ical pattern of reduced TM protein expression in CM. Persistence of staining for von Willebrand factor, which is stored in intracellular Weibel-Palade bodies, provided evidence that decreased TM staining was not due to endocardial loss. To assess the physiologic consequence of reduced TM expression, we measured in situ formation of APC using a chromogenic assay. Segments of control LV (n = 4) generated 2,100 ± 138 fmol APC/min/cm^2. In contrast, LV harvested after 8 and 15 weeks of pacing generated only 329 ± 35 and 307 ± 78 fmol APC/min/cm^2, respectively (p < .0001 vs control LV). **Conclusion:** Endocardial TM protein expression is reduced by 90% at 8 weeks and 92% at 15 weeks following rapid pacing. This decrease in TM results in an 85% reduced capacity of the EE to generate activated protein C compared to normal LV. Reduced TM expression in CM markedly impairs APC formation, thereby contributing to a significant loss of endocardial thromboresistance.

27

PROTECTIVE EFFECT OF PURINERGIC AGONIST ATPγS AGAINST ACUTE LUNG INJURY. L.A. Kolosova, T. Mirzapoiazova, L. Moreno, S. Sammani, J.G. Garcia, A.D. Verin, 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 of the lung and protein-rich pulmonary edema. The pharmacological treatment of ALI/ARDS is nonspecific and relies on good supportive care and control of initiating cause. ALI/ARDS pathogenesis is still only partly understood; however, pulmonary endothelium plays a major role by changing its barrier permeability, thus promoting pulmonary edema formation. Pulmonary endothelial functional and structural alterations are key components of increased vascular leak. Consequently, endothelium-related therapies may have beneficial effects in ALI/ARDS. Recently, much attention has been given to the therapeutic potential of purinergic agonists and antagonists for the treatment of cardiovascular and pulmonary diseases. Extracellular purines (adenosine, ADP, and ATP) and pyrimidines (UDP and UTP) are important signaling molecules that mediate diverse biological effects via cell-surface P2Y receptors. We have shown that ATP promotes endothelial cell (EC) barrier enhancement via a complex cell signaling. We hypothesize that activation of endothelial purinoreceptors would exert anti-inflammatory barrier-protective effect. To test this hypothesis we used two model systems: cultured pulmonary EC and murine model of ALI induced by intratracheal administration of endotoxin/lipopolysaccharide (LPS). In cell culture model, ATP inhibited intracellular gap formation and junctional permeability induced by inflammatory mediator thrombin. In murine model of ALI, nonhydrolyzed ATP analogue ATPγS (50 μM final blood concentration) administered intravenously attenuated inflammatory response by decreasing accumulation of cells (48%, p < .01) and proteins (57%, p < .01) in bronchioalveolar lavage (BAL) and by reducing neutrophil infiltration into alveoli. ATPγS slightly reduced LPS-induced release of inflammatory cytokines (tumor necrosis factor alpha and interleukin-6) into BAL. These findings suggest that purinergic receptor stimulation exerts a protective role against ALI, probably via decreasing endothelial junctional permeability.

28

THE REGULATION OF NEURONAL OUTGROWTH AND RETRACTION BY SEROTONIN RECEPTORS 5-HT4a AND 5-HT7: THE ROLE OF LIM KINASE 1. A. Krbanjevic, G. Liu, J. Profirovic, T. Voyno-Yasenetskaya, Department of Pharmacology, University of Illinois at Chicago, Chicago, IL.

Serotonin is a neurotransmitter with a prominent role in the central nervous system. The higher mammals express seven families of serotonin receptors. Functions of only some of the receptors have been determined. Most of the 5-HT functions are mediated through G protein–coupled receptors. We have shown that 5-HT$_{4a}$ serotonin receptor couples $G_{\alpha S}$ and $G_{\alpha 13}$ protein, causing RhoA-dependent neurite retraction and cell rounding (Ponimaskin et al, 2002). We also found that 5-HT$_7$ serotonin receptor couples to $G_{\alpha s}$ and $G_{\alpha 12}$ protein. 5-HT$_7$ receptor-$G_{\alpha 12}$ protein pathway induces activation of two small GTPases, RhoA and Cdc42, leading to pronounced cell rounding and filopodia formation, respectively (Kwachnina et al, 2005). LIM kinase 1 (LIMK1) is a serine-threonine kinase that negatively regulates function of actin depolymerizing protein cofilin. Therefore, activation of LIMK1 leads to actin polymerization. The small GTPases of Rho family are shown to stimulate LIMK1. In the present study, we are showing for the first time that 5-HT$_{4a}$ and 5-HT$_7$ receptor phosphorylate LIMK1. Therefore, this could be mechanism for regulation of cytoskeleton of cells transfected with 5-HT$_{4a}$ and 5-HT$_7$ receptors. When we down-regulated endogenous LIMK1 using siRNA technology we almost totally lost rounded cells. Also, when we cotransfected 5-HT$_{4a}$R and 5-HT$_7$ receptor with LIMK1-siRNA, we observed that the 5-HT$_7$ receptor led to an increase in the number of rounded cells and a decrease in the number of process-bearing cells. We are suggesting here that LIMK1 influences activity of small GTPases through the feedback loop between LIMK1 and small GTPases. This mechanism is important for precise control of LIMK1 activity in cells transfected with 5-HT$_{4a}$ and 5-HT$_7$ receptors. Our data give a new insight into serotonin, not only as a well-known neurotransmitter but also as a modulator of neuronal plasticity.

