937 EXPRESSION AND CHARACTERIZATION OF TRPM8 RECEPTORS IN THE LUNG EPITHELIUM.

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Several members of the Transient Receptