moniae by acid and physiologic concentrations of nitrite. Mice received subcutaneous pantoprazole or saline every 12 hours for 3 days followed by orogastric inoculation of 100 colony-forming units of VRE or *K. pneumoniae* in combination with subcutaneous clindamycin. The presence and density of the pathogens in stool were monitored by plating samples onto selective media. Student's *t*-test was performed to compare the pH of gastric contents from pantoprazole versus saline-treated mice and chi-square test was performed to compare the frequency of acquisition of stool colonization with the pathogens. **Results:** Exposure to pH 2 buffer or fasting human gastric contents resulted in significant killing of the pathogens and physiological concentrations of nitrite-enhanced killing. In comparison to saline controls, pantoprazole-treated mice had elevated gastric pH (mean ± SD, 4.8 ± 0.87 versus 2.3 ± 0.3; $p < .001$) and increased frequency of establishment of intestinal colonization with the pathogens (75% versus 25% for VRE, $p = .01$; 100% versus 40% for *K. pneumoniae*, $p < .001$). For all mice that developed colonization, high densities of the pathogens were measured (range 6–9.8 $\log_{10}$CFU/g of stool). **Conclusions:** Proton pump inhibitor administration facilitates establishment of intestinal colonization by VRE and *K. pneumoniae* in clindamycin-treated mice. These data suggest that the widespread use of proton pump inhibitors in health care facilities could contribute to the dissemination of nosocomial pathogens.

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PHOSPHORYLATION OF β-CATENIN BY PROTEIN KINASE A IN VASCULAR SMOOTH MUSCLE CELLS. S. Taurin, N. Sandbo, Y. Qin, N.O. Dulin, Department of Medicine, The University of Chicago Chicago, IL.

Beta-catenin is a transcriptional coactivator that promotes cell proliferation by induction of genes such as cyclin D1, c-myc, and others. Enhanced β-catenin signaling was observed in many cancer cells and in vascular smooth muscle cells (VSMC) during restenosis. The canonical mechanism of regulation of β-catenin involves its phosphorylation by glycogen synthase kinase 3 (GSK3) that targets β-catenin to ubiquitination and degradation by the proteasome system. Mitogenic factors promote β-catenin signaling through inhibition of GSK3, resulting in reduced β-catenin phosphorylation, its stabilization, and subsequent accumulation in the nucleus, where it stimulates gene transcription. In the present study, we investigated the mechanism of VSMC proliferation induced by extracellular ATP (a VSMC mitogen relevant to the pathogenesis of atherosclerosis and restenosis). We found that (1) ATP potently induces the cyclin D1 transcription in VSMC; (2) stimulation of cyclin D1 transcription by ATP is dependent on β-catenin signaling and on protein kinase A (PKA) activity; (3) β-catenin is phosphorylated by PKA in vitro and in intact cells at two novel sites, Ser-552 and Ser-675; (4) phosphorylation by PKA promotes the transcriptional activity of β-catenin; (v) mutation of Ser-675 attenuates the promoting effect of PKA; (6) phosphorylation by PKA does not affect the GSK3-dependent phosphorylation of β-catenin, its stability or intracellular localization; but (7) phosphorylation at Ser-675 site promotes the binding of β-catenin to its transcriptional coactivator, CREB-binding protein (CBP). In conclusion, this study identifies a novel, noncanonical mechanism of modulation of β-catenin signaling through direct phosphorylation of β-catenin by PKA, promoting its interaction with CBP.

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SPHINGOSINE-1-PHOSPHATE-MEDIATED ENDOTHELIAL CELL MOTILITY IS REGULATED BY $S1P_1$ RECEPTOR, LIPID PHOSPHATE PHOSPHATASE-1, AND SPHINGOSINE KINASE 1. I. Gorshkova, E. Berdyshev, B. Saatian, Y. Zhao, S. Pendyala, J.G.N. Garcia, V. Natarajan, The University of Chicago, Chicago, IL.

The extracellular effects of S1P, a naturally occurring and platelet-derived bioactive lipid molecule, are mediated by $S1P_{1-5}$ family of G protein–coupled receptors. The exact role of different S1P receptors and signaling pathways regulating S1P-induced cell motility is not completely defined. Human pulmonary artery endothelial cells (HPAEC) expressed $S1P_1$, $S1P_3$, and $S1P_4$ receptors with a predominance of $S1P_1$, as determined by RT PCR. Exposure of HPAEC to S1P resulted in a potent induction of cell motility as determined by a scratch wound healing assay and by monitoring changes in transendothelial electrical resistance of wounded HPAEC by ECIS. Inverse agonist of $S1P_1$ receptor, SB649146, and $S1P_1$ siRNA attenuated S1P-induced EC motility. Furthermore, overexpression of sphingosine kinase 1 wild type and $S1P_1$ wild type increased intracellular content of S1P as well DHS1P (DHS1P > S1P) with a concomitant loss of cell motility in response to exogenous S1P. Overexpression of lipid phosphate phosphatase (LPP)1 wild type that enhanced hydrolysis of exogenous S1P blocked S1P-induced cell motility. These results suggest that a balance between $S1P_1$ expression and intracellular content of S1P and DHS1P seems to be critical for maintaining cellular sensitivity to external S1P stimulation.