29

REGULATION OF APOPTOSIS SIGNAL-REGULATING KINASE 1 BY α SUBUNITS OF HETEROTRIMERIC G PROTEINS G12 AND G13. M.A. Kutuzov, A.V. Andreeva, T.A. Voyno-Yasenetskaya, Department of Pharmacology, University of Illinois at Chicago, Chicago, IL.

Heterotrimeric G protein α subunits Gα12 and Gα13 are able to stimulate c-Jun N-terminal kinase (JNK) and induce apoptosis via more than one MAP kinase pathways. Gα12 and Gα13 have been reported to stimulate apoptosis signal-regulating kinase (ASK1), one of MAPKK kinases upstream of JNK; the mechanism of this effect has not been studied in detail. Here we report that Gα12 and Gα13 associate with ASK1, as demonstrated by immunoprecipitation assays. Gα12 and Gα13 binding to ASK1 occurs independently of their activation state, as evidenced by ASK1 binding to wild-type Gα subunits both in the presence and in the absence of AlF_4^-, as well as its similar binding to mutationally activated as compared to wild-type Gα12/Gα13. Using deletion mutants of ASK1, we show that both N- and C-terminal regulatory domains of ASK1 may be involved in the interaction with Gα12/Gα13, while its kinase domain is not required. Coexpression of ASK1 with wild-type Gα13 in COS-7 cells leads to strongly decreased levels of ASK1 protein. In contrast, coexpression of ASK1 with mutationally activated Gα13 increases ASK1 levels as compared to control. Analysis of ASK1 polyubiquitinylation shows that polyubiquitinylated high molecular mass forms of ASK1 are more pronounced in the presence of wild-type Gα13 but are alleviated in the presence of activated Gα13. These results indicate that, in addition to the stimulation of ASK1 kinase activity by Gα12/Gα13 reported previously, ASK1 expression levels can be regulated by these G proteins via control of polyubiquitinylation and following degradation of this kinase.

30

NOVEL REGULATION OF LIPID SYNTHESIS: THE SPOT 14 PROTEIN CONTROLS FATTY ACID SYNTHASE ACTIVITY IN VIVO AND IN VITRO. L.T. LaFave, L.B. Augustin, S. Chohnan, C.N. Mariash, University of Minnesota, Minneapolis, MN, and Ibaraki University, Ibaraki, Japan.

Spot 14 (S14) is a 17 kD protein located in tissues that synthesize fatty acids. In the lactating mammary glands of S14 null mice there is significantly reduced triglyceride content due to a 70% reduction in lipogenesis with a 90% reduction in medium-chain fatty acids. However, the site and role of the S14 protein in the regulation of lipid synthesis remains unknown. We observed no difference in gene expression or in vitro activity of all tested lipogenic enzymes in wild-type vs null mice. A sensitive recycling assay measuring mammary gland malonyl-CoA revealed that malonyl-CoA is significantly increased in S14 null mice vs wild type (7.19 ± 0.68 vs 5.44 ± 0.43 nmol/g, p < .05). Therefore, the block in lipogenesis occurs at the terminal step, fatty acid synthase (FAS). Based on this evidence, we hypothesized that S14 is integral to the function of FAS, which catalyzes the production of fatty acids from malonyl-CoA. We first tested whether S14 acts by binding fatty acids during their production or transport. Recombinant Spot 14 produced from baculovirus-infected insect cells was used to investigate whether S14 acts as a fatty acid binding protein (FABP). We utilized a well-established assay that binds a hydrophobic fluorescent probe, 1-anilinonaphthalene 8-sulfonic acid (ANS), to putative FABPs. We found that the S14 protein bound to ANS with positive cooperativity (Hill coefficient = 2.9), suggesting that S14 is a multimer, and the formation of the multimer creates the hydrophobic pocket for ANS binding. However, the fluorescent probe was not displaced by fatty acids or their CoA derivatives of varying lengths. Therefore, to investigate further which specific lipogenic proteins associate with S14 in vitro, we created an affinity agarose column to which we bound the purified S14. Elution at 3M NaCl followed by SDS-PAGE showed only a single protein of molecular weight greater than 200 kD. This high-affinity interaction is consistent with S14 binding to FAS. Our data suggest that S14 possesses a hydrophobic binding pocket and interacts with FAS in vivo and in vitro to regulate the rate of fatty acid synthesis in mammary tissue.

