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CALCIUM/CALMODULIN-DEPENDENT KINASE IV MEDIATES G13-STIMULATED MYOCYTE ENHANCE FACTOR 2–DEPENDENT GENE TRANSCRIPTION. G. Liu, J. Han, T.A. Voyno-Yasenetskaya, University of Illinois at Chicago, Chicago, IL.

G13 and G12 belong to the same subfamily of heterotrimeric G proteins. The major physiological difference between them is revealed by knockout mice. Mice with null mutant of G13 alpha subunit (Gα13) are embryonic lethal, while those with null mutant alpha subunit of G12 (Gα12) are alive. This difference suggests that G13 and G12 may differently regulate gene transcription during embryonic development. Muscle enhance factor 2C (MEF2C) is an important transcription factor during angiogenesis. Here we characterized MEF2-dependent gene transcription by constitutively activated Gα13 (Gα13Q226L) and Gα12 (Gα12Q229L). We found that Gα13Q226L was more potent than Gα12Q229L to stimulate MEF2-dependent gene transcription in NIH 3T3 cells and only Gα13Q226L was able to stimulate MEF2-dependent gene transcription in HUVEC cells. Repression of MEF2C-mediated gene transcription by HDAC4 and HDAC5 was abolished by Gα13Q226L but not by Gα12Q229L. In addition, Gα13Q226L but not Gα12Q229L was able to induce the translocation of histone deacetylase 5 (HDAC5) from nucleus to cytoplasm. Gα13Q226L stimulated MEF2-dependent gene transcription was inhibited by dominant negative mutant of calcium/calmodulin-dependent kinase IV (CaMKIV). Furthermore, Gα13Q226L but not Gα12Q229L was able to increase Ca^{2+}/CaM-independent CaMKIV actvity. Our studies indicate the different regulation of CaMKIV activity may result in the different functions of G13 and G12 in MEF2-dependent gene transcription.

2

THE RELATIONSHIP OF AGE AND LEFT VENTRICULAR DIASTOLIC FUNCTION. A. Maksoud, I. Porter, K. Schneider, R. Joseph, P. Lebourveau, D. Meyers, Kansas University Medical Center. Kansas City, KS.

Purpose: To determine the frequency and severity of left ventricular (LV) diastolic dysfunction with increasing age. **Methods:** 103 consecutive patients (55 females and 48 males) referred for echocardiography who had sinus rhythm and no mitral stenosis or severe regurgitation, AV block, intraventricular conduction delay, anemia, thyrotoxicosis, AV shunt, or electronic pacemaker had Doppler interrogation of mitral, pulmonary vein diastolic flows and mitral annulus motion (tissue Doppler), and measurement of ejection fraction (EF) by standard protocol. Diastolic function was graded as normal or abnormal (mild, moderate, severe) by the consensus of three observers using predetermined criteria. Presence of cardiovascular disease (CVD) was defined as the patient-recalled history of myocardial infarction, valvular disease, or hypertension; the current use of antihypertensive agents; or investigator-determined blood pressure > 140/90 mm Hg.

Results:

				Diastolic Function				
Age (years)	*n*	*CVD*	*EF*	*Normal*	*Mild*	*Moderate*	*Severe*	
< 40	22	45%	.57 ± 8	82%	14%	0	4%	
40–49	19	58%	.58 ± 6	68%	32%	0	0	
50–59	18	67%	.57 ± 8	56%	44%	0	0	$p < .0001$
60–69	26	73%	.55 ± 14	19%	58%	15%	8%	
≥ 70	18	100%	.56 ± 10	0	67%	33%	0	

The older the patient, the more advanced the diastolic dysfunction ($p < .0001$). On the other hand, the age-related increase in frequency of CVD was significant ($p = .005$), as was the association of CVD to the presence of diastolic dysfunction (dysfunction present in 69% with CVD and 27% without CVD, $p = .002$). **Conclusions:** The prevalence and severity of diastolic dysfunction, but not systolic dysfunction, increase with advancing age and correlate with the increasing prevalence of cardiovascular disease.

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SUPPRESSION OF LIPOPOLYSACCHARIDE-INDUCED ACUTE LUNG INJURY BY TAXOL. T. Mirzapoiazova, L. Moreno, S. Sammani, I. Kolosova, J.G. Garcia, A.D. Verin, The University of Chicago, Chicago, IL.

Our prior study demonstrated that similar to actin cytoskeleton, mictotubule (MT) network is a key participant in the regulation of endothelial (EC) permeability. Disassembly of MT by pharmacological inhibitors nocodazole and vinblastine results in rearrangement of actin cytoskeleton, stress fiber formation, EC contraction, and increased permeability. MT-stabilizing agent, Taxol, prevented EC barrier dysfunction induced by MT inhibitors and significantly attenuated permeability induced by proinflammatory agonists such as thrombin in cell culture model. We hypothesized that Taxol may prevent inflammation and vascular leak associated with lung injury. The effect of Taxol was assessed employing a model of murine lung injury induced by intratracheal LPS administration (2.5 mg/kg). Our data demonstrate that intravenous Taxol (8.5 mg/kg) injected simultaneously with LPS administration dramatically reduced inflammatory histological changes in lung parenchyma, decreased infiltration of proteins (40%, $p < .001$) and inflammatory cells in bronchoalveolar lavage (BAL) (51%, $p < .01$) and extravasation of Evans blue albumin dye into lung tissue. Taxol also significantly reduced LPS-induced release of inflammatory cytokines (tumor necrosis factor α and interleukin-6) into BAL (30% for both, $p < .05$). At the same time Taxol alone had no appreciable effect on the parameters described above. These data suggested that MT destabilization may be involved in LPS-induced lung injury in vivo.

