coexpression of Wnt 7a and Frizzled 9 (Fzd 9) has antitumorigenic effects in non–small cell lung cancer (NSCLC) through ERK5-dependent activation of peroxisome proliferator–activated receptor γ (PPARγ); PPARγ acts as a tumor suppressor in NSCLC. A known PPARγ inhibitor (SR202) blocked the increase in PPARγ activity and restored tumorigenic anchorage-independent growth in NSCLC expressing Wnt 7a and Fzd 9. Interestingly, the combination of Wnt 7a and Fzd 9 in NSCLC cell lines also induced protein expression of Spry 4, a putative receptor tyrosine kinase inhibitor (ie, inhibitor of EGF and FGF). In spite of this, the role of Spry 4 in lung tumorigenesis has not been established in NSCLC. Thus, the goal of this study was to define the mechanism of Spry 4 activation by the Wnt 7a/Fzd 9 signaling pathway. We found that Spry 4 did not lead to increased activation of PPARγ or E-cadherin (which is activated by PPARγ), both established downstream targets of the Wnt 7a/Fzd 9 growth inhibitory pathway. However, wild-type PPARγ led to significant activation of Spry 4, suggesting that Spry 4 is a downstream target of PPARγ. In addition, PD98059, an inhibitor of MEK5/ERK5, also decreased Spry 4 activation by Wnt 7a/Fzd 9, further suggesting Spry 4 as a downstream target of ERK5 activation of PPARγ. These data demonstrate that the activation of Spry 4 by Wnt 7a/Fzd 9 signaling pathway is via ERK5-dependent activation of PPARγ and represents a major effector pathway mediating the antitumorigenic effects of Wnt 7a and Fzd 9 in NSCLC. Thus, Spry activation by Wnt 7a and Fzd 9 may represent a novel approach in molecular targeting and could have usefulness in the treatment of NSCLC.

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PULMONARY PNEUMOCOCCAL SPECIFIC IMMUNOGLOBULIN G1 AND G2 RESPONSES IN HIV-POSITIVE MALAWI PATIENTS. D.K. Wyler, Y. Wang, R.B. Day, R.E. Eagan, S.B. Gordon, H.L. Twigg, Indiana University Medical Center, Indianapolis, IN; Blantyre, Malawi.

Human immunodeficiency virus (HIV)-infected patients in Malawi have a high incidence of pneumococcal pneumonia. Since IgG2 is known to be protective against *Streptococcus pneumoniae*, we measured pneumococcal-specific IgG (IgG-pn), IgG1 (IgG1-pn), and IgG2 (IgG2-pn) in bronchoalveolar lavage (BAL) fluid to see if a quantitative deficit might explain this observation. IgG-pn, IgG1-pn, and IgG2-pn were measured to determine if there was a change in the ratio of immunoglobulin isoforms. Fifty-two BAL samples were collected from 26 HIV-infected HAART-naive Malawi patients and 26 HIV-negative volunteers. IgG-pn, IgG1-pn, and IgG2-pn were determined using sandwich ELISA techniques. ELISA wells were coated with pneumococcal vaccine (Pneumovax 23). Biotinylated IgG, IgG1, and IgG2 were used as the secondary antibodies. Data are expressed as ng/mL BAL ± SEM. Total IgG-pn was elevated in HIV+ patientss compared with HIV− patients ($p = .0098$), as were IgG2-pn ($p = .0002$) and IgG1 ($p = .0067$). HIV-infected patients did not have a statistically significant difference in IgG1/IgG2 ratio compared with HIV-negative patients ($p = .3804$). In conclusion, despite the high incidence of invasive pneumococcal disease in Malawi, HIV-infected Malawians had higher total IgG-pn-, IgG1-pn-, and IgG2-pn-specific antibodies in BAL than uninfected subjects. We speculate that this may be related to elevated proinflammatory cytokines in HIV BAL, including IFN-γ, which is known to stimulate IgG2 production in particular. These results suggest that the high incidence of pneumococcal pneumonia in Malawi is not due to a quantitative deficit in IgG levels but rather points to potential functional antibody defects.

	IgG-pn	*IgG1-pn*	*IgG2-pn*	*IgG1-pn/ IgG2-pn*
HIV+	93 ± 25*	19 ± 2*	25 ± 4	1.6 ± 0.3
HIV−	18 ± 3	11 ± 1	9 ± 2	2.1 ± 0.4

Data expressed as ng/mL BAL ± SEM.

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LYSOPHOSPHATIDIC ACID INDUCES CYCLOOXYGENASE 2 EXPRESSION AND PROSTAGLANDIN E_2 PRODUCTION IN HUMAN PRIMARY BRONCHIAL EPITHELIAL CELLS. Y. Zhao, D. He, R. Stern, S. Kalari, E.W. Spannhake, V. Natarajan, The University of Chicago, Chicago, IL; Baltimore, MD.

