (MLP−/−, n=6), and wild-type (WT) mice (n=6), strains were measured at 3 cross-sectional levels (base, mid, apex) and averaged to obtain global strains.

Results: Global strains were significantly decreased in both homozygous knockouts compared to WT, however LV1 strain was more variable than MRI (table). Despite normal EF, the heterozygous cMyBP-C+/− mice could be distinguished by decreased CS using VVI but not with MRI. Bland-Altman analysis comparing VVI and MRI strain showed τ = 0.39 for RS and τ = 0.50 for CS.
Conclusions: Echocardiographic and MRI strain correlated modestly and was greater for CS than RS. VVI underestimated strain compared to MRI and showed greater variability. However, measuring CS using VVI detects subtle changes in contractility as seen in heterozygous cMyBP-C and may be useful in early disease detection.

Table 1: Global strain measurements by VVI and MRI. LV ejection fraction measured by MRI

<table>
<thead>
<tr>
<th></th>
<th>Wild-Type</th>
<th>cMyBP-C(−/−)</th>
<th>eMyBP-C(−/−)</th>
<th>MLP(−/−)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV EF (%)</td>
<td>69 ± 6</td>
<td>68 ± 5</td>
<td>53 ± 8*</td>
<td>29 ± 5*</td>
</tr>
<tr>
<td>Global RS (µV)</td>
<td>20 ± 15</td>
<td>16 ± 4</td>
<td>15 ± 5</td>
<td>9.4 ± 1*</td>
</tr>
<tr>
<td>Global PS (µV-MRI)</td>
<td>25 ± 1</td>
<td>22 ± 2</td>
<td>13 ± 2*</td>
<td>15 ± 3*</td>
</tr>
<tr>
<td>Global CS (µV-Echo)</td>
<td>-21 ± 4</td>
<td>-15 ± 3*</td>
<td>-14 ± 4*</td>
<td>-7 ± 4*</td>
</tr>
<tr>
<td>Global CS (µV-MRI)</td>
<td>-18 ± 1</td>
<td>-7 ± 2</td>
<td>-9 ± 2*</td>
<td>-10 ± 2*</td>
</tr>
</tbody>
</table>

Measurements shown as Mean ± SD, (*P < 0.05 vs. Wild-Type, †P < 0.05 vs. eMyBP-C(−/−), §)P < 0.05 vs. cMyBP-C(−/−). 87

87 GENE-EXPRESSION GUIDED SELECTION OF CANDIDATE LOCI AND MOLECULAR PHENOTYPE ANALYSES ENHANCE GENETIC DISCOVERY IN SYSTEMIC LUPUS ERYTHEMATOSUS

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CURRENT CATEGORY: Rheumatology/Immunology/Allergy

ABSTRACT BODY: Objective: Systemic lupus erythematosus (SLE) is a highly heterogeneous autoimmune disease characterized by differences in autoantibody profiles, serum cytokines, and clinical manifestations. This study aims to use gene expression data to rationally select new candidate genes from a previous genome-wide association study (GWAS) of SLE.

Methods: We devised an algorithm to select additional candidate SNPs from an existing case-case GWAS of SLE patients stratified by autoantibody profile and serum interferon alpha. The top 200 SNPs from the GWAS were searched in the SCAN database, which compares genome-wide expression data to genome-wide SNP genotype data in HapMap cell lines. SNPs were chosen if they were associated with differential expression of 20 or more genes at a significance of p < 10⁻⁵, and in all cases at least one of the 20 differentially expressed genes had known immune function. This resulted in 13 SNPs which were genotyped in 416 SLE patients and 526 matched controls. Logistic regression models were used to detect associations between the candidate SNPs and anti-Ro, La, Sm, RNP, and DNA autoantibodies in SLE patients.

Results: 10 of 13 SNPs showed associations with specific autoantibody classes within either the European- or African-American ancestral backgrounds, including several SNPs that were associated with combinations of autoantibodies (e.g., anti-Sm and anti-RNP). A Q-Q plot demonstrated clear deviation from the null distribution in favor of positive findings in our autoantibody-SNP association results (p = 1 x 10⁻⁵ for a difference in slope by sum of squares F test between the observed vs. null distribution regression lines). Case-control analysis showed no large differences in allele frequencies, supporting the idea that the associations we detected are strongest in subgroups of patients defined by molecular phenotypes.

Conclusions: This study provides an appreciation for the complexity of macrophage/microglia activation plasticity, such that there are at least two distinct phenotypes: M1 ‘classically activated’ and M2 ‘alternatively activated’. An emerging principle in miRNA biology is that a single or multiple miRNAs can provide regulation of target genes, which could lead to complete phenotypic switching. Thus, we hypothesize that these core pro-inflammatory ‘M1’ miRNAs, miR-146a/b, miR-155, and miR-155. Amyloid-beta peptide (Aβ)42, commonly found in the senile plaques of Alzheimer’s disease brain, also stimulates TLR4 in microglia, although its activation of miRNAs is unknown. There is a growing appreciation for the complexity of macrophage/microglia activation plasticity, such that there are at least two distinct phenotypes: M1 ‘classically activated’ or M2 ‘alternatively activated’. An emerging principle in miRNA biology is that a single or multiple miRNAs can provide regulation of target genes, which could lead to complete phenotypic switching. Thus, we hypothesize that these core pro-inflammatory ‘M1’ miRNAs, miR-146a/b, miR-155, and miR-132, others and act in coordination to suppress alternatively activated M2 phenotype functionality thereby further promoting the M1 classically activated phenotype. To demonstrate that the phenotypical M2 function of the disease is inhibited by M1 stimulation, we have skewed a population of primary microglia to an M2 skew by a pre-treatment of IL-4, followed by stimulation with fibrillar Aβ42, as a M1 stimulant, and measured the phagocytic process by the administration of fluorescent labeled microparticles. We found that IL-4 pretreatment of primary microglia enhanced the phagocytic process by over 40% relative to control. Further, the enhanced phagocytosis was significantly inhibited with Aβ42 stimulation by over 67%. We further validated the M2 skewing of IL-4-pretreated primary M2 microglia.
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GENOMIC EVALUATION OF PULMONARY HYPERTENSION IN SICKLE CELL DISEASE


ABSTRACT BODY: Rationale: Pulmonary hypertension (PH) is associated with increased mortality in patients with sickle cell disease (SCD). Our aim was to identify PH patients in SCD using transthoracic echocardiography (TTE) imaging. We performed microarray analysis of isolated peripheral blood mononuclear cells (PBMCs) on a subset of patients to identify novel gene expression pattern and signaling pathways that could be associated with PH susceptibility in SCD.

Methods: Thirty-one SCD stable outpatients (mean age 33 +/- 7yrs) prospectively underwent TTE with Doppler color and tissue Doppler imaging. Diastolic dysfunction was based on mitral valve inflow pattern, tissue Doppler, and left atrial (LA) volumes. PH was defined as right ventricular (RV) systolic pressures >30mmHg. Microarray expression analysis of PBMC mRNA utilized an FDR <0.1 analysis.

Results: Patients with SCD exhibited preserved left ventricular (LV) ejection fraction (55.8 +/- 8%) with dilated LV (178 +/- 53mL, normal-56 -155mL) and end diastolic volumes (EDV) and LA volumes (52 +/- 16mL, normal- 22 +/- 6mL). Diastolic dysfunction was present in 7/31 (30%), PH in 14/31 (45%), while both were present only in 4/31 (13%). PH tended to be more common in female patients (11/14 vs 3/14 for males), patients with a body surface area (BSA) less than 1.75m2 (9/14 vs 5/14 for BSA>1.75m2), and patients younger than 35 years old (11/14 cases vs. 3/14 for age>35).

Conclusion: Consistent with previous reports, cardiac chamber dilation, diastolic dysfunction and PH are common in patients with SCD. PH tended to be more common in patients younger than 35 years, with BSA<1.75, and in females. Patients with PH also demonstrated a unique gene expression profile representing specific metabolic pathways that may be involved in susceptibility to PH in SCD.

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HSA-MIR-374A AND HSA-MIR-568 ATTENUATE LPS- AND CYCLIC STRETCH-INDUCED PBEF GENE EXPRESSION IN HUMAN LUNG ENDOTHELIUM

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ABSTRACT BODY: Rationale: We previously demonstrated that the pro-inflammatory cytokine pre-B cell colony enhancing factor (PBEF) is a viable candidate gene in acute lung injury (ALI) and ventilator-induced lung injury (VILI) syndromes. PBEF is highly expressed in LPS- and cyclic stretch (CS)-stimulated endothelial cells (EC) and is up-regulated in murine, canine and human ALI, with PBEF spatially localized to lung endothelium. MicroRNAs (miRNAs), widely known to regulate gene expression post-transcriptionally through binding to 3' UTR of mRNA, are linked to a variety of diseases, such as inflammation, cancer, and cardiovascular diseases. We performed in silico analysis (Miranda-microrna.org, TargetScan) which identified two miRNA candidates, hsa-mir-374a and hsa-mir-568 as potentially binding the 3'UTR of PBEF and therefore regulate expression. We investigated whether these miRNAs participate in regulation of LPS- and CS-induced PBEF gene and protein expression in vitro.

Methods: To study the function of hsa-mir-374a and hsa-mir-568 in the regulation of PBEF in LPS- and CS-stimulated cultured human pulmonary artery EC (HPAEC) in vitro, we used dual luciferase assays of reporter constructs (SwitchGear Genomics, Menlo Park, CA), containing the luciferase gene fused to the PBEF 3'UTR (luc-PBEF-3'UTR) and immunoblotting.

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microglia through qRT-PCR of key M2 markers (YM1 and Arg1) and M1 markers (TNF-α and IL-1β). In accord, we confirmed the upregulation of core M1 miRNAs (miR-132, 155, and 146a-b) in microglia via locked nucleic acid (LNA)-based miRNA qRT-PCR. Lastly, to investigate the possibility that coordinated core M1 miRNA activity can suppress M2 functionality, the molecular underpinnings of phagocytosis were identified through a bioinformatical approach, utilizing the miranda database, for potential target identification. We observed a systematic targeting of multiple proteins involved in the phagocytic process, with a few key proteins targeted multiple times or by multiple M1 miRNAs. Interestingly, we found similarities and differences in the targeting selection between humans and mice. Both humans and mice appear to target the initial recognition, as well as the intracellular signaling pathways to activate engulfment. In the mouse additional targeting is more focused on membrane/vesicle transit, whereas in the human additional targeting are focused on the Rac1-Cdc42 complex and other members of the RhoA GTPase switching family that trigger engulfment. These studies suggest that pro-M1 miRNA collectively suppress core M2 functionality and promote the M1 phenotype. Further, this suggests that a therapeutic approach of systematic targeting of pro-M1 miRNA could potentially enhance Aβ clearance by microglia in an Alzheimer’s disease brain.
Results: In HPAEC transfected with the luc-PBEF-3’UTR reporter construct, stimulation with LPS (20 hrs) resulted in 1.5±0.1 fold increase in luciferase activity, while untreated EC had basal activity 2.6±0.1 fold (4 replicate experiments) as compared with unstimulated cells transfected with same construct. Both synthetic precursor miRNAs, hsa-miR-374a and hsa-miR-568, attenuate LPS-induced increase in PBEF 3’UTR mediated luciferase activity, and hsa-miR-374a reduced PBEF 3’UTR mediated luciferase activity in CS treated cells. Cotransfections of EC with this construct along with hsa-miR-374a or hsa-miR-568 followed by LPS stimulation resulted in a decrease of the luciferase activity (~65±10% for hsa-miR-374a and ~60±11%, for hsa-miR-568, n=3) as compared with the stimulated EC transfected with this construct. Cotransfection of cells with hsa-miR-374a and luc-PBEF-3’UTR attenuate CS-induced increase in luciferase activity (~40±4%, n=3), compared with CS treated EC, transfected with luc-PBEF-3’UTR alone. LPS induced 2.65±0.1 fold increase in endogenous PBEF expression at 48 hrs post stimulation, this increase was essentially attenuated by transfection of EC with hsa-miR-374a (38±13%, n=3) or hsa-miR-568 (~25±2%, n=3) compared with stimulated cells. CS induced 1.2±0.1 fold increase in endogenous PBEF expression at 10 hrs post stimulation, which was attenuated by transfection of EC with hsa-miR-374a (8±1%, n=3) and hsa-miR-568 (8±1%, n=3) compared with CS treated cells.

Conclusion: These data suggest that hsa-miR-374a and hsa-miR-568 are novel candidates for regulating the LPS- and CS-induced expression of the important ALI and VILL-associated cytokine, PBEF. Synthetic miRNAs have potential as novel class of therapeutic molecules with potentially profound clinical impact.

Funded by NIH HL080042

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PEGLOTICASE DOES NOT CAUSE QT INTERVAL PROLONGATION

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CURRENT CATEGORY: Cardiology/Cardiovascular Disease

ABSTRACT BODY: Background: Pegloticase is a PEGylated uricase that is under development for the treatment of patients with chronic gout refractory to conventional therapy. Uricase converts uric acid into allantoin, which is more water soluble and better excreted than uric acid. The purpose of this investigation was to determine if pegloticase caused QT and QTC prolongation in patients with severe gout and coronary artery disease.

Methods: There were 212 patients who were randomized to receive either 8 mg pegloticase every 2 weeks (q2wk; N=84), or every 4 weeks (q4wk; N=85), or placebo (N=43). ECGs were obtained at baseline and at study end and analyzed post hoc by two blinded observers. The QT (longest QT in the six frontal plane leads) and RR intervals were measured in duplicate. The QT was corrected for heart rate using the Bazett (B QTC) and Fridericia (Fi QTC) formulas. Differences between groups were analyzed by ANOVA, changes within each group from baseline to study end were analyzed by paired sample t test. A two-sided alpha error of <0.05 was considered statistically significant.

Results: Of the 212 patients, 4 were excluded due to poor ECG quality. There was no significant increase in the rate corrected QT intervals when baseline was compared to study end. Baseline vs. study end in the q2wk dose group; Fi QTC: 412±34 vs. 416±37 ms (p=0.236), B QTC: 423±36 vs. 428±39 ms (p=0.225). Baseline vs. study end in the q4wk dose group; Fi QTC: 407±26 vs. 413±30 ms (p=0.079), B QTC: 419±30 vs. 424±38 ms (p=0.208). Baseline vs. study end in the placebo group; Fi QTC: 404±30 vs. 414±31 ms (p=0.069), B QTC: 414±33 vs. 423±33 ms (p=0.095). A trend toward QTC increase was the greatest in the group receiving placebo. The QTC d (Fi) did not exceed 2 ms at study end between the placebo and the low or high dose groups. Placebo vs. q2wk dose: 415±31 vs. 417±34 ms, placebo vs. q4wk dose: 415±31 vs. 413±30 ms.

Conclusion: No QT prolonging effect of pegloticase was detected in this study. Repolarization changes are unlikely in patients taking pegloticase.

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LEPTIN SIGNALING AND OXALATE HOMEOSTASIS: RELevANCE TO CALCIUM OXALATE KIDNEY STONE DISEASE


CURRENT CATEGORY: Mechanisms of Disease

ABSTRACT BODY: The vast majority of kidney stones are composed of calcium oxalate, and minor changes in urinary oxalate affect stone risk. Intestinal oxalate secretion mediated by anion exchanger Slc26a6 plays a major constitutive role in limiting net intestinal absorption of ingested oxalate, thereby preventing hyperoxaluria and calcium oxalate urolithiasis. Leptin is emerging to play an important role in the regulation of intestinal transport through signaling pathways including PKC activation, which also regulates intestinal oxalate transport. We therefore examined whether leptin affects intestinal oxalate transport using the human intestinal Caco2-BBE cells, which express leptin receptors and in which ≥50% of oxalate transport is mediated by Slc26a6. We measured apical [14C]oxalate uptake in the presence of an outward Cl gradient as an assay of Cl-oxalate exchange activity. Interestingly, we found that apical or basolateral leptin (0.2 nM x 6 hours) significantly inhibited [14C]oxalate uptake by ~30-40%, without affecting the transepithelial resistance. Importantly, leptin-inhibition of oxalate transport is completely blocked by preincubation of the cells with the leptin receptor antagonist (leptin mutein D39A/I40A/F41A), indicating that leptin binding to its receptors is necessary for the observed regulation. In addition, Leptin inhibition of oxalate transport is partially but significantly blocked by preincubation of the cells with the PKC inhibitor Go6983, suggesting that leptin signaling involves PKC activation. To confirm these findings in native epithelium, mouse duodenal segments were isolated and mounted in Ussing chambers. Following a 30-minute control period, the
effects of mucosal or serosal leptin (100 nM) on transepithelial unidirectional $^{14}$C-oxalate fluxes (mucosa to serosa, $J_{MS}$, and serosa to mucosa, $J_{SM}$) was assessed over another 30-minute period. Mucosal leptin significantly inhibited oxalate secretion (by >29%), a process largely mediated by Slc26a6, without affecting absorption (Control: $J_{MS} = 32.11 \pm 3.70$, $J_{SM} = 41.95 \pm 5.71$, $J_{SM} = -10.56 \pm 6.59$ pmol/cm²/h; Leptin: $J_{SM} = 35.22 \pm 2.99$, $J_{SM} = 29.73 \pm 4.19$, $J_{MS} = 5.49 \pm 5.13$ pmol/cm²/h), resulting in conversion of oxalate transport from net secretion in control tissues to net absorption in leptin-treated tissues. Using KEPCR, the observed leptin-induced inhibition of oxalate uptake by CaCo2 cells is not due to reduced SLC26A6 mRNA expression. However, we observed in preliminary experiments that leptin treatment led to redistribution of some of the SLC26A6 protein to an intracellular space, suggesting that leptin inhibits oxalate uptake by CaCo2-BBE cells by reducing SLC26A6 surface expression. These findings are of potential importance because intestinal cells are known to be exposed to leptin under physiological conditions. Moreover, luminal colonic leptin concentration is >15-fold higher in patients with inflammatory bowel disease, a condition associated with hyperoxaluria and a high incidence of related kidney stones. We conclude that leptin negatively regulates intestinal oxalate transport and that the observed inhibition is likely due to reduced SLC26A6 surface expression in CaCo2-BBE cells.

### 97 CHRONIC KIDNEY DISEASE AS A RISK FACTOR FOR ACUTE CORONARY SYNDROMES IN PATIENTS PRESENTING TO THE EMERGENCY ROOM WITH CHEST PAIN

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**CURRENT CATEGORY:** Cardiology/Cardiovascular Disease

**ABSTRACT BODY:** Atherosclerosis is the leading causes of death in western countries and lipoprotein metabolism is closely related with the disease. ApoB-containing lipoproteins (BLp) are atherogenic particles; while, high density lipoproteins (HDL) are anti-atherogenic ones. Phospholipid Transfer Protein (PLTP) activity has important impact on both BLp and HDL homeostasis. Animal studies, including general knockout and transgenic approaches, showed that PLTP has pro-atherogenic properties. However, less is known about tissue specific function of PLTP. To address the impact of liver-expressed PLTP on lipoprotein metabolism, we created a mouse model which expresses PLTP specifically in the liver on the PLTP-null background. We found that liver-specific expression of PLTP is responsible for the PLTP activity in the circulation and significantly increases plasma BLp levels. We also found that HDL levels are also slightly but significantly increased. In order to unravel the mechanism, we checked the apob and triglyceride production rates after blocking the clearance of the particles. We found that liver-expressed PLTP increases apob, both apoB100 and apoB48 and triglyceride secretion. Our results clearly showed that the liver-expressed PLTP promotes atherogenic potentials, by increasing BLp and triglyceride production and provided a rationale for seeking liver PLTP inhibition as a therapeutic approach for the treatment of atherosclerosis.