31

EFFECT OF HIGHLY ACTIVE ANTIRETROVIRAL THERAPY ON BRONCHOALVEOLAR LAVAGE IMMUNOGLOBULIN G SUBCLASS LEVELS IN HIV-POSITIVE PATIENTS. S. Lodhi, R.B. Day, P.A. Smith, C. Huffer, H.L. Twigg III, Indiana University Medical Center, Indianapolis, IN.

Background and Objective: HIV infection in the lung is characterized by lumphocytic alveolitis and a state of chronic alveolar macrophage and lung lymphocyte activation resulting in enhanced cytokine secretion and hypergammaglobulinemia. It has been shown that HAART is associated with a generalized decline in cytokine secretion, especially interferon-gamma (IFN-γ), and reduced IgG and IgM production. We examined the effect of HAART on IgG subclasses, including the IFN-γ-dependent subclass IgG2. **Methods:** IgG subclass levels were measured in BAL of 20 HIV-infected patients before and at 24 weeks after initiation of HAART using a human IgG subclass ELISA kit. Cytokine concentrations were measured using a cytokine bead array assay. IgG and cytokine concentrations were corrected for BAL dilution by determining epithelial lining fluid (ELF) using the urea dilution technique. **Results:** Data expressed as mean ± SEM μg IgG/mL ELF (*p value ≤ .05).

	IgG1	*IgG2*	*IgG3*	*IgG4*	*IgG1:IgG2*
Entry	3.71 ± 0.44	7.55 ± 1.58	0.72 ± 0.17	0.86 ± 0.22	0.90 ± 0.12
Week 24	3.30 ± 0.34	5.32 ± 0.90*	0.29 ± 0.05*	0.76 ± 0.19	0.82 ± 0.17

HAART was associated with a significant decline in IgG2 and IgG3 levels. Unlike normal subjects, BAL IgG2 levels were higher than IgG1 levels, a difference persisting even after 24 weeks of HAART. A significant correlation was found between IFN gamma levels and IgG1 (R = .47, p = .03), IgG2 (R = .53, p = .02) and IgG3 (R = .53, p = .02) before initiation of HAART. This did not persist at week 24. IL-6 levels and IgG1 levels were found to be positively correlated at the time of entry (R = .46, p = .04). None of the other subclasses demonstrated any significant correlation with IL-6 levels. **Conclusion:** HIV infection is associated with increased IgG2 in the lungs of HIV infected patients, possibly due to elevated IFN-γ concentrations in the lung. While this is partially correcting after 6 months of HAART, IgG2 levels still remain elevated in the lung, probably due to the long half-life of IgG.

32

EFFECT OF HIGHLY ACTIVE ANTIRETROVIRAL THERAPY ON BRONCHOALVEOLAR LAVAGE PNEUMOCOCCAL-SPECIFIC IMMUNOGLOBULIN A LEVELS IN HIV-POSITIVE PATIENTS. S. Lodhi, Y. Wang, R.B. Day, P.A. Smith, T. Lahm, C. Huffer, H.L. Twigg III, Indiana University Medical Center, Indianapolis, IN.

Background and Objective: The incidence of invasive pneumococcal disease among HIV-infected persons has declined in the post HAART era. Changes in the humoral immune responses have been implicated in this change. It has already been shown that HAART is associated with a decline in BAL total IgG and IgM concentrations and a decrease in BAL pneumococcal-specific IgG concentrations. We measured total and pneumococcal-specific IgA concentrations in BAL of 24 HIV-infected patients before and at 4 and 24 weeks after initiation of HAART, anticipating a similar response. **Methods:** BAL pneumococcal-specific IgA levels were measured using ELISA. The IgA concentrations were corrected for dilution by determining epithelial lining fluid (ELF) using the urea dilution technique. **Results:** Data expressed as mean ± SEM μg IgA/mL ELF (*p value ≤ .05).

	Baseline	*Week 4*	*Week 24*
Total IgA	495.13 ± 88.47	514.16 ± 145.07	389.59 ± 67.27
Pneumococcal-specific IgA	3.57 ± 0.82	2.96 ± 0.57	2.68 ± 0.66
Pneumococcal-specific IgA%	1.35% ± 0.004	1.16% ± 0.003	1.85% ± 0.009