Rationale: We have demonstrated that transactivation of EGF-R by lysophosphatidic acid (LPA) partly regulates IL-8 secretion in human bronchial epithelial cells (HBEpCs). The present study provides evidence that crosstalk between G protein–coupled LPA receptors and EGF-R regulates LPA-induced cyclooxygenase 2 (COX-2) expression and prostaglandin E_2 (PGE_2) production in HBEpCs. **Methods/Results:** LPA (1 μM) treatment induced COX-2 expression at mRNA and protein levels but had no effect on COX-1 expression. Down-regulation of COX-2 by transfection of COX-2 siRNA blocked LPA-induced PGE_2 release in HBEpCs. Pretreatment of HBEpCs with pertussis toxin (PTx), intracellular calcium chelator (BAPTA-AM), overexpression of dominant negative PKC delta, IKK inhibitor (Bay11-7082), JNK inhibitor (JNKi), transfection of c-Jun siRNA, or C/EBPβ siRNA blocked LPA-induced COX-2 expression. Further, down-regulation of EGF-R by EGF-R siRNA or pretreatment with EGF-R tyrosine kinase inhibitor (AG1478) partly attenuated LPA-induced phosphorylation of C/EBPβ, COX-2 expression, and PGE_2 release but not phosphorylation of IκB, JNK1/2, and nuclear localization of NF-κB. **Conclusions:** We show here that LPA induces COX-2 expression through intracellular calcium, activation of PKC delta, NF-κB, JNK/AP-1, C/EBPβ, and EGF-R transactivation. Since COX-2 is anti-inflammatory in the airway, the present results suggest that LPA plays a protective role in airway inflammation and remodeling.

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PROTEOME ANALYSIS OF SPHINGOSINE 1-PHOSPHATE–MEDIATED LIPID RAFTS IN HUMAN PULMONARY ENDOTHELIAL CELLS. J. Zhao, P.A. Singleton, S.M. Dudek, L. Natarajan, J.G. Garcia, University of Chicago, Chicago, IL.

Rationale: Lipid rafts are detergent-insoluble plasma membrane microdomains implicated in membrane signaling and trafficking. We have demonstrated that the lysophospholipid sphingosine 1-phosphate (S1P) plays a critical regulatory role in the maintenance and enhancement of pulmonary vascular barrier function, which is dependent on lipid raft–mediated signaling events (Singleton et al. FASEB J 2005). In this study, we used two-dimensional electrophoresis analysis to determine the protein profiles in S1P-challenged lipid rafts of human pulmonary artery endothelial cells (HPAECs). **Methods and Results:** HPAECs were treated with S1P (1 μM) for 5 minutes and solubilized with 1% Triton X-100 at 4°C. The Triton X-100 insoluble and light density fractions were collected after discontinuous OptiprepTM gradient centrifugation. The isolated lipid rafts pellets were resolubilized in 50 μL of isoelectric focusing (IEF) buffer (7 M urea/2 M thiourea, 4% CHAPS). IEF was carried out on a linear electrophoresis gel strip (pH 3–10, 7 cm), with the second dimension performed on 4 to 20% SDS-PAGE gel. Analytic gels were stained using Imperial blue (Pierce). We observed several novel protein bands in lipid rafts isolated from S1P versus control-treated cells (1 μM, 5 minutes). To identify these novel proteins, analytic gels were stained with Sypro Ruby and images were analyzed using PDQuest (Bio-Rad). Novel protein spots were sliced from the gel, and gel pieces were swelled in 20 ng/μL modified trypsin and incubated at 37°C overnight after protein reduction and alkylation. The digested peptides were extracted with 5% formic acid and 50% acetonitrile. Protein identification was performed on ABI 4700 Maldi TOF/TOF MS. Two-dimensional gel image analysis results indicated that about 50 protein spots (such as discoidin domain receptor tyrosine kinase, myosin light chain kinase, pp60src, phosphodiesterase 6C, etc.) have over a threefold change after S1P (1 μM, 5 minutes) treatment compared with control. Further, antiphosphotyrosine immunoblots of two-dimensional gels indicated that S1P increased tyrosine phosphorylation of over 20 proteins, including the actin regulatory protein cortactin. **Conclusions:** Taken together, these results indicate that S1P induces recruitment of novel tyrosine kinases (ie, discoidin domain receptor tyrosine kinase, pp60src), with multiple tyrosine phosphorylation events in lipid rafts. These results suggest that recruitment of tyrosine kinases to lipid rafts may participate in S1P-induced signaling in HPAECs and could potentially play important roles in regulating S1P-induced pulmonary endothelial cells barrier function.

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POSSIBLE NEW TREATMENT MODALITY FOR ATRIAL FIBRILLATION. N. Agarawal, M.Z. Fissha, R. Baltazar, C. Cummings, M.M. Mower, Sinai Hospital, Baltimore, MD.

Application of cold to the epicardial surface of the atrium was shown to reverse acetylcholine-induced atrial fibrillation to sinus rhythm in animal models. Varying the intensity of the cold gives a graded response with respect to time, and it appears that about one-sixth of the atrial surface is needed to effect this response. Intravenous cold saline solution has also been found to be effective for cardioversion of atrial fibrillation in dogs induced by atrial pacing in the presence of increased adrenergic tone. The following clinical episode supports the concept that the use of cold could be a possible new treatment modality for atrial fibrillation in humans. A 30-year-old African American male with ventricular septal defect was brought to the catheterization laboratory for left and right heart catheterization and ventriculography. Prior to the procedure, he was placed on telemetry, which revealed atrial fibrillation. This was a new finding as multiple prior ECGs had consistently shown sinus rhythm. During the catheterization, he was injected twice with a bolus of cold saline for cardiac output determination using thermal dilution. After the second injection, it was observed that the atrial fibrillation instantly terminated and did not recur. Coronary angiography revealed normal coronary arteries. The patient was discharged on Coumadin as prophylaxis against stroke and was discontinued after 6 months. This episode suggests that intravenous injection of cold saline may be effective in cardioversion of human atrial fibrillation. An implanted device therapy based on application of cold to the atrium may be possible from either the endocardial or the epicardial surfaces.

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DIFFUSE LARGE B-CELL LYMPHOMA MIMICKING ADVANCED BASAL CELL CARCINOMA. F. Akinyemi, M. Le, P. Sircar, A. Maini, A. Barua, S. Niranjan, Coney Island Hospital, Brooklyn, NY.

Introduction: Primary cutaneous B-cell lymphomas (PCBCLs) are made up of a heterogeneous group of B-cell lymphoproliferative diseases confined to the skin at the time of diagnosis with no evidence of extracutaneous involvement. With early diagnosis and adequate treatment, PCBCLs as a group have an excellent prognosis, with about a 95% survival rate at 5 years. There is no universally acceptable method of treatment and classification. Diagnosis can be made with the use of immunohistochemical staining and histologic appearance. We report a case of diffuse large B-cell lymphoma (DLBCL) in a 52-year-old woman presenting as a fungating skin ulcer mimicking advanced basal cell carcinoma. **Case Report:** A 52-year-old female originally from Ukraine presented to our emergency room with a 7-month history of circumscribed, reddish ulcerations and nodules over the left scapular region with extension into the left axillary region. According to the patient, the lesion started as a small dark pigmented spot 7 years prior to admission into our service and began to grow rapidly 9 months ago. The lesion was slow-growing, nontender, and nonpruritic. There was no history of fever, weight loss, or night sweats. Past medical history was noncontributory except that she lived close to the Chernobyl nuclear disaster of 1986. Physical examination revealed a 15 × 15 cm ulcer over the serratus anterior muscle with elevated borders and another 6 × 6 cm ulcer in the left axilla. The ulcers had central areas of necrosis with malodorous greenish discharge. No evidence of peripheral lymphadenopathy was found. Routine laboratory tests such as hemogram, serum chemistry, and chest radiography were within normal limits. Computed tomographic scans of the chest, abdomen, and pelvis showed no evidence of lymphadenopathy or systemic involvement, and a nuclear bone scan failed to reveal any evidence of metastatic bone disease, thus ruling out extracutaneous involvement. Biopsy of the ulcer showed dermis filled with sheets of large cells with vesicular nuclei and prominent nucleoli. Mitotic admixtures were numerous with areas of necrosis. The epidermis was free. Immunohistochemical stains favored the diagnosis of DLBCL. Therapy was initiated with rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) and radiotherapy. After three cycles of chemotherapy, the lesion became smaller, with a flat border and dry and healing skin, with disappearance of the axillary nodules. **Discussion:** PCBCL is a rare group of lymphoproliferative disorders. It is a distinct subclass of non-Hodgkin's lymphoma that originates in the skin and comprises the second largest group of extranodal B-cell lymphomas (after gastrointestinal). It is estimated to be about 20 to 25% of all cutaneous lymphomas. Extracutaneous dissemination is rare, with most disease having a favorable prognosis. Various treatment options are available and depend on the histologic subtype and number of lesions. CHOP with and without radiotherapy has been used extensively. Remission has been achieved in 92% of cases studied, with a median follow-up of 28 months and only one patient with DLBCL of the leg dying from progressive cutaneous disease. PCBCLs are highly radiosensitive. Radiation therapy can be used as an adjunct with chemotherapy at presentation or relapse. It is frequently used for localized disease. Treatment of PCBCL with anti-CD20 monoclonal antibody (rituximab) was recently introduced. It is usually combined with cytotoxic therapy and has shown mixed results.