### 98 RAG-1 MUTANT DAHL SALT SENSITIVE RATS ARE RESISTANT TO NACL-INDUCED HYPERTENSION

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**CURRENT CATEGORY:** Mechanisms of Disease

**ABSTRACT BODY:** Introduction: Various studies have demonstrated the role that the immune system plays in the development and progression of hypertension and chronic kidney disease. Previous studies from our laboratory have demonstrated that hypertension and renal disease in the Dahl Salt-Sensitive (SS) rat is accompanied by the infiltration of macrophages and T lymphocytes into the kidney (Hypertension 48:149; Am J Physiol 298:R1136). To assess the importance of infiltrating cells in the development of hypertension, a mutation in the Recombination Activating Gene 1 (RAG-1) was induced in Dahl SS rats. The recombination activating genes are vital for the development of diverse immunoglobulins and T-cell receptors. Specifically, RAG-1 is a gene that encodes a protein that orchestrates the development of mature T-cells through a series of DNA rearrangements. A RAG-1 homozygous mutant was produced by injecting zinc finger nucleases targeting the sequence gtctacgcaaggttggtgacctgagtggtgca into Dahl SS rat embryos. The resulting mutation is a 13-bp frameshift deletion in exon1, resulting in a RAG-1 mutant strain.

**Methods:** Dahl SS rats and SS-RAG1-/- rats (n=5/group) were fed a low salt diet (0.4% NaCl) from weaning until 9 weeks of age. At 9 weeks, the rats were placed on a high salt diet (4.0% NaCl) and maintained on that diet throughout the experiment. After two weeks of the high salt diet, the rats underwent a surgical protocol to chronically place femoral arterial catheters for the measurement of arterial blood pressure. The animals were permitted to recover from surgery for a week and daily blood pressure measurements were obtained on seven consecutive days. At the conclusion of the experiment, the kidneys were removed from the rats, digested in collagenase, the infiltrating T lymphocytes were isolated with a magnetic separation method and counted. **Results:** As indicated in the table, the MAP (mean ± standard error) averaged $174 \pm 8$ mmHg in the control Dahl SS rats. In contrast, MAP in the RAG1-/- rats following 3 weeks of the 4.0% NaCl diet was significantly less, averaging $136 \pm 4$ mmHg. Consistent with the differences in MAP, the number of infiltrating T-cells in the kidneys were significantly (p < 0.05) reduced in the RAG1-/- rats (1.8 ± 0.5 × 105 T cells per kidney) compared to the number Dahl SS (5.6 ± 0.8 × 105 T cells per kidney). Though the number of white blood cells in whole blood tended to be lower in the RAG1-/- (9.15 ± 1.29 × 103 cells per µL) than in Dahl SS (13.74 ± 1.88 × 103 cells per µL), these differences were not statistically significant.

**Conclusions:**
1. RAG-1 deficient Dahl SS rats exhibit resistance to salt induced hypertension when compared to normal Dahl SS rats.
2. The numbers of T-cells infiltrating RAG-1 deficient kidneys are markedly lower than normal Dahl SS kidneys after four weeks of a high NaCl diet.

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3. The WBC counts of RAG-1 deficient and Dahl SS rats are not statistically different.

4. These data support a role for infiltrating T lymphocytes in the kidney in the development of salt-sensitive hypertension.

Mean Arterial Pressures (MPas)

<table>
<thead>
<tr>
<th>Rat Strain</th>
<th>Day 8</th>
<th>Day 9</th>
<th>Day 10</th>
<th>Day 11</th>
<th>Day 12</th>
<th>Day 13</th>
<th>Day 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAG1(-/-)</td>
<td>132</td>
<td>137</td>
<td>140</td>
<td>137</td>
<td>132</td>
<td>130</td>
<td>129</td>
</tr>
<tr>
<td>Dahl SS (mmHg)</td>
<td>165</td>
<td>178</td>
<td>175</td>
<td>167</td>
<td>168</td>
<td>171</td>
<td>174</td>
</tr>
</tbody>
</table>

P-values for the above seven days were (p < 0.05)

MOUSE MODEL OF PREMATURITY INDUCED REDUCTION IN NEPHRON NUMBER AND HYPERTENSION

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CURRENT CATEGORY: Nephrology

ABSTRACT BODY: Prematurity is a risk factor development of hypertension, metabolic syndrome, and kidney disease later in life. In the USA, one of every 8 children is born prematurely, and incidence in blacks of non-Hispanic origin is twice the incidence in white population. Worldwide, there are 12.5 million premature births annually. The consequences of prematurity are dependent on gestational age as well as other factors including genetic, environmental, and socioeconomic variable. Animal models provide an invaluable resource to study the effect of prematurity on organ development. Kidney is an attractive organ to study organ development. Besides, the central role of the kidney in homeostasis and blood pressure control, the nephron number is a tangible qualitative trait which correlates with kidney development. Premature infants are at risk of reduced nephron number and epidemiologic data support that reduced nephron number (Oligonephronia) is a risk factor for hypertension and chronic kidney disease (CKD).

In this report we describe the development of a mouse model for prematurity with evidence of renal developmental abnormality. Adult mice born one or two days early have 18-25% reduction of nephron number compared to full term controls (figure 1) which was statistically significant. However, when the nephron number was adjusted for body weight, only the 2 day premature mice have statistically significant reduction in nephron number (figure 2). The premature mice have statistically significant lower Glomerular filtration rate, as measured by real-time inulin clearance (figure 3). Premature mice also have higher urinary protein excretion compared to full term mice (figure 4). The premature mice were found to have a statistically significant higher systolic and diastolic blood pressure (figure 5). Moreover, the glomeruli of the premature mice at 9 month of age are enlarged and show significant mesangial expansion and increased matrix deposition (figure 6).

The renal consequences of prematurity in human has been attributed to organ sparing due (diversion of resources away from kidney to spare other organs mainly heart and brain) due to lack of resources during pregnancy. The animal model developed in this study shed a different light on the process and will provide a very useful resource to study effect of prematurity on kidney development. These mice were carried through a normal pregnancy till the day of premature delivery. We hypothesis that loss of placental support prematurity leads to arrest and/or abnormal programming of the developing kidney by inducing a termination of nephrogenesis signaling events which leads to reduced nephron number and hypertension in adult animals.

GADD45A MEDIATES AKT SIGNALING IN MECHANICAL STRESS-INDUCED LUNG INJURY: ROLE OF TRAF6 AND UCHL1

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CURRENT CATEGORY: Pulmonary/Critical Care

ABSTRACT BODY: Objective: GADD45a, a stress-induced nuclear protein involved in cell cycle regulation, DNA repair, apoptosis and maintenance of genomic stability, was previously identified as a significantly up-regulated gene in our pre-clinical model of ventilator induced lung injury (VILI). Compared to wildtype (WT), GADD45a(-/-) mice demonstrated enhanced susceptibility to VILI and significantly reduced levels of total and phosphorylated Akt in association with increased Akt proteasomal degradation. In this study we report that GADD45a regulates Akt lysine 63 (K63) ubiquitination and subsequent phosphorylation in mechanical stress via the cumulative effects of the deubiquitinating enzyme (DUB) ubiquitin carboxyl terminal hydrolase 1 (UCHL1) and TNF receptor associated factor 6 (TRAF6).

Methods and Results: Mechanically stretched (18% cyclic stretch; 4 h) human pulmonary artery endothelial cells (EC) revealed significant and time-dependent increases in Akt phosphorylation compared to static controls. However, EC transfected with GADD45a siRNA exhibited reduced Akt phosphorylation compared to untransfected cells. Subsequently, EC transfected with WT or mutant ubiquitin (K48R and K63R) vectors were subjected to cyclic stretch (CS) and immuno-precipitates of Akt exhibited increased Akt ubiquitination in cells transfected with WT or K48R mutant ubiquitin vectors. However, Akt ubiquitination was abolished in EC transfected with the mutant K63 ubiquitin providing evidence of differential specific-site Akt ubiquitination in cyclic stretch. Immunoprecipitation of Akt following the onset of mechanical stress demonstrated increasing association of the E3 ligase TRAF6 with Akt, which is known to mediate Akt K63 ubiquitination, while TRAF6 siRNAs attenuated Akt phosphorylation under static and CS conditions compared to untransfected controls. Separately, microarray analysis and real time PCR revealed VILI-induced upregulation of UCHL1 but reduced expression of UCHL1 in GADD45a(-/-) mice. Silencing UCHL1 in EC subjected to CS resulted in significantly reduced p-Akt and Akt levels compared to controls. Treatment with proteasome inhibitor, MG132 rescued Akt from proteasomal degradation in UCHL1 silenced cells. Finally, methyl-specific PCR using bisulfite modified genomic DNA from murine lungs demonstrated hypermethylation of the UCHL1 promoter in GADD45a(-/-) mice compared to WT animals.

Conclusion: These studies confirm a key role of UCHL1 and TRAF6 in the regulation of Akt ubiquitination and activation by mechanical stress and GADD45a and thus, aberrations in GADD45a signaling may implicate with clinical predisposition to acute inflammatory lung injury.

HGF-MEDIATED LUNG ENDOTHELIAL BARRIER REGULATION: ROLE OF SIPRI TRANSDUCTION, INTEGRIN BETA 4 AND Gab-1

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CURRENT CATEGORY: Pulmonary/Critical Care

ABSTRACT BODY: Rationale: The preservation of vascular endothelial cell (EC) barrier integrity is critical to normal vascular homeostasis, and barrier dysfunction a feature of inflammation, tumor angiogenesis, atherosclerosis, and acute lung injury. We have previously shown that hepatocyte growth factor (HGF) binding to its cell surface receptor tyrosine kinase, c-Met, promotes c-Met recruitment into specialized caveolin-1-enriched plasma membrane microdomains known as lipid rafts, transactivates the receptor for sphingosine 1-phosphate (SIPRI) and CD44 (a major glycoprotein receptor for hyaluronan), and increases EC barrier function via cytoskeletal rearrangement. In this study, we further explored the regulation of HGF-mediated enhancement of EC barrier function via SIPRI.

Methods/Results: In cultured human pulmonary artery EC, HGF-induced c-Met/SIPRI receptor interactions in cross immunoprecipitation experiments as well as recruitment of c-Met, integrin beta 4 (ITGB4) and SIPRI to caveolin-enriched lipid rafts. HGF ligation of c-Met transactivation evidenced by threonine phosphorylation. Silencing of SIPRI expression via siRNA (~90% silencing) attenuated HGF-induced Rac-1 activation and increases in trans-EC electrical resistance (~90% and 80% of attenuation for each). In addition, SIP and HGF-induced increases in EC barrier function were reduced by silencing of the adapter protein Gab-1 (~80% silencing, 25% of attenuation), ITGB4 (~25% silencing, 20% of attenuation) or the key cytoskeletal tyrosine kinase, c-Abl (~60% silencing, 30% of attenuation).

Conclusion: These results suggest that SIPRI is a critical regulator of HGF/ c-Met-mediated vascular EC barrier enhancement, with the essential involvement of Gab-1, ITGB4 and c-Abl. Supported by HL58064
We simultaneously performed right heart catheterization and 723
0.7 and 4.7 0.001). Fick CO and PA saturation were significantly correlated with
1.0 pump speeds per patient. Intra-
23 consecutive SLE and CL patients seen in our lupus clinic were
0.6, respectively. Interm
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We conducted a retrospective review of 102 patients who had
1.2 L/min by thermodilution (TD CO), 5.3 11.3 years. The majority of
0.4, 4.8
12.3, and 11.0
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Volume 59, Number 4, April 2011
index to determine if there was a correlation.
CARDIAC HEMODYNAMICS WERE COMPARED TO PUMP FLOW, POWER AND PULSATILITY
METHODS: We simultaneously performed right heart catheterization and transesophageal echocardiography in 13 patients with HMII LVAD. Measurements were obtained at a mean of 3.0 ± 1.0 pump speeds per patient. Intra-cardiac hemodynamics were compared to pump flow, power and pulsatility index to determine if there was a correlation.
RESULTS: The mean age of patients was 57.1 ± 11.3 years. The majority of patients were men (84.5%) and implanted as bridge to transplant (76.9%). The mean time between pump implantation and hemodynamic assessment was 108 ± 55.5 days. Baseline heart rate, mean Doppler pressure and hemoglobin were 82.7 ± 10.2, 91.3 ± 12.3, and 11.0 ± 1.9, respectively. Baseline pump speed (RPM), power (watts), pulsatility index and flow (L/min) were 9000 ± 200, 5.9 ± 0.4, 4.8 ± 0.7 and 4.7 ± 0.6, respectively. Intermittent aortic valve (AV) opening occurred in 66.7% of patients. Baseline cardiac output (CO) was 4.9 ± 1.2 L/min by thermodilution (TD CO), 5.3 ± 1.1 L/min by Fick (Fick CO) and the mean pulmonary artery saturation (PA sat) was 60.9 ± 7.3%. Pump power was strongly correlated with pump flow (p < 0.001). Fick CO and PA saturation were significantly correlated with pump flow only in patients with AV opening (Table 1). After controlling for power, there was no correlation between any hemodynamic measurement and displayed pump flow.
CONCLUSIONS: Estimated device pump flow correlates poorly to measured cardiac output in patients with HMII LVAD. Future studies are warranted to determine the clinical significance of estimated pump flow and its relationship to standard hemodynamic assessment.

OUTCOMES AFTER TRANSHIATAL AND TRANSTHORACIC ESOPHAGECTOMY FOR ESOPHAGEAL CANCER
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G.V. Aranha Department of Surgery, Loyola University Medical Center, Stritch School of Medicine, Maywood, IL.
CURRENT CATEGORY: Hematology/Oncology
ABSTRACT BODY: Background: Today, the preferred operative approach to esophageal cancer has not been fully elucidated as no single technique has been clearly shown to be superior in terms of morbidity and survival. Therefore, we aimed to investigate the peri-operative, short-term, and mid-term outcomes between transthiatal esophagectomy (THE) and transthoracic esophagectomy (TTE) at our institution.
METHODS: We conducted a retrospective review of 102 patients who had undergone esophagectomy for esophageal cancer, by 2 experienced surgeons, between March 1, 2002 and March 5, 2010. Among those patients who underwent THE or TTE we compared: a) clinical characteristics; b) pathologic findings; and c) outcomes. Parametric and non-parametric tests of significance were performed, and survival was determined by Kaplan-Meier analysis.
RESULTS: We identified 29 patients who underwent THE and 73 patients who underwent TTE. Age, gender, race/ethnicity, alcohol and tobacco use, weight loss and body mass index at the time of surgery, operative risk, chemoradiation regimen, tumor stage, and pathologic findings were similar between groups. However, survival at 1- and 3-years was significantly shorter after THE than after TTE (52% vs. 76%, p = 0.032 and 20% vs. 53%, p = 0.021), though no difference was noted at 5-years. In addition, those who underwent THE had a greater intra-operative blood loss (p = 0.002), required more intra-operative blood transfusions (< 0.0001), spent a longer time on the ventilator (p = 0.0001) and in the intensive care unit (p = 0.002), and had a higher 30-day mortality (p = 0.023). Finally, those who underwent THE also had a greater prevalence of post-operative vocal cord dysfunction (17% vs 3%, p = 0.027) and anastomotic leak (30% vs. 3%, p = 0.0001).
CONCLUSIONS: These preliminary results demonstrate a short-term survival advantage and lower morbidity of TTE as compared to THE. We speculate that the higher morbidity after THE may account for the worse outcomes associated with this approach. Future studies will be directed at subgroup analyses to account for potential selection bias and response to chemoradiotherapy.

AN ESSENTIAL ROLE FOR A PHOSPHATIDYLINOSITOL 4-KINASE IN HEPATITIS C VIRUS REPLICATION COMPLEX ASSEMBLY
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CURRENT CATEGORY: Gastroenterology/Clinical Nutrition
ABSTRACT BODY: Hepatitis C virus (HCV) chronically infects about 170 million people worldwide, and its complications account for the leading indications for liver transplantation. Many HCV-infected individuals will fail to respond to current and even next-generation HCV therapy. By understanding the host factors that support the viral life cycle, we may identify novel targets for antiviral therapy. HCV, like all positive-sense ssRNA viruses, replicates on an altered host membrane compartment that has been termed the "membranous web." However, the molecular mechanisms that support web formation are poorly understood. Whole-genome RNA screens have identified PI4KA, a phosphatidylinositol 4-kinase, as an essential host cofactor of HCV replication. Using cell culture models of HCV replication, we show PI4KA is essential for replication of an HCV replicon as well as of cell culture infectious HCV. The ability of PI4KA to support HCV replication requires its lipid kinase activity, suggesting that its product, PI 4-phosphate, also plays a role in HCV replication. Indeed, we find that the HCV membranous web is enriched in PI 4-phosphate. Using a nonreplicative model of HCV web assembly, we find that PI4KA silencing impairs formation of the HCV membranous web. Finally, we demonstrate that PI4KA associates with the HCV nonstructural protein NS5A. In summary, our data suggest a model in which recruitment of PI4KA to HCV nonstructural proteins leads to the local synthesis of PI 4-phosphate, which in turn is essential for web formation.

VALIDATION OF THE CUTANEOUS LUPUS DISEASE AREA AND SEVERITY INDEX (CLASI) FOR LUPUS
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CURRENT CATEGORY: Rheumatology/Immunology/Allergy
ABSTRACT BODY: Objective: Systemic Lupus Erythematosus (SLE) is an autoimmune disease that can affect multiple systems; one variant, Cutaneous Lupus (CL), is restricted to the skin. Recently, the Cutaneous Lupus disease Area and Severity Index (CLASI) was developed to facilitate objective quantification and assessment of cutaneous lupus. The purpose of this study was to validate CLASI against physician assessments of cutaneous or overall disease activity and damage.
METHODS: 23 consecutive SLE and CL patients seen in our lupus clinic were self-administered a demographics form after providing informed consent; the physician then completed the CLASI, and assessed disease activity (SLEDAI) and damage (SLICC/SDI) during the study visit. SLEDAI includes physician's global assessment (PGA), itemized scores and total score. SDI includes irreversible damage from disease/treatment in itemized system involvement based and a total score. CLASI provides a total activity and damage score, along with itemized activity and damage scores; higher scores indicate worse disease.
RESULTS: Mean age (SD) was 44.8 (11.8) yrs. 20/24 had SLE. Ninety six percent were women and 46% had a flare at the time of the study. Mean (median, IQR) total scores were: CLASI activity 8.9 (6.0, 7.0, 9.0), CLASI damage 10.2 (9.5, 7.0, 13.0), PGA 1.1 (0.8, 1.0, 0.5), SLEDAI 3.9 (4.0, 3.0, 2.0), SDI 1.7 (2.0, 1.0, 3.0). CLASI activity score correlated with SLEDAI rash item (r = 0.51, p = 0.01), but not with PGA (**, p = 0.002) or total SLEDAI (r **, p **). CLASI damage score correlated with SDI items on cutaneous skin scarring/alopecia (r = 0.45, p = 0.01), skin extensive scarring/panniculitis (r = 0.41, p = 0.05) and total SDI score (r = 0.64, p < 0.001). interestingly; CLASI scalp activity and CLASI scalp damage were associated (r = 0.79, p < 0.001). CLASI scalp...
activity \( (r = 0.79, p = 0.001) \) and CLASI scalp damage \( (r = 0.80, p = 0.001) \) correlated with SLE skin scarring/alopecia.