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IMPACT OF HEPATITIS C VIRUS INFECTION ON INCIDENCE OF ERYTHROCYTE AUTOANTIBODIES IN PATIENTS WITH HUMAN IMMUNODEFICIENCY VIRUS INFECTION. M. Razaq,[1,2] Y. Patsiornik,[2] Z. Hussain,[2] A. Ohri,[1,2] [1]Division of Hematology, [2]Department of Medicine, Coney Island Hospital, Brooklyn, NY.

Background: Human immunodeficiency virus (HIV) is associated with numerous hematological disorders—anemia, pancytopenia, and hemostatic abnormalities being the most common ones. Direct antiglobulin test has frequently been observed in the clinical syndrome of HIV, but autoimmune hemolytic syndrome (AIHA) is rare. Review of other reports of DAT and AIHA in patients with HIV infections suggests that these autoantibodies may be associated with anemia in this population. Many of these patients are also concurrently infected with hepatitis C virus (HCV). We recently reported a significantly higher incidence of autoantibodies to red blood cells in patients with HCV infection as compared to healthy blood donors. We also noted an even higher incidence of autoantibodies to red blood cells in patients concurrently positive for both HCV and HIV. We hypothesized that HCV infection in patients with HIV confers a much higher risk of developing autoantibodies against red blood cells. **Method:** Sixty-eight patients with HCV and HIV were randomly screened for DAT and AIHA in our hospital from June 2001 to June 2004. **Results:** Thirty-four patients were positive for HIV. Among them 16 were positive for HCV and 18 were negative. Thirty-four patients were positive for HCV only.

	HIV +ve/HCV +ve	*HIV +ve/HCV 2ve*	*HIV 2ve/HCV +ve*
DAT +ve	3	1	5
DAT −ve	13	17	29

We noted an incidence of 18.8% of DAT positivity in patients with concurrent HIV and HCV infection. One of these three patients had clinical AIHA, with high LDH, indirect bilirubinemia, and low haptoglobin. Five percent of the patients with HIV in the absence of HCV had positive DAT. There was no clinical AIHA in this group, and 14.7% of patients with HCV infection alone had positive DAT. One of these five patients had clinical AIHA. **Conclusion:** Our study indicates that HIV-positive patients who are concurrently infected with HCV have a higher incidence of autoantibodies against erythrocytes.

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SERUM FERRITIN MAY NOT BE A RELIABLE PREDICTOR OF TISSUE IRON CONCENTRATIONS. M. Siddique, M.C. Delano, K. Schwartz, Departments of Medicine and Radiology, Michigan State University, East Lansing, MI.

Tissue iron concentrations in patients suspected of iron overload are currently estimated using the concentration of ferritin in serum. Because serum ferritin is an acute-phase reactant, we hypothesized that it is not a reliable predictor of amount of tissue iron. MRI relaxation times decrease as the concentration of iron in tissues increases. The MRI T2* and T2 relaxation times evaluated from liver and myocardial interventricular septum will be measured in approximately 30 iron patients with primary or secondary hemochromatosis. MRI measurements from the interventricular septum will be correlated with MRI results from the liver, serum ferritin, serum iron (SI), total iron binding capacity (TIBC), and percent iron binding (SI/TIBC × 100). To date we have enrolled seven patients and shown that it is feasible to study both myocardial and liver iron in one MRI session. Our preliminary findings suggest that serum ferritin may not be an accurate predictor of amount of iron in liver and heart as measured by MRI. We conclude that (1) MRI measurements to determine amount of myocardial and liver iron can be obtained in one session and (2) our preliminary findings suggest that serum ferritin may not be an accurate predictor of amount of iron in liver and heart as measured by MRI, but these initial data will be verified via study of additional iron overload patients.

6

IDENTIFICATION OF PROTEINS IN THE LUNG POTENTIALLY INTERACTING WITH HUMAN TIMAP. D.M. Adyshev, I.A. Kolosova, A.D. Verin, Department of Medicine, Section of Pulmonary and Critical Care Medicine, The University of Chicago, Chicago, IL.

We have previously shown that myosin light chain phosphatase (MLC PPase) is a key determinant in the regulation of endothelial cell (EC) permeability. Most of the functional characteristics of MLC PPase are determined by its regulatory subunit-MYPT1 and include binding to the catalytic subunit and targeting it to MLC insuring substrate specifity. In addition, it was recently discovered that MLC PPase activity can be regulated by several endogenous inhibitory proteins. The inhibitory subunits/proteins include MYPT3, protein inhibitor CPI-17, and a recently discovered TIMAP (TGF-β-inhibited membrane-associated protein). Hence regulation of EC MLC PPase may involve several targeting subunits and endogenous inhibitory proteins, as well as other cytoskeletal proteins. TIMAP shares significant domain homology with MYPT3, which specifically inhibits PPase activity toward MLC and myosin in vitro. TIMAP is highly expressed in EC and may be involved in endothelial cytoskeletal and barrier regulation. However, the exact role of TIMAP in regulation of MLC PPase activity has not been yet reported. BacterioMatch Two-Hybrid System (Stratagene) was applied for screening of human lung cDNA library in order to identify potential human TIMAP interaction proteins in the lung. Seven potential TIMAP interacting partner proteins were identified. Four of identified proteins, cysteine- and glycine-rich protein 1, eukaryotic translation elongation factor 2, U5 snRNP-specific protein 116 kD, and solute carrier family 3 member 2, are involved in actin cytoskeleton organization, cell adhesion or translation, and transcriptional regulation. However, the exact role of these regulatory proteins in endothelial cytoskeletal and barrier regulation is yet to be found. Collectively, these data suggest involvement of TIMAP in the regulation of EC cytoskeleton.

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