necrosis factor α and interleukin-1β generation of ROS and subsequent activation of the transcription factor NF-κB. We propose that ClC-3 functions as a chloride-proton exchanger and thereby influences ROS production via charge neutralization of the electron flow generated by Nox1 in the endosome. These findings identify ClC-3 as a critical component of the signaling endosome and a novel intermediate in redox-dependent control of gene expression.

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A NOVEL MYOSIN LIGHT CHAIN KINASE INHIBITOR, PIK, PROTECTS FROM LIPOPOLYSACCHARIDE-INDUCED ACUTE LUNG INJURY. T. Mirzapoiazova, S. Sammani, L. Moreno, S.M. Dudek, J.R. Turner, J.G. Garcia, University of Chicago, Chicago, IL.

Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are major causes of acute respiratory failure that are associated with high morbidity and mortality. These disorders are characterized by a significant pulmonary inflammatory response resulting in injury to alveolar epithelial and endothelial barriers followed by pulmonary edema. Our prior studies demonstrated that myosin light chain kinase phosphorylation of myosin light chains (MLC) is associated with increases in both epithelial and endothelial barrier permeability. We previously have identified a novel oligopeptide, PIK, that inhibits MLC kinase in vitro, is membrane permeant, decreases intracellular MLC phosphorylation, and causes increased intestinal epithelial cell barrier function (Turner et al. Gastroenterology 2002). We hypothesized that PIK-mediated inhibition of MLC kinase activity would attenuate inflammation and vascular leak associated with acute lung injury. To test this hypothesis, we used a murine acute lung injury model with intratracheal administration of endotoxin/lipopolysaccharide (LPS, 2.5 mg/kg). Optimal PIK responses were observed at 125 μM PIK when tested over a range of PIK concentrations. PIK administered intravenously simultaneously with LPS resulted in significantly attenuated lung inflammation reflected by decreasing accumulation of bronchoalveolar lavage (BAL) proteins (25% reduction, $p < .02$) and BAL cells (25% reduction, $p < .05$). IV administration of PIK decreased LPS-induced tissue MPO activity (a reflection of leukocytes in lung tissue) (15% reduction) and LPS-mediated MLC phosphorylation in lung homogenates (25% reduction). Our data suggest that PIK stabilizes epithelial-endothelial permeability and has significant therapeutic potential in ALI.

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CARDIOVASCULAR EVENTS ≤ AGE 45: ATHEROTHROMBOSIS. J. Munjal, G.J. Charles, D.M. Aregawi, P. Wang, The Jewish Hospital, Cincinnati, OH.

The population that develops occlusive arterial circulatory events at a young age (≤ 45 years), particularly if they are normolipidemic, represents a rare population in which a heightened hemostatic system may play an important mechanistic role in thrombus formation and in arterial events. We assessed whether thrombophilia-hypofibrinolysis contributed to premature atherothrombotic cardiovascular disease (ATCVD) in 78 men and 40 women with 230 ATCVD events (≤ age 45 years). ATCVD events included ≥ 1 myocardial infarction ($n = 60$), CABG ($n = 33$), angioplasty ($n = 52$), chronic angina ($n = 41$), ischemic stroke ($n = 11$), TIA ($n = 24$), and claudication ($n = 9$). Hereditary thrombophilia was assessed: G1691A factor V Leiden, G20210A prothrombin, MTHFR C677T-A1298C, and platelet glycoprotein PL A1/A2 mutations, with serologic measures of ACLA IgG and IgM, lupus anticoagulant, proteins C and S, antithrombin III, homocysteine, and factors VIII and XI. Hypofibrinolysis was assessed: 4G4G plasminogen activator inhibitor 1 mutation (PAI), PAI activity (PAI-Fx), and Lp(a). Cases were compared with healthy normal controls (149 men for PCR, 40 men for serologic tests, 109 women for PCR and serologic tests). Cases did not differ from controls by race or age ($p > .05$) Hypertension was present in 44% of men and 25% of women, diabetes in 17% and 8%, and cigarette smoking in 25% and 20%. In the 78 male cases, mean ± SD age was 48 ± 11 years, BMI 29.5 ± 4.5, LDLC 103 ± 44, HDLC 41 ± 14, and TG 195 ± 219 mg/dL. In 40 female cases, mean ± SD age was 38 ± 10 years, BMI 28.3 ± 7.7, LDLC 105 ± 31, HDLC 50 ± 13, and TG 147 ± 121 mg/dL. High factor VIII (> 150%) was present in 15 of 59 (25%) male cases versus 1 of 38 (3%) male controls, $p = .003$. High factor XI (> 150%) was present in 9 of 56 (16%) male cases versus 0 of 38 (0%) male controls, $p = .01$. High PAI-Fx (> 21.1 U/mL) was present in 24% of male cases (15 of 63) versus 8% (3 of 39) of male controls, $p = .038$. Low protein C (< 73%) was present in 4 of 25 (16%) female cases versus 2 of 107 (2%) female controls, $p = .012$. Low free protein S (< 66%) was present in 5 of 27 (19%) female cases versus 3 of 107 (3%) female controls, $p = .009$. High factor XI (> 150%) was present in 3 of 26 (12%) female cases versus 2 of 107 (2%) female controls, $p = .051$. The lupus anticoagulant was present in 9 of 37 (24%) female cases versus 6 of 84 (7%) female controls, $p = .014$. In patients with ATCVD ≤ age 45, thrombophilias (factor VIII, factor XI, protein C and S deficiency, and the lupus anticoagulant) and hypofibrinolysis (PAI-Fx) may promote arterial thrombosis, synergistic with atherosclerotic endothelial injury. If thrombophilia-hypofibrinolysis accompanies ATCVD at ≤ age 45, thromboprophylaxis may have value in secondary prevention of subsequent ATCVD.