**Conclusions:** CLASI provides a better quantification and assessment of cutaneous damage than SLEDAI and SLE items. It uniquely allows simultaneous measurement of disease activity and damage within the same lesion. It has good validity against physician assessed cutaneous disease activity, cutaneous damage and overall damage in SLE.

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**106 HISTONE DEACETYLASE 11 AS A NOVEL REGULATOR OF T-CELL REACTIVITY**


**CURRENT CATEGORY:** Rheumatology/Immunology/Allergy

**ABSTRACT BODY:** Histone deacetylases (HDACs), classic epigenetic silencers, have been demonstrated to regulate a variety of immune-related targets including cytokines and transcription factors. HDACs consist of a family of 18 characterized members, grouped into four classes. HDAC inhibitors (HDIs) are a diverse family of chemical compounds known to inhibit the zinc-dependent HDACs (classes I, II, and IV). HDIs have demonstrated conflicting effects in vivo, some groups demonstrating an anti-inflammatory role, while others ascribing a more pro-inflammatory, anti-tumor effect. In light of these discrepancies, we sought to investigate potential unique roles of individual HDACs. To this end, we have recently defined a novel role for HDAC11, the newest member of the HDAC family and the sole member of Class IV, as a transcriptional repressor of IL-10 in macrophages, a potent anti-inflammatory cytokine.

In an effort to better define the physiologic role of HDAC11 in other immune cells in vivo, we analyzed T lymphocytes from HDAC11KO mice in which exon 3 of this gene has been deleted. Grossly, we did not notice any differences when comparing the proportions of CD4+ and CD8+ T-cells as well as naive and memory subsets in spleen and lymph nodes of HDAC11KO versus wild type mice. Surprisingly, upon stimulation with eCD3 and eCD28 in vitro, we noticed that T-cells lacking HDAC11 proliferate more rapidly and secrete more pro-inflammatory cytokines such as TNF-α, IFN-γ, and IL-2. In vivo, adoptive transfer of these T-cells in a murine model of bone marrow transplantation induced more severe and rapid graft versus host disease, leading to increased mortality. The apparent contradictory role of HDAC11 in macrophages and T-cells as promoting both anti- and pro-inflammatory properties, respectively, still needs to be reconciled. Nevertheless, these data demonstrate a hitherto undescribed role for HDAC11 in regulating T-cell mediated immunity.

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**107 REGULATION OF STRESS-INDUCED CHANGES IN PFC SYNAPTIC FUNCTION BY HDAC6**

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**CURRENT CATEGORY:** Molecular Medicine and Bioinformatics

**ABSTRACT BODY:** The prefrontal cortex (PFC) is responsible for high-order cognitive functions such as decision-making, attention and working memory. Our understanding of how stress and corticosteroid stress hormones affect the PFC is limited. It is critical to expand our understanding because alterations in PFC neural activity can impair the ability to cope with stressors and are strongly linked to the cognitive deficits seen in depression, schizophrenia, bipolar disorder and post-traumatic stress disorder. Recent studies in our lab have shown that acute stress potentiates the membrane trafficking of glutamate receptors and glutamatergic transmission in the PFC via a mechanism dependent on the activation of glucocorticoid receptor (GR) and the up-regulation of its downstream immediate early gene SGK (serum & glucocorticoid-inducible kinase). Since GR signaling relies on its chaperon (C/EBPα) and the activity of the p38 MAPK, whose activity is regulated via the reversible acetylation by histone deacetyase 6 (HDAC6), we examined the role of HDAC6 in regulating the effects of stress on glutamatergic transmission in PFC. We found that trichostatin A (TSA, a pan HDAC inhibitor) or tubacin (a selective HDAC6 inhibitor) blocked the corticosterone-induced enhancement of miniature excitatory postsynaptic currents (mEPSC), a response from quanta release of single glutamate vesicles, in cultured PFC neurons. Trapaconin A (TpxA), a HDAC inhibitor that does not block HDAC6, failed to block this mEPSC enhancement. Cellular knockdown of HDAC6 with RNA interference also blocked the enhancing effect of corticosterone on mEPSC, confirming the specific involvement of HDAC6. Furthermore, TSA but not TpxA blocked the acute stress-induced potentiation of AMPA-mediated synaptic transmission in PFC pyramidal neurons in vivo. In addition, we found that TSA but not TpxA blocked the up-regulation of SGK in animals exposed to acute stress. Using combined electrophysiological, biochemical and molecular approaches, we have identified HDAC6 as a key molecule regulating the effects of stress on synaptic functions in the PFC. Insight gained from this work will be valuable for developing novel and effective pharmacological interventions for stress-related disorders.

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**108 MITOCHONDRIAL SOD PROTECTS HYPEROXIA-INDUCED DYSFUNCTION OF LUNG ENDOTHELIAL**

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**CURRENT CATEGORY:** Pulmonary/Critical Care

**ABSTRACT BODY:** We have earlier demonstrated that generation of reactive oxygen species (ROS), particularly by the endothelial NADPH oxidase, plays a major role in the pathophysiology of hyperoxia-induced lung injury. Here we hypothesized that prolonged exposure of vascular endothelium to hyperoxia will alter mitochondrial electron transport leading to excess of superoxide production which in turn affects lung endothelial function.

**Methods and Results:** Exposure of human pulmonary artery endothelial cells (HPAECs) to hyperoxia (95% O2/5% CO2) resulted in a time-dependent (24, 48 and 72 h) increase in superoxide/ROS generation, as measured by HE/DCFDA oxidation, and enhanced cytokine IL-8 production. Further, the ratio of red/green fluorescence, an index of mitochondrial membrane potential, decreased after 72 h hyperoxia suggesting cell apoptosis. Over expression of adenoviral mitochondrial superoxide dismutase wild type (MnSOD, Wt), which converts superoxide to H2O2, significantly decreased hyperoxia-induced superoxide production and increased total ROS generation including hydrogen peroxide. Further, MnSOD (Wt) over expression abolished hyperoxia-induced depolarization of mitochondrial membrane potential and attenuated IL-8 secretion. Exposure of HPAECs to hyperoxia had no effect on caspase-3, caspase-9 and PARP cleavage, but increased association of Annexin Alexa 488 to plasma membrane suggesting early stage of apoptosis which were attenuated in MnSOD (Wt) infected cells.

**Conclusion:** These results suggest a role for mitochondrial superoxide in hyperoxia-induced p73phox activation and IL-8 secretion. This work was supported by NIH grant P01 HL8064 to VN.
110 SPHINGOSINE KINASE 1 DEFICIENCY PROTECTS BLEOMYCIN INDUCED MOUSE LUNG FIBROSIS

L. Huang, P. Fu, W. Ma, D. He, V. Natrajan Department of Pharmacology, The University of Illinois at Chicago, Chicago, IL; J. Garcia College of Medicine, The University of Illinois at Chicago, Chicago, IL; B. Matthew Institute for Personalized Respiratory Medicine, The University of Illinois at Chicago, Chicago, IL.

CURRENT CATEGORY: Pulmonary/Critical Care

ABSTRACT BODY: Rationale: Idiopathic pulmonary fibrosis (IPF) is characterized by alveolar epithelial cell injury, areas of type II cell hyperplasia, accumulation of fibroblasts and myofibroblasts, and the deposition of extracellular matrix proteins such as collagen and fibronectin. The cyto-kine transforming growth factor beta (TGF-beta) plays a critical role in the bleomycin induced pulmonary fibrosis through up-regulation of extracellular matrix proteins. Similar to TGF-beta, sphingosine kinase 1 (SphK1) has been shown to regulate the differentiation of human and mouse lung fibroblasts via activation of S1P receptors 2 and 3. Here, we hypothesized that SphK1 deficiency may protect bleomycin induced lung fibrosis.

Methods: Bleomycin (2 unit/Kg) in PBS was administered intratracheally to SphK1–/– mice or c57BL/6j mice. After 14 days, the mice were sacrificed, lungs were perfused and fixed for histology and immunohistochemical analysis. Expression of SphK1, alpha-SMA, collagen and fibronectin were analyzed by Western blotting, and real time RT-PCR. Reactive oxygen species, acyclic acid collagen and cytokines were quantified by commercial kits.

Results: Bleomycin challenge showed significant increase in fibrosis as measured by acid-soluble collagen as well as enhanced alpha-SMA, and fibronectin in whole lung tissue. Additionally, bleomycin stimulated TNF-alpha and TGF-beta levels in bronchialveolar lavage (BAL) fluids and expression of SphK1, but not SphK2, in lung tissue. Genetic deletion of SphK1 protected mice from bleomycin-induced fibrosis and mortality and attenuated enhanced expression of alpha-SMA, fibronectin and collagen. Mice lacking SphK1, compared to wild type, exhibited relatively lower phosphorylation of Smad2/3. In vitro, TGF-beta up-regulated expression of SphK1 in human lung fibroblasts.

Conclusion: These studies support a potential role for SphK1 in lung fibrosis and a link between TGF-beta signaling and enhanced SphK1 expression. This work was supported by R01 HL087936 to VN.

111 IRON-CONTAINING PARTICLES INDUCE GROWTH OF P. AERUGINOSA

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CURRENT CATEGORY: Infectious Disease

ABSTRACT BODY: Background: Iron is an essential for all living organisms, including bacteria. Pathogens colonizing respiratory epithelium live in an environment with very low iron content, which limits its growth and the development of infection. However, inhaled air pollution, composed of particles rich in iron, is potentially an important source iron that favors the development of respiratory infections. We set out to determine if iron-containing particles favor growth and pathogenicity of P. aeruginosa in vitro.

Methods: Iron-containing particles of various sizes, composition and iron content were used. These include alpha and gamma Fe2O3, metallic iron nanoparticles and Fly Ash. Soluble FeCl3 was used as a positive control and TiO2 particles were used as a negative control. P. aeruginosa (PA01 strain) were grown in iron-deficient media (M9 minimal media + succinate) and different iron-containing particles were then added. Growth was monitored by optical density (OD) measurement. Dissolved iron in the media was also measured using ICP-OES (inductively coupled plasma – optical emission spectrometry).