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IDENTIFICATION OF ORGAN-SHARED AND STRAIN-INDEPENDENT CANDIDATE GENES DURING COMBINED VENTILATOR-ASSOCIATED LUNG AND KIDNEY INJURY. D.N. Grigoryev,[1] S. Sammani,[2] S.-F. Ma,[2] S.Q. Ye,[2] K.C. Barnes,[1] H. Rabb,[1] J.G.N. Garcia,[2] [1]Johns Hopkins University, Baltimore, MD; [2]The University of Chicago, Chicago, IL.

Combined ventilator-induced lung injury (VILI) and acute renal failure (ARF) have 80% mortality in the ICU. We hypothesized that analysis of global genetic changes in lung and kidney tissues during developing of ALI will identify similar genomic responses to injury that could underlie injurious effects in both organs. We performed gene expression profiling of lung and kidney tissues in three different mouse strains in a model of VILI, speculating that key transcriptional changes would be tissue and strain independent. Three inbred mouse strains C57BL/6J (B), C3H/HeJ (H), and DBA/2J (D) were selected and young 4-week-old mice were mechanically ventilated (V_t = 17 mL/kg, 110 breaths/min) for 2 hours (n = 6–8) and compared to spontaneous ventilated (n = 6) mice. Analysis of bronchoalveolar lavage proteins demonstrated a significant ($p < .05$) increase in B, H, and D strains by 133 ± 47SE μg/mL, 125 ± 28SE μg/mL, and 108 ± 53SE μg/mL, respectively, with no significant differences between groups at this age. Gene expression profiles of VILI-affected lung and kidney tissues were generated using MG-U74A (12,488 genes) GeneChips (n = 12) and simultaneously analyzed using *GC-Robust Multichip Average* and *Significance Analysis of Microarrays* softwares. Genes with the lowest false discovery rate (q = 0.092%) and ± 50% fold change (FC) were considered affected by VILI in both tissues. Gene Ontology (GO) analysis of identified genes was conducted by GenMAPP and MAPPFinder tools and biological processes with z score > 1.96 were considered significantly affected by VILI. This cross-tissue and cross-strain analysis identified 58 genes that behaved similarly in lung and kidney tissues during VILI. Gene ontology analysis of these genes revealed significant up-regulation of genes involved in response to stress (z = 2.51), including Mig-6 ($5.97FC_{Lung\,(L)}$, $3.02FC_{Kidney\,(K)}$) and Gadd-45{γ ($3.68FC_L$, $6.06FC_K$); and transcription activity (z = 4.04), including Ceb/pδ} ($3.97FC_L$, $3.74FC_K$) and Ceb/pβ ($2.87FC_L$, $4.58FC_K$). These data demonstrate that VILI activates specific transcription factors and stress responsive genes in both lung and kidney in all tested strains. Detailed studies of these pathways can reveal organ-shared VILI targets for the development of new therapeutic strategies for this devastating disorder.

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ZYXIN IS INVOLVED IN THROMBIN SIGNALING VIA INTERACTION WITH PROTEASE-ACTIVATED RECEPTOR 1. J. Han, J. Niu, G. Liu, T. Voyno-Yasenetskaya, University of Illinois at Chicago, Chicago, IL.

Introduction: Thrombin-induced increase in endothelial monolayer permeability depends on reorganization of cytoskeletal structures. Protease-activated receptor 1 (PAR-1) is the main receptor responsible for thrombin signaling in endothelial cells. Zyxin, a low-abundance phosphoprotein, serves as a molecular adaptor that mediates the productive juxtaposition of protein partners. Zyxin may participate in aspects of cytoskeletal assembly and dynamics by recruiting components of actin assembly machinery to specific sites in the cells. The subcellular distribution of zyxin is critical to its functions. In human umbilical vein endothelial cells (HUVECs), thrombin stimulation induces zyxin targeting to actin stress fibers. We proposed that zyxin participates in thrombin induced cytoskeletal reorganization by interaction with PAR-1. **Methods:** Coimmunoprecipitation and GST fusion protein pull-down assay were performed to identify the domains of zyxin to interact with carboxyl-terminal PAR-1 in COS-7 cells. Confocal immunofluorescence microscopy was utilized to observe the subcellular distribution of zyxin, PAR-1, actin stained with appropriate antibodies against these proteins. Zyxin-specific siRNA duplexes are generated to knock down the expression of endogenous zyxin. **Results:** We showed that PAR-1 interacted with zyxin both in vitro and in vivo. We have shown that LIM domains 1 and 2 of zyxin interacted with C-terminal domain of PAR-1. In human umbilical vein endothelial cells (HUVECs), thrombin promotes zyxin targeting to actin stress fibers. Down-regulation of zyxin using siRNA and disruption of the interaction between zyxin and PAR-1 resulted in reduced cellular responses to thrombin stimulation: actin stress fiber formation and serum response element (SRE)-dependent gene transcription. Zyxin-specific siRNA and C terminus of zyxin overexpression did not inhibit thrombin-induced RhoA activity, which plays a key role in thrombin-induced stress fiber formation and SRE activity and knockdown of endogenous zyxin could still attenuate the active RhoA-induced SRE activity, suggesting that zyxin may regulate thrombin-induced actin reorganization and SRE activity in an RhoA-independent manner. These results suggest that zyxin is involved in thrombin signaling by direct interaction with PAR-1.

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EFFECT OF CONCENTRATED BRONCHOALVEOLAR LAVAGE FROM HIV-POSITIVE SUBJECTS TO PRIME ALVEOLAR MACROPHAGES AGAINST *MYCOBACTERIUM TUBERCULOSIS*. C.J. Huffer, R.B. Day, S. Lodhi, Y. Wang, P.A. Smith, H.L. Twigg III, Indiana University School of Medicine, Indianapolis, IN.

Background and Objective: *Mycobacterium tuberculosis* (MTB) is a common cause of pulmonary disease in HIV-infected patients. Normal alveolar macrophages (AM) phagocytize MTB and are then activated by cytokines to kill ingested MTB. We have previously shown that HIV alveolar AM are intrinsically resistant to MTB infection compared to normal AM, likely due to an AM-activating factor secreted by alveolar T cells. In this work, we examined the ability of concentrated BAL from HIV-infected subjects to activate AM to kill MTB. **Methods:** BAL from HIV-infected subjects before and after starting antiretroviral therapy were concentrated 5 times using 10 kD spin columns and then cultured with normal AM for 24 hours. Virulent and avirulent MTB at an MOI of 1:1 was then added and subsequent infection determined using a BACTEC methodology. BAL cytokine concentrations were measured using a cytometric bead array assay system. Time to initial and maximal infection of the AM by MTB was assessed. **Results:** Virulent and avirulent MTB grew equally well in AM not treated with HIV BAL. However, pretreatment with HIV BAL significantly delayed AM infection (both initial and maximal) by avirulent MTB compared to virulent MTB (initial infection: 12.2 ± 0.3 days vs 9.8 ± 0.3 days, $p < .001$; maximal infection measured at day 16: 0/39 avirulent vs 34/39 virulent, $p < .001$). There was not a significant correlation between levels of IFN-γ in BAL and time to initial infection by MTB. While treatment with antiretroviral agents lowered IFN-γ concentrations in BAL, there was no difference in BAL from untreated compared to treated subjects in the ability to prime AM against MTB. **Conclusions:** BAL from HIV-infected subjects primes AM against infection against avirulent MTB but not virulent MTB. This is consistent with the known sensitivity of avirulent MTB to macrophage activating cytokines. The lack of correlation between BAL IFN-γ concentrations and the ability to prime AM against MTB suggests that other soluble factors are involved and are currently under investigation.

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CARDIOMYOPATHY RESULTS IN DYSREGULATION OF ENDOCARDIAL THROMBOMODULIN AND AN ALTERED PROTEIN C ACTIVATION PATHWAY. A.Y. Kim, H.M. Russell, J. Raman, M. Gupta, X. Tian, R.M. Lang, J.L. Miller, The University of Chicago, Chicago, IL.

Cardiomyopathy (CM) is associated with a significant risk for thrombosis and an increased risk for thromboembolic events. Early studies invoked blood stasis or flow disturbance as the principal underlying mechanisms. More recent evidence has linked CM with a hypercoagulable state, as demonstrated by elevated levels of thrombin-antithrombin complexes, prothrombin fragment F1+2, and fibrinopeptide A, all indices of thrombin activation. Thrombomodulin (TM), an integral membrane protein found on the endocardial endothelium (EE), is a potent thrombin inhibitor via activation of the protein C pathway. We hypothesized that the increased ventricular wall tension characteristic of CM would result in reduced TM protein expression on the surface of EE, which would, in turn, result in an impairment of local activated protein C (APC) activity, thereby increasing the risk for thrombosis. Using a rabbit pacing-induced CM model, we quantified TM protein expression on EE over time by digital immunohistochemical analysis (n = 4/time point), expressed as arbitrary units (AU)/mm of left ventricular (LV) perimeter. Baseline TM staining of LV endocardial segments was 1,669 ± 128 AU/mm. After 8 weeks of rapid pacing, TM protein expression on EE was markedly decreased (175 ± 41 AU/mm, $p < .0001$ vs control LV). TM protein expression remained significantly low after 15 weeks of pacing compared to control LV (142 ± 32 AU/mm, $p < .0001$). Western blot analysis confirmed the immunohistochem-

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.

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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.

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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.

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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.

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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.

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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.

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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