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EXPRESSION OF mRNAs ENCODING GAP JUNCTION AND CELL ADHESION PROTEINS IN TYPE II CELLS EXPOSED TO COAL DUST IN VITRO. D. E. Rannels D. C. Carey and Y. C. Lee. Dept. of Cellular & Molecular Physiology, Penn State College of Medicine, Hershey PA 17033 and Weis Center for Research, Geisinger Clinic, Danville PA 17822.

Type II pulmonary epithelial cells (T2P) in primary culture express mRNAs encoding gap junction (GJ) proteins, connexins (Cx), and establish functional GJ intercellular communication (GJIC) (AJP 272: L1105, 1997). Cx43 mRNA and protein increase 10- and 30-fold, respectively, by culture *day 3* in parallel with increased matrix fibronectin (FN) content and elevated GJIC. Exposure of T2P to anthracite coal dust 867 further increases FN mRNA and matrix FN content, but has little effect on Cx43 mRNA. FN promotes cell spreading required to establish cell contact and to initiate GJIC. Syndecans are transmembrane heparin sulfate proteoglycans that function in cell adhesion. Expression of syndecan (Syn) mRNAs was thus compared to those of FN and Cx43. T2P express mRNA for Syn-1, -2, -3 and -4, with Syn-1 and -4 being most abundant. Neither Syn-1 nor Syn-4 mRNA changes substantially with culture time, whereas mRNAs encoding Syn-2 and -3 increase at differing rates. Exposure to 867 elevates Syn-3 and Syn-4 mRNAs on *day 3* and *day 6*, but not those for Syn-1 or Syn-2. Parallel changes in matrix FN and in expression of mRNA encoding Syn-2, Syn-3 and Cx43 are consistent with the hypothesis that as T2P spread in culture, syndecans may mediate cell binding interactions that accompany initiation of functional GJIC. The issue of whether 867 plays a significant role to modulate these effects remains to be resolved along with the underlying regulatory mechanisms. Supported by US Bureau of Mines G1145242; NS21925; HL31560.

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LIGATION OF FAS ANTIGEN INDUCES APOPTOSIS IN A HUMAN ALVEOLAR EPITHELIAL CELL LINE INDEPENDENTLY OF NITRIC OXIDE GENERATION A. Jankov, I. Joshi, A. Bifero, C. Leche and B. Uhal. Cardiovascular Institute, Michael Reese Hospital, Chicago, IL 60616

Recent work has demonstrated the expression of functional FAS (APO1, CD95) by pulmonary alveolar epithelial cells of the mouse and rat lung. Our laboratory had earlier demonstrated that human lung fibroblasts synthesize a low-MW protein capable of inducing apoptosis in a human alveolar epithelial cell line (A549) by a mechanism which was sensitive to inhibitors of nitric oxide synthase (NOS). To determine if the FAS-activated pathway is active and also sensitive to NOS inhibitors in A549 cells, we examined the influence of the NOS substrate analog N-nitro-L-arginine methyl ester (L-NAME) on A549 cell apoptosis induced by an activating antibody to FAS (anti-FAS, clone CH-11). Over 20 hours incubation, 500ng/ml anti-FAS induced a 2.1-fold increase ($p<0.001$) in the percentage of apoptotic cells identified conservatively by the presence of nuclear fragmentation under fluorescence microscopy. The anti-FAS-mediated increase was unaffected by 0.5mM L-NAME, a concentration which inhibited fibroblast-induced apoptosis of A549 cells by 50%. Western blotting of A549 cell lysates with the same anti-FAS antibody identified a single band of 55kDa; by immunocytochemistry, the antibody labeled 5-6% of adherent A549 cells in a pattern consistent with cell surface labeling. These data suggest that a subset of A549 cells expresses functional FAS, which induces apoptosis by a mechanism independent of nitric oxide generation. Supported by HL-45136 and by Michael Reese Hospital.

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EPITHELIAL DERIVED H_2O_2 MAY ACT AS A TONIC INHIBITORY FACTOR IN ISOLATED BOVINE BRONCHI. M. A. Sergi, M. Eter and T. Burke-Wolin (SPON: M. Wolin). NYMC, Valhalla, NY 10595.

We have previously found that hydrogen peroxide (H_2O_2) relaxes isolated bovine bronchi only in the absence of the epithelial layer. The purpose of this study was to determine the role of H_2O_2 as a potential epithelial dependent inhibitory factor (EpDIF) and if this response is cyclic GMP dependent. Isolated bovine bronchi were cut into rings and the luminal surface of alternate rings was rubbed to remove the influence of the epithelium. In one group of tissues, lucigenin enhanced chemiluminescence (LEC) was used as a measure of superoxide anion (O_2^-) production which could act as a source of H_2O_2. Removal of the epithelium decreased LEC 53±7%. Pretreatment with 100μM nitro-L-arginine (NLA) also decreased LEC 43±12% suggesting that O_2^- may be produced through nitric oxide synthase. We next examined the role of H_2O_2 in basal guanylate cyclase (sGC) activation in these tissues. In intact bronchi, basal cGMP levels were 808±235fmole/g tissue. This was reduced to 413±90fmole/g in rubbed bronchi. NLA also inhibited cGMP in both intact but not rubbed tissue (cGMP in intact bronchi=328±68fmole/g). H_2O_2 was scavenged using the glutathione mimetic ebselen (EBS: 1μM). Pretreatment of the bronchi with EBS reduced cGMP levels in the intact airway to 199±52fmole/g. No effect of EBS was seen in rubbed tissue (cGMP=415±108fmole/g). We conclude that the epithelial layer in the bronchi acts as a significant source of O_2^- derived H_2O_2, which stimulates sGC in the smooth muscle and regulates airway reactivity.
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REGULATION OF DISTAL AIRWAY ION TRANSPORT BY EXTRACELLULAR NUCLEOTIDES S.K. Inglis and R.E. Olver. Lung Membrane Transport Group, Department of Child Health, University of Dundee, Dundee, Scotland, DD1 9SY