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MYOCARDIAL INFARCTION-ANGIOPLASTY-CORONARY ARTERY BYPASS GRAFTS ≤ AGE 45: ATHEROTHROMBOSIS. J. Munjal, C.J. Glueck, D.M. Aregawi, P. Wang, The Jewish Hospital, Cincinnati, OH.

Although heritable thrombophilias and hypofibrinolysis predominantly cause venous thrombosis, they may also promote arterial thrombosis, synergistic with atherosclerosis, producing atherothrombotic cardiovascular (ATCVD) events. In 70 men and 16 women with ≥ 1 premature myocardial infarction ($n = 60$)-angioplasty ($n = 52$)-coronary artery bypass grafts ($n = 33$) (≤ age 45 years), we assessed whether and to what degree heritable thrombophilia-hypofibrinolysis contributed to 145 ATCVD events ≤ age 45. Hereditary thrombophilias studied by PCR included the G1691A factor V Leiden, G20210A prothrombin, MTHFR C677T-A1298C, and platelet glycoprotein PL A1/A2 mutations, with serologic studies of ACLA IgG and IgM, the lupus anticoagulant, proteins C and S, antithrombin III, homocysteine, and factors VIII and XI. Hypofibrinolysis studies included the 4G4G plasminogen activator inhibitor 1 mutation (PAI), PAI activity (PAI-Fx), and Lp(a). Cases were compared with healthy normal controls (149 men for PCR, 40 for serologic tests, 109 women for PCR and for serologic tests). At entry, hypertension was present in 46% of men and 31% of women, diabetes in 19% and 13%, and cigarette smoking in 26% and 13%, respectively. In the 70 male cases, mean ± SD age was 49 ± 11, BMI 29.5 ± 4.2, LDLC 102 ± 45, HDLC 41 ± 15, and TG 204 ± 229 mg/dL. In 16 female cases, mean ± SD age was 43 ± 7, BMI 28.1 ± 5.5, LDLC 103 ± 32, HDLC 45 ± 11, and TG 191 ± 170 mg/dL. Male cases were more likely than male controls to have factor V Leiden (6 of 60, 10% vs 4 of 149, 3%, $p = .035$), high Lp(a) (≥ 35 mg/dL) (25 of 69, 36% vs 7 of 40, 18%, $p = .039$), high PAI-Fx (> 21.1 U/mL) (15 of 61, 25% vs 3 of 39, 8%, $p = .036$), high (> 150%) factor VIII (15 of 57, 26% vs 1 of 38, 3%, $p = .003$), and high (> 150%) factor XI (9 of 55, 16% vs 0 of 38, 0%, $p = .0096$). Female cases were more likely then female controls to have high factor VIII (5 of 14, 36% vs 13 of 109, 12%, $p = .033$) and were more likely to have low free protein S (3 of 14, 21% vs 3 of 107, 3%, $p = .02$). In patients sustaining MI-angioplasty-CABG events ≤ age 45, we speculate that hereditary thrombophilias (factor V Leiden, factor VIII, factor XI, low free protein S) and hypofibrinolysis (PAI-Fx, Lp(a)) promote arterial thrombosis that may be synergistic with atherosclerotic endothelial injury. In patients with MI-angioplasty-CABG events ≤ age 45 and concurrent hereditary thrombophilia, we speculate that thromboprophylaxis may have value in secondary prevention of subsequent ATCVD.

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UNEXPLAINED SPORADIC FIRST-TRIMESTER MISCARRIAGE: FACTOR V LEIDEN GENE MUTATION. J. Munjal, L. Rovner, S. Gogineni, T. Tracy, P. Wang, C.J. Glueck, The Jewish Hospital, Cincinnati, OH.

Background: The propensity to form thrombi is physiologically increased in normal pregnancy secondary to reduction in naturally occurring anticoagulants, an increase in coagulation factors, and a reduction in fibrinolysis. We hypothesized that when the physiologic hypercoagulability of pregnancy is superimposed on the thrombophilic G1691A factor V Leiden mutation, sporadic first-trimester miscarriage and repetitive pregnancy loss are promoted. We hypothesized that the thrombophilic G1691A factor V Leiden gene mutation was a common, significant, treatable cause of sporadic first-trimester miscarriage. **Methods:** We compared the frequency of the G1691A factor V Leiden mutation in women with ≥ 1 pregnancy and 1 miscarriage with women having ≥ 1 pregnancy and 0 miscarriages. In 848 Caucasian women with consecutive measures of the factor V Leiden mutation, we compared the frequency of the V Leiden mutation in 136 women with ≥ 1 pregnancy and 1 miscarriage (260 live births, 136 miscarriages), 50 women with ≥ 1 pregnancy and 2 miscarriages (83 live births, 100 miscarriages), 53 women with ≥ 1 pregnancy and ≥ 3 miscarriages (recurrent pregnancy loss) (109 live births, 227 miscarriages), and 609 women with ≥ 1 pregnancy and 0 miscarriages (1,473 live births). We used PCR techniques to characterize the thrombophilic G1691A V Leiden [FV] gene mutation. **Results:** Of the 609 controls, 41 (6.7%) had FV heterozygosity versus 15 heterozygous and 2 homozygous FV cases (17 of 136, 12.5%) with 1 sporadic miscarriage, $\chi^3 = 5.2$, $p = .023$, vs 8 of 53 (15%, 7 heterozygous and 1 homozygous) with ≥ 3 miscarriages, Fisher's $p = .048$, vs 2 of 50 (4%) with 2 miscarriages. The V Leiden frequency in cases with 1 sporadic miscarriage (17 of 136, 12.5%) did not differ from recurrent pregnancy loss cases with ≥ 3 miscarriages (8 of 53, 15%), $\chi^2 = 0.22$, $p = .64$. **Conclusions:** After unexplained sporadic first-trimester miscarriage, as well as after recurrent pregnancy loss, to provide the option to prospectively optimize subsequent live birth outcomes with low molecular weight heparin throughout pregnancy, we suggest that measurements be done of the FV mutation, a treatable etiology for sporadic miscarriage, as well as for recurrent pregnancy loss.

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PROLACTIN ALTERS BCR-MEDIATED APOPTOSIS AND RECEPTOR EDITING. E. Peeva, G.E. Rosenfeld, J. Gonzalez, S. Saha, Albert Einstein College of Medicine, Bronx, NY.

Background: Autoreactive lymphocytes are constantly generated and eliminated to maintain tolerance. Two major mechanisms for tolerance induction are deletion and receptor editing. In the spleen, the majority of negative selection occurs in transitional B cells, mainly in the T1 B-cell subset, resulting in a T1:T2 ratio > 1. We have shown that prolactin-altered negative selection of autoreactive specificities allows for survival and activation of DNA-reactive B cells and development of lupus in mice that do not develop lupus spontaneously. In BALB/c mice, treatment with prolactin decreases the number of T1 B cells and increases the number of T2 B cells leading to a T1/T2 ratio < 1, which indicates that prolactin impairs negative selection. **Purpose:** The aim of this study is to characterize the mechanisms by which prolactin breaks B-cell tolerance. Deletion and receptor editing as means of negative selection were examined using a combination of flow cytometry and real-time PCR experiments. **Methods:** Eight- to 10-week-old female BALB/c mice were treated with prolactin (100 μg/d) or placebo (normal saline) for 1 month. Splenocytes were isolated, and RBC lysis was performed. Flow cytometric measurement of anti-IgM Ab-induced apoptosis was used to determine the effect of prolactin on BCR-mediated deletion in transitional T1 ($CD19^+AA4.1^+CD21^-CD23^-$) and T2 ($CD19^+AA4.1^+CD21^+CD23^+$) B-cell subsets. The effect of prolactin on receptor editing was evaluated by RAG-1 and RAG-2 mRNA expression and the presence of kappa/lambda-positive B cells. RNA was isolated by RNAeasy kit from B cells purified with Dynabeads. RAG-2 mRNA expression was determined by real-time PCR. Flow cytometry was used to determine the number of B cells coexpressing kappa/lambda light chains in the transitional T1 and T2 and mature marginal zone (MZ) ($CD19^+AA4.1^-CD21^+CD23^-$) and follicular (Fo) ($CD19^+AA4.1^-CD21intermediateCD23^+$) B-cell subsets. The real-time data were obtained by a Light Cycler real-time PCR machine. Flow cytometry data were acquired by an LSRII flow cytometer, and data analysis was done by *FlowJo* software. **Results:** After BCR stimulation with anti-IgM antibody (10 μg/mL) as a surrogate antigen, T1 B-cell subset from prolactin-treated mice showed significantly less annexin V–positive B cells than the T1 subset from placebo-treated mice ($p = .023$). As per our microarray data, this effect of prolactin on B-cell deletion may be mediated by prolactin-induced overexpression of the antiapoptotic molecules Bcl-2, Birc-1, and/or IFNalfaR. In addition, treatment with prolactin induced a two- and threefold increase in RAG-1 and RAG-2 mRNA expression in B cells ($p = .012$), as well as an elevated number of kappa/lambda-expressing B cells with T2 ($p = .028$) and Fo ($p = .001$) phenotype, an indication of continued receptor editing. **Conclusion:** Treatment with prolactin increases the resistance to anti-IgM-mediated apoptosis of T1 B-cell subsets, which, under normal circumstances, is a commonplace for negative selection of the autoreactive specificities. The failed ability to delete by apoptosis may explain the increased survival of self-reactive B cells destined for deletion. In addition, increased serum prolactin levels lead to increased receptor editing in splenic B cells, and the V(D)J recombination in the peripheral lymphoid organs has been associated with generation of pathogenic autoantibodies and autoimmunity. Thus, alterations of both BCR-mediated deletion and receptor editing may be implicated as mechanisms in prolactin-induced breakdown of B-cell tolerance.

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NRF2 REGULATES HYPEROXIA-MEDIATED NOX4 EXPRESSION AND REACTIVE OXYGEN SPECIES PRODUCTION. S. Pendyala, I.A. Gorshkova, D. He, H. Cho, S.R. Kleeberger, V. Natarajan, University of Chicago, Chicago, IL; Research Triangle Park, NC.

Rationale: We have demonstrated earlier that Nox4, a homologue of Nox2 (gp91phox), is highly expressed in human pulmonary artery endothelial cells (HPAECs) and involved in hyperoxia-induced reactive oxygen species (ROS) production and signal transduction. Nrf2 is a transcriptional factor that is activated in hyperoxia and is known to regulate a number of genes involved in antioxidant defense mechanisms in the lung. Here we have investigated the role of Nrf2 in regulating hyperoxia-induced Nox4 expression and ROS generation in HPAECs. **Methods/Results:** In HPAECs, mRNA expression of Nox4 is several-folds higher compared with Nox2 (gp91phox), and exposure of cells to hyperoxia (95% O2) resulted in up-regulation of expression of Nox4 and p22phox but not Nox1 or Nox3. Nrf2 is up-regulated in short-term (3 hours) hyperoxia as much as twofold. Down-regulation of Nrf2 mRNA with siRNA attenuated Nox4 expression in normoxic HPAECs; however, enhanced ROS generation under both normoxia and hyperoxia (3 hours). Exposure of Nrf2 wild-type mice to hyperoxia (100% O_2) for 24 and 48 hours resulted in enhanced Nox4 expression in the lung compared with normoxia. Further, $Nrf2^{-/-}$ mice exposed to hyperoxia (24 and 48 hours) showed decreased Nox4 expression in the lung