Results: Iron-containing particles increased PAO1 growth. Different particles induced different levels of growth, where smaller particles (~20 nm), such as gamma Fe2O3 and iron nanoparticles, confer a greater growth compared with larger particles. Conversely, the effects on growth did not correlate with soluble iron released in the media.

Conclusion: Iron-containing particles induce bacterial growth. This effect seemed to be dependent of particle size. This study provides evidence that inhaled particles rich in iron, are a potential source of iron for bacteria in the respiratory system.

112 ETHANOL ALTERS EXPRESSION OF CYTOCHROME P450 (CYP) ISOFORMS IN PODOCYTES

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CURRENT CATEGORY: Nephrology

ABSTRACT BODY: Moderate ethanol intake is associated with salutary effects on cardiovascular function, as well as unwanted effects such as hyper-tension when taken in higher doses. Likewise, 20-hydroxyeicosatetraenoic acid (20-HETE) has both anti- and pro-hypertensive effects. The effect of ethanol on podocyte expression of the CYP isoforms that make 20-HETE, namely CYP4a and 4f isoforms, has not been described. We incubated immortalized podocytes with ethanol (1, 2.5, 10 and 20 µl/ml) for 1, 8 or 24 hrs. We then examined expression of CYP4a12a, CYP4a12b and CYP4f13 using quantita-tive RT-PCR. Results were expressed as fold change over appropriate time controls. In preliminary studies we examined the effect of ethanol on podocyte cytoskeleton using fluorescence microscopy following actin staining. Ethanol (2, 5, 10 and 20 µl/ml) significantly increased expression of CYP4a12a in podocytes at 8 hr (P<0.05 vs control). The highest concentration of ethanol (20 µl/ml) caused a significant decrease in expression of CYP4a12a at 24 hr (P<0.02 vs control). Expression returned to baseline values by 24 hr at other concentrations. A similar pattern was seen in expression of CYP4a12b in podocytes, namely increased expression at 8 hr (5 and 10 µl/ml, P<0.01 vs control) and suppression by 20 µl/ml at 24 hr (P<0.001 vs control). Ethanol (20 µl/ml) significantly increased expression of CYP4f13 in podocytes with a 2-fold increase being seen at 24 hr, though this did not reach statistical significance. Fluores-cence microscopy showed marked derangement of the actin cytoskeleton after ethanol treatment (10 and 20 µl/ml) for 24 hr. In summary, we have shown that ethanol in meaningful concentrations decreases podocyte expression of CYP450 isoforms capable of 20-HETE synthesis, namely CYP4a and 4f. We hypothesize that ethanol alters eicosanoid metabolism in glomerular cells, and that this effect varies with levels of ethanol exposure. The long-term effects of chronic ethanol exposure on podocytes is unknown. The effect of ethanol on eicosanoid metabolism in renal cells may in part explain the cardiovascular impact seen in humans.
Pelvic Pain disorders, e.g. cystitis. Colitis induced in rats by trimethobenzene-sulfonic acid (TNBS), causes sensitization of the urinary bladder as evidenced by pathologic functional changes. This study examined the role of mast cell recruitment and/or activation and mast cell secretory products acting on the PAR-2 receptor (PAR-2) in this type of cross-sensitization in rats and mice.

**Methods:** 7-12 days after intraocular application of TNBS in female rats, functional pathologic changes including changes in voiding interval in met- abolic cages, urethelial permeability and afferent nerve firing were measured. To determine the role of mast cells, voiding interval and urethelial perme- ability were also measured in a mast cell deficient mouse model and in rats that were fed a mast cell stabilizing agent (ketotifen 10 mg/kg/day for 5 days in the drinking water). PAR-2 expression in TNBS rats was measured by immunohistochemistry and whole bladder PAR-2 mRNA levels. Functional interactions of mast cells with PAR-2 were determined by measuring bladder strip contractility in response to the PAR-2 agonist, SLIGRL (100μM), compound 48/80 (50μg/ml), a mast cell degranulating agent, and subse- quent 48/80 responses after PAR-2 desensitization. Voiding interval was also examined in a separate group of TNBS mice that were injected in- traperitoneally with either control liposomes or liposomes containing PAR-2 siRNA

**Results:** In TNBS treated rats, voiding intervals decreased from 287 ± 41 seconds in controls to 181 ± 8 seconds in TNBS (p<0.015), and afferent nerve firing induced by capsaicin or urinary bladder distension was enhanced 2-3 fold (p<0.05). Voiding interval normalized in both the mast cell deficient mouse and in rats pre-treated with Ketotetin. Ketotetin did not reverse urinary bladder mastocytosis but normalized urethelial permeability measured by fluorescein uptake which increased from 0.99 ± 0.22 μg/ml of plasma in controls to 3.01 ± 0.64 μg/ml in TNBS (p<0.012). In TNBS, PAR-2 immu- noreactivity increased in the urethrole, whole bladder mRNA increased by 60 percent, and the facilitatory effect of a PAR-2 agonist (SLIGRL) on uri- nary bladdr capsaicin-sensitive afferents significantly increased two fold. Bladder strip contractility induced by SLIGRL (100 μM) (p<0.004) and 48/80 (50μg/ml) (p<0.047) were significantly enhanced in TNBS, respectively. PAR-2 desensitization with prolonged SLIGRL application normalized bladder strip contractivity induced by 48/80 in TNBS rats. Mice treated with TNBS and control liposomes had a >40% decrease in bladder voiding interval, while mice treated with liposome-containing PAR-2 siRNA had normal voiding interval.

**Conclusions:** These data indicate that urinary bladder hyperactivity in the TNBS-induced, cross-sensitization model is mediated in part by increased release of inflammatory mediators from mast cells that activate PAR-2 and enhance sensory mechanisms in the urinary bladder.

**115 GAIN OF EXPRESSION OF ALDO-KETO REDUCTASE FAMILY 1 B10 (AKR1B10) PROTEIN IN PanCREATIC ADENOCARCINOMA: AN EARLY EVENT INVOLVED IN PanCREATIC CARCINOGENESIS AND A POTENTIAL THERAPEUTIC TARGET**

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**CURRENT CATEGORY:** Mechanisms of Disease

**ABSTRACT BODY:** Background: Aldo-keto reductase family 1 B10 (AKR1B10) protein acts as an enzyme capable of reducing aliphatic alde- hydes and ketones; it detoxifies reactive free radical carbonyl compounds and is involved in retinoid metabolism, thus modulating cell proliferation, differentiation and tumorgenesis. Expression of AKR1B10 protein is norm- ally present in the intestine and found to be expressed in liver and lung carcinomas. Several studies have identified upregulation of AKR1B10 in the normal respiratory epithelium of smokers, suggesting cigarette smoking as an inciting event. Protein prenylation is a process of lipid modification which involves the covalent addition of either farnesyl or geranylgeranyl isoprenoids. Prenylation appears to be a crucial event in carcinogenesis involved in cell signal transduction, cell proliferation and apoptosis. Proteins that undergo prenylation include Ras and Ras-related GTP-binding proteins. Up to as many as 15% of all human cancers harbor Ras mutations. In contrast, more than 95% of human pancreatic cancers carry a Kras gene mutation; the Kras protein requires prenylation for its activity. The development of pancreatic cancer is firmly linked to cigarette smoking and many reports have demonstrated the central role of Ras activation in pancreatic can- cer, yet the role of AKR1B10 expression or protein prenylation in pancre- atic cancer have not been studied. Thus, the aim of this study is to identify a molecular target with diagnostic and therapeutic values for pancreatic adenocarcinoma.

**Design:** To investigate the application of AKR1B10 as a clinical marker in pancreatic adenocarcinoma, we determined the expression of AKR1B10 at the transcript and protein levels using cultured cells and formalin fixed, paraffin-embedded human pancreatic adenocarcinoma samples. In addition, to more accurately determine the role of AKR1B10 in pancreatic cancer we performed protein prenylation, siRNA knock-down studies and enzyme activity assays.

**Results:** We investigated the expression levels of AKR1B10 by qPCR in cultured human pancreatic adenocarcinoma cell lines and compared to a normal pancreatic ductal epithelial cell line and a positive control hepatocel- lular carcinoma line. Eight pancreatic cell lines (8/12; 66.7%) displayed significant over-expression of AKR1B10; three of the cell lines exhibited 3-10 fold higher expression of AKR1B10 when compared to the positive control cell line. AKR1B10 expression was co-expressed with estrogen receptor staining in 35/50 (70%) of carcinomas and 60% of patients with AKR1B10 over-expression were smokers. AKR1B10 is over-expressed in well-differentiated carci- nomas, but not in adjacent normal ductal epithelial cells or normal acinar cells. Further analysis showed that pancreatic intraepithelial neoplasia (PanIN) also over-expressed AKR1B10. Silencing of AKR1B10 dynamic expression in a human pancreatic adenocarcinoma cell line demonstrated increased apoptosis-related cleaved caspase-8 and non-farnesylated protein. A decrease
of enzyme activity that paralleled AKR1B10 expression levels was observed in silna treated cells.

Conclusion: The over-expression of AKR1B10 in PanIN and carcinoma demonstrate that AKR1B10 upregulation is an early event in pancreatic carcinogenesis. AKR1B10 may contribute to disease development via effects of smoking by dysregulated apoptosis and cellular proliferation, and should be considered a diagnostic marker and candidate for further therapeutic investigations.