The lung abnormalities characteristic of cystic fibrosis are manifest initially in distal airways and they are thought to result from defective regulation of Cl^- secretion. Proximal airways express at least 2 apical P2Y receptor subtypes that may allow nucleotides to stimulate Cl^- secretion in CF epithelia: $P2Y_2$ receptors sensitive to both UTP and ATP; $P2Y_6$ receptors insensitive to ATP, but sensitive to UDP (Lazarowski *et al* Proc. Natl. Acad. Sci. 94:2599-2603, 1997). We have used cable analysis to determine the effects of luminal nucleotides on the bioelectric properties of isolated, perfused, porcine distal bronchi (3.5mm diameter). Addition of 100μM UTP increased transepithelial potential difference (PD) from 3.4±0.8mV to 6.4±1.4mV (n=6). This increase in PD was accompanied by an increased short circuit current (I_{SC}) (from 30±7μA.cm^{-2} to 54±13μA.cm^{-2}), and was almost completely abolished by pretreatment with 200μM bumetanide, an inhibitor of Cl^- secretion. UDP (50μM) did not affect either PD (control 4.1±0.8mV, UDP 4.2±0.9mV, n=3) or I_{SC} (44±9μA.cm^{-2} vs 45±11μA.cm^{-2}) although subsequent treatment with UTP evoked a clear response. The epithelia of the distal bronchi thus appear to express apical $P2Y_2$ receptors but not the $P2Y_6$ receptors that have been found in proximal airways. (Supported by Wellcome Trust grant 049986/Z/96/Z).

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Measurement of Mitochondrial Function in Human Epithelial Cells
Katherine A. Heminger and Nicholas S. Gantenberg
Procter and Gamble Co., Health Care Research Center, Mason, Ohio 45040

Mitochondrial changes in cellular function may drive the cell's response to cytokine stimulation and pathogens. The objective of these studies was to determine the sensitivity and specificity of the Alamar Blue™ (AB) assay in predicting mitochondrial changes. This assay may be useful for measuring mitochondrial function in cultured epithelial cells. AB incorporates an oxidation-reduction indicator that fluoresces in response to metabolic reduction via the mitochondrial electron transport chain. Cell viability depends on mitochondrial health, and the ability of the cell to sustain metabolic reduction.

Mitochondrial inhibitors for complexes I (rotenone) and III (antimycin A), arrest the flow of electrons through the electron transport chain. Rotenone caused a slight reduction (9-10%) in fluorescence intensity (FI) relative to vehicle-treated (control) cells, while antimycin A treatment resulted in a 27% reduction. Succinate, an electron donor for complex II, and carnitine, important for the oxidation of fatty acids, were investigated as possible modulators of mitochondria. Cells treated with 500 μM succinate produced a 6% increase in AB FI compared to a 15% increase with 400 μM carnitine. Catalase, an oxidoreductase, (2000 Units/ml) enhanced FI by 11%. A catalase inhibitor, aminotriazole (400 μM) reduced FI by 11%.

Thus, AB assay can predictably measure changes in mitochondrial function, however, it is not specific for mitochondria only. Changes in cellular oxidoreductase activity can also affect the FI measurements.

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ALTERATIONS IN O_3-INDUCED AIRWAY REACTIVITY OF GUINEA-PIG AIRWAYS TO METHACHOLINE *IN VIVO* AND *IN VITRO*: ROLE OF EPITHELIUM-DERIVED RELAXING FACTOR (EpDRF). J.S. Fedan, L.L. Millecchia, and D.G. Frazer. HELD, NIOSH, Morgantown, WV 26505.

We examined the hypothesis that, after ozone (O_3) inhalation exposure, changes in the production of EpDRF by respiratory epithelium could affect reactivity of guinea pigs to inhaled methacholine (MCh). Dose-response curves for enhanced pause (Penh) responses to inhaled MCh were obtained from conscious animals before, immediately after, and 24 hr after inhalation of 3 ppm O_3 for 1 hr. Airway reactivity *in vitro* was examined using the perfused trachea preparation to apply MCh separately to the serosal surface (extraluminal (EL) bath) or to the mucosal surface (intraluminal (IL) perfusing perfusate); EpDRF release was examined using IL NaCl-stimulated relaxant responses of MCh-contracted preparations. Immediately after O_3-exposure, reactivity to inhaled MCh was significantly increased, whereas by 24 hr reactivity declined to the control level. Immediately after O_3 delivery, reactivity to IL MCh was increased in the presence but not in the absence of the epithelium, and responsiveness to EL MCh was unchanged; by 24 hr post-exposure reactivity to IL MCh had returned to the control level. Immediately after O_3 exposure, IL NaCl-induced, EpDRF-mediated relaxation responses were inhibited, but by 24 hr responses to IL NaCl were normal. Throughout the post-exposure periods the epithelium was substantially damaged. These finding indicate that the development of airway hyperreactivity to inhaled MCh occurs in association with decreased EpDRF release, whereas recovery to normal reactivity is accompanied by the return of EpDRF release to normal.
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ABSTRACTS

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