116 ASSOCIATION OF SOCIAL SUPPORT AND MORTALITY IN AFRICAN AMERICANS WITH CHRONIC KIDNEY DISEASE


CURRENT CATEGORY: Nephrology

ABSTRACT BODY: Previous studies have shown that end-stage renal disease (ESRD) patients with poor social support have higher mortality than those with good social support. The association between social support and mortality of end-stage renal chronic kidney disease (CKD) has not been studied. We hypothesized that CKD patients with poor social support would have higher mortality than those with good social support. To examine this, we conducted an analysis of social support in participants of the African American Study of Kidney Disease and Hypertension (AASK) Cohort Study, which included 691 African American patients with hypertensive CKD. The Interpersonal Support Evaluation List-16 (ISEL-16) was used to measure social support. Social support scores were available for 659 participants. Cohort mean ISEL-16 score was 36 (the maximum score is 48). The relative risk of all-cause mortality was assessed for participants with ISEL-16 scores below the mean using participants with ISEL-16 scores above the mean as the referent category. After adjustment for age, gender, estimated GFR, and proteinuria, the relative risk of mortality was 1.52 (95% confidence interval 1.01-2.30, p=0.046).

In conclusion, low social support was independently associated with increased all-cause mortality in African Americans with hypertensive CKD. Future work should focus on possible mechanisms for this association and ways to improve social support. (No Table Selected)

ABSTRACT FINAL ID: 117

118 SERUM FREE LIGHT CHAINS, INTERFERON-α AND INTERLEUKINS AS BIOMARKERS OF DISEASE ACTIVITY IN SYSTEMIC LUPUS ERYTHEMATOSUS

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CURRENT CATEGORY: Rheumatology/Immunology/Allergy

ABSTRACT BODY: Objective: Interleukin-6 (IL-6), Interleukin-10 (IL-10) and Interferon-α (IFN-α) are associated with etio-pathogenesis of Systemic Lupus Erythematosus (SLE). B cell activity and antibodies production is the hallmark of SLE. Herein, we compare the utility of serum Free light chains (FLC), a marker of B cell activity, with IL-6, IL-10 and IFN-α as a biomarker of disease activity in SLE.

Methods: 134 SLE patients underwent assessments of disease activity using Physician Global Assessment (PGA) and SLE disease Activity Index (SLEDAI). Serum total FLC, IL-6, IL-10 and IFN-α were quantitated. Demographics, clinical-serological characteristics and medications data were collected by medical chart reviews. Statistical analysis included correlation analysis and Mann Whitney test. Adjusted SLEDAI was obtained by deleting DsDNA and complement items from total SLEDAI score.

Results: Mean age of the patients was 43±12 yrs; PGA (mean: SD) was 0.5±0.6, while SLEDAI was 3±3.6, 2.0. The (mean: SD) values were: Total FLC mg/L (46.5±22.8), IL-6 pg/ml (6.2±6.8), IL-10 pg/ml (14.1±21.1) and IFN-α activity (14.3±22.6). Total FLC correlated with PGA (r=0.29, p=0.001), and adjusted SLEDAI (r=0.29, p=0.001). IL-10, IL-6 and IFN-α correlated with adjusted SLEDAI (r=0.23, p=0.008), (r=0.20, p=0.02) and (r=0.19, p=0.02) respectively.

Conclusion: Serum total FLC correlated with PGA and adjusted SLEDAI. Strength of total FLC association with adjusted SLEDAI is greater than observed with IL-6, IL-10 or IFN-α. Longitudinal studies to determine if changes in disease activity result in changes in total FLC are indicated.

ABSTRACT BODY: Background: Pulmonary disease is the major cause of morbidity and mortality in scleroderma, and pulmonary vascular disease is the most injurious of the lung disease processes. A major feature of pulmonary vascular disease is gas exchange abnormality, especially with exertion. Telangiectasias are abnormally dilated capillaries, which bypass the normal capillary bed, where substances are exchanged with tissues. Telangiectasias are often detected on the skin of patients with scleroderma, but can be found in internal organs including the lung and pleural surface. In the lung, these arteriovenous bypasses can contribute to hypoxia. We hypothesized that the amount of skin telangiectasias would correlate with markers of hypoxia especially with exertion. The primary endpoint was desaturation on six minute walk test; other markers of hypoxia and lung disease were secondary endpoints.

Methods: We prospectively studied 51 patients with scleroderma and scored the presence of telangiectasias on a scale we developed. It is as follows: 0 for no telangiectasias, 1 for less than 10 telangiectasias, 2 for more than 10 telangiectasias confined to one surface area, 3 for more than 10 telangiectasias involving two surface areas, 4 for more than 10 telangiectasias involving more than two surface areas, 5 for more than 10 telangiectasias involving more than two surface areas and confluent telangiectasias. This score was correlated with the variables of gas exchange including diffusing capacity, oxygen saturation, and oxygen saturation with exercise. Other factors
measured included gender, age, subtype of scleroderma, duration of the disease, Raynaud's extent, severity and duration, presence of digital pitting scars, loss of digital pad tissue, medications, alcohol intake, modified Rodnan skin score, severity of sclerodactyly, and other pulmonary functions.

**Summary of Results:** We found no significant correlation between telangiectasia score and diffusing capacity, oxygen saturation, and oxygen saturation with exercise. A negative relationship was noted with forced expiratory volume in one second (FEV1). Telangiectasia score did positively correlate with age, Raynaud's duration, scleroderma duration, and anticytomegaly antibodies.

**Conclusions:** The number of telangiectasias on exam did not correlate with measures of pulmonary disease but did correlate with other clinically important factors. A significant relationship between telangiectasias and pulmonary disease may not have been seen secondary to the small number of patients enrolled in our study.

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**120 BALANCING MEDICAL INFORMATICS AND MEDICAL "IGNORAMICS": CURRICULUM, ASSESSMENT, AND THE VIRTUAL CLINICAL RESEARCH CENTER/QUESTIONARIUM**

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**CURRENT CATEGORY:** Molecular Medicine and Bioinformatics

**ABSTRACT BODY:**

**Objectives:**

1. Develop and assess a summer medical research training curriculum to educate student researchers about the expanding “Unknowns” (unanswered questions and unquestioned answers) as well as the exploding “Knowns” (current information, facts/data) in medical practice and research;

2. Design a virtual space to deliver this curriculum balancing medical ignoramics and medical informatics.

**Methods:**

**Subjects:** Medical students (n=734) (1981-2010) and disadvantaged high school students (n=474) (1987-2010) conducting research in the University of Arizona and Midwestern Regional Programs.

1. Student activities included summer basic, translational, and clinical biomedical research accompanied by seminars and novel question answering activities designed to stimulate basic, clinical and societal questions about their particular research and more generally. Finally, students prepared oral and written research reports which were framed by beginning and ending questions. Various instruments assessed their progress during the summer with long-term followup.

2. Several designs for the virtual space were produced and tested for user friendliness, functionality, and efficacy in transferring the face-to-face curriculum to a more versatile and transportable online format.

**Results:**

1. Subjective surveys indicated that students gained an appreciation of ignorance and the unknown, and feedback identified the “single most important things learned” were overcoming reluctance to ask questions, recognition that teachers and researchers don’t have all the answers, and that asking questions is important in school, science, and everyday life and even enjoyable. Long-term followup showed sustained career impact of the questioning approach on medical students as well as disadvantaged high school students, a remarkable number of whom subsequently entered medical school and graduate biomedical science programs, many attributing their interest and motivation to their summer experience in medical ignoramics.

2. A Virtual Clinical Research Center/Questionarium (VCRC/Q) was created that is scalable, adaptable, and contemporary in appearance. Research presentations (unknowns and knowns) and student questions saved to a database are featured along with functionalities that parallel effectively the original summer research training curriculum.

**Significance:** The Curriculum on Medical Ignorance and the VCRC/Q, which balance Ignoramics and Informatics, can be used to introduce research trainees at all levels to “what we know we don’t know, don’t know we don’t know, and think we know but don’t.” This curriculum fills in a major gap in medical education and yet represents the endless frontier of science and the thrill of discovery.

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**121 EXOGENOUS TESTOSTERONE OR ARIMIDEX AND OSTEONECROSIS OF HIPS-JAW, PULMONARY EMBOLISM, AND AMAUROSIS FUGAX IN MEN WITH PREVIOUSLY UNDIAGNOSED FAMILIAL THROMBOPHILIA**

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**CURRENT CATEGORY:** Endocrinology/Metabolism

**ABSTRACT BODY:** Our specific aim was to describe thrombosis (osteonecrosis of the hips and jaw, pulmonary embolism, amaurosis fugax) after exogenous testosterone or Arimidex were given to men with no antecedent thrombosis and previously undiagnosed familial thrombophilia. Two men developed bilateral hip osteonecrosis and 1 pulmonary embolism, 6, 5, and 7 months after starting testosterone gel, 50 mg/d or intramuscular testosterone 400 mg/d. One man developed amaurosis fugax 18 months after starting testosterone gel, 50 mg/d. One man developed osteonecrosis of the jaw 6 months after being given Arimidex, 1 mg/d. Of these 5 men, four were found to have previously undiagnosed thrombophilic Factor V Leiden heterozygosity, two of whom had MTHFR C677T homozygosity, and two with MTHFR C677T-A129C compound heterozygosity. One man had thrombophilic high Factor VIII (195%), high Factor XI (179%), and high homocysteine (29.3 umol/L). We speculate that when exogenous testosterone is aromatized to estradiol (E2), and E2 (or Arimidex) -induced thrombophilia is superimposed on familial thrombophilia, thrombosis occurs. Testosterone or Arimidex should be given cautiously or not at all to men with known thrombophilia. Screening for the Factor V Leiden and MTHFR mutations, Factors VIII and XI, and homocysteine should be considered before giving testosterone or Arimidex to men.
Our data indicate that volume-induced hypertension increases BBB permeability in the striatum of PE rats and that urinary excretion of MBG is increased before the onset of hypertension and proteinuria. Angiogenic imbalance occurs at the onset of hypertension and proteinuria and may correlate to extent of vascular injury progression. MBG induced an increase in monolayer of HBMEC permeability within 6 hours (1.5 fold). MBG caused a significant decrease in the phosphorylation of ERK1/2 and activated the phosphorylation of Jnk, p38, and Sre at 10-60 min. MBG significantly increased the expression of caspases 3/7, indicating the activation of apoptosis (1.5 fold). Apoptotic signaling was not observed in MBG treated cells that were pretreated with a p38 inhibitor, as evaluated by annexin-V staining. MBG causes the disruption of endothelial adherent tight junction proteins. The urinary excretion of MBG is higher in PE patients compared to normal pregnant patients. An angiogenic imbalance was observed in PE patients compared to normal pregnancy.

Conclusion: We propose the novel hypotheses that MBG precedes PE; MBG causes the disruption of tight junction proteins leading to BBB hyperpermeability via activation MAPK which triggers apoptotic mechanisms resulting in further disruption of the BBB leading to cerebral edema that is a common feature of PE; and angiogenic factors released in this process serve as biomarkers of the extent of vascular imbalance. This study provides new evidence about the role of MBG in the pathogenesis of the neurologic sequelae to PE and may reveal new therapeutic targets for the prevention of neurologic symptoms which are among the most severe PE sequelae.

123 CRITICAL ROLE OF SPHINGOLIDIP PATHWAY COMPONENTS IN MURINE RADIATION-INDUCED LUNG INJURY: PROTECTION BY SPHINGOSINE 1 PHOSPHATE ANALOGUES


CURRENT CATEGORY: Disease Modulation/Intervention

ABSTRACT BODY: Clinically significant radiation-induced lung injury (RILI) is associated with significant morbidity and mortality and a common toxicity in patients administered thoracic radiotherapy. While the molecular etiology of RILI is poorly understood, we previously characterized a murine model of RILI in which alterations in lung endothelial barrier integrity surfaced as a potentially important pathobiologic event in these studies. inhibition of HMGI-CoA reductase activity (simvastatin) reduced murine RILI-associated lung inflammation and vascular leak and attenuated radiation-induced dysregulation of sphingolipid metabolic pathway genes identified by genome-wide lung gene expression profiling. In the present study we test the hypothesis that sphingolipid signaling components serve as important modulators of RILI pathobiology and novel therapeutic targets. Sphingolipid involvement in murine RILI was confirmed by radiation-induced increases in lung expression of sphingosine kinase (Sphk) isoforms 1 and 2 and increases in the ratio of ceramide to cumulative sphingosine 1-phosphate (S1P) and dihydrosphingosine (DHSP) levels in plasma, bronchoalveolar lavage (BAL) fluid and lung tissue following 25 Gy exposure (6 weeks). Moreover, genetically engineered mice with either targeted deletion of Sphk1 (Sphk1-/-), or with reduced expression of selective members of the S1P receptor family (S1PR1-/-, S1PR2-/-, S1PR3-/-), exhibited marked susceptibility to RILI-mediated lung inflammation. Finally, we assessed the efficacy of three potential vascular barrier-protective S1P analogues FTY720 (FTY), fTYsiperonate (fTys), and SEW2871 (SEW) in attenuating indices of RILI. The phosphonate analogue, fTys, and to a lesser degree SEW, significantly attenuated the extent of RILI and RILI-induced gene dysregulation compared to control RILI-challenged mice (6 weeks). In contrast, FTY failed to significantly alter physiologic or genomic changes compared to RILI-challenged controls. Together, these results support the targeting of sphingolipid components as a novel and effective therapeutic strategy in RILI.

124 TRANSCRIPTION FACTOR STAT3 IS INVOLVED IN THE REGULATION OF LIPOPOLYSACCHARIDE-INDUCED SPHINGOSINE-1-PHOSPHATE LYASE EXPRESSION IN LUNG ENDOTHELIUM

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CURRENT CATEGORY: Pulmonary/Critical Care

ABSTRACT BODY: Background: Sphingosine-1-phosphate lyase (SGPL1) is a membrane associated enzyme that belongs to the pyridoxal-dependent decarboxylase family. This enzyme irreversibly degrades S1P, a naturally occurring bioactive sphingolipid metabolite, to hexadecenal and phosphoethanolamine in the terminal step of S1P catabolism. Recent evidence suggests a role for SGPL1 in lipopolysacharide (LPS)- and cecal puncture ligation-induced lung injury. This study provides insights into the regulation of Sgpl1 in human lung microvascular endothelial cells (HLMVECs) and a murine model of LPS-induced acute lung injury (ALI).

Methods/Results: The expression of Sgpl1 is increased at both the mRNA and protein levels in the mouse lung in a murine model of sepsis induced by either intraperitoneal injection (15mg/kg, 48 h) or intratracheal instillation (5mg/kg, 24 h) of LPS. In vitro, challenge of HLMVECs to LPS (100 ng/ml) up-regulated Sgpl1 expression as evidenced by Western blotting, real time RT-PCR and luciferase reporter assay (using a Sgpl promoter construct). Treatment of cells with actinomycin D had no effect on Sgpl1 mRNA degradation, and decreased the radiation rate either in the absence or presence of LPS. The proximal promoter of Sgpl1 is located approximately 250 bp upstream of the transcript start site as evidenced by luciferase reporter assay using serially deleted Sgpl1 promoter constructs. LPS stimulated phosphorylation of STAT3, but not other members of STAT family proteins. Knocking down of STAT3 by siRNA did not affect the basal expression of Sgpl1 but abolished the LPS-induced up-regulation of Sgpl1 by luciferase reporter assay and Western blotting.

Conclusion: These results provide evidence for the first time that increased expression of Sgpl1 by LPS is regulated by transcription factor STAT3, but not other members of STAT family proteins.

125 THE SHORT TERM EFFECTS OF OMEGA-3 POLYUNSATURATED FATTY ACIDS ON HEART RATE VARIABILITY IN A CONTROL POPULATION

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CURRENT CATEGORY: Cardiology/Cardiovascular Disease

ABSTRACT BODY: Background: The use of omega-3 polyunsaturated fatty acids (n-3 PUFA) has been shown to decrease rates of sudden cardiac death (SCD) in patients with congestive heart failure (CHF) and most significantly in the first three to four months post-myocardial infarction (MI). Improved heart rate variability (HRV) indices have been shown to correlate to the quantity of n-3 PUFA consumption. We studied the short term effects of n-3 PUFA on HRV in a control population.

Methods: In this prospective, double blinded, placebo controlled trial we studied 46 patients randomized to either 3.0 grams or 1.5 grams of Omegabrite fish oil or placebo. HRV recordings were obtained at baseline and then monthly for a period of five months. Analysis of variance for repeated measures was used to compare HRV over the five visits.

Results: 1.5 gram Omegabrite showed no improvements in HRV indices compared to placebo. 3.0 gram Omegabrite had the greatest effects compared to placebo in the time period from baseline to three months as measured by total HRV (p<0.001) and low frequency HRV (p=0.008), which is related to sympathetic activation. These effects are diminished by five months. Near significant increases in high frequency HRV, which is reflective of parasympathetic activity, were seen at five months (p=0.06).

Conclusion: Our data suggest that the reduction in sudden cardiac death seen in CHF and post-MI patients taking n-3 PUFA may be due in part to beneficial changes in autonomic balance. While this study is underpowered to show significant differences in the treatment groups, trends suggest that the
early effects of n-3 PUFA on SCD in post-MI patients may be related to initial, and perhaps transient, improvements in the total heart rate variability and decreases in sympathetic activity as reflected by the increase in high frequency HRV at five months. Our data further shows that these benefits are likely dose dependent and future studies in patients with cardiovascular disease must take into account this apparent dose response relationship.

126 NUCLEAR LOCALIZATION OF TGF BETA RECEPTORS IN MESENCHYMAL DERIVED CELLS FROM THE LUNG

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CURRENT CATEGORY: Pulmonary/Critical Care

ABSTRACT BODY: Transforming growth factor beta (TGBF) is an important mediator of the repair process of tissue after injury. TGFβ has pleiotropic effects including regulation of cell differentiation, proliferation, apoptosis and migration. The lack of control of TGFβ action is thought to play a key role in lung remodeling observed in asthma and in other respiratory and non-respiratory disorders. TGFβ effects are mediated by its binding to the type I (TβRI) and type II (TβRII) serine/threonine receptors that localize on the cell membrane. Upon ligand binding, the kinase function of TβRI and TβRII causes phosphorylation and activation of the Smad2 and Smad3 signaling transducers. Activated Smad2 and Smad3 interact in the cytoplasm with Smad4, and this complex enters the nucleus to transactivate TGFβ responsive genes, frequently in association with co-activators or corepressors. Using confocal microscopy, we showed that in addition to their localization at the cell membrane, the TβRI and TβRII are also found in the nucleus of airway smooth muscle (ASM) cells from normal donors. We hypothesized that additional cell types of the lung also exhibit nuclear localization of TβRs, including parenchymal fibroblasts from asthmatics, and that TβRs nuclear import into the nucleus is mediated by their association with Smad2/3. To test these hypotheses, we prepared nuclear (NE) and cytosolic (CE) extracts from cultured human ASM cells and from cultured lung parenchyma fibroblasts from asthmatics and non-asthmatic donors. We examined the localization of TβRI, TβRII and Smad1/2/3 by western analysis. We found that while TβRI is present mainly in the CE of asthmatics mesenchymal derived cells, TβRII is localized in both the cytosolic and the nuclear compartments. To analyze the potential association of TβRs with Smad proteins inside the nucleus, we transiently transfected HEK293T cells with TβRI, TβRII and Smad 3 expression vectors and we performed immunoprecipitation using equal amount of NE or CE protein. Extracts were assessed for phosphorylation of type I (TβRI) and type II (TβRII) serine/threonine receptors. We observed a significant increase of the binding to the type I (TβRI) and type II (TβRII) serine/threonine receptors. In the absence of heparin, TβRII is also found in the nuclear compartments. To analyze the potential association of TβRs with Smad proteins inside the nucleus, we transiently transfected HEK293T cells with TβRI, TβRII and Smad 3 expression vectors and we performed immunoprecipitation using equal amount of NE or CE protein. Extracts were assessed for phosphorylation of type I (TβRI) and type II (TβRII) serine/threonine receptors. We observed a significant increase of the binding to the type I (TβRI) and type II (TβRII) serine/threonine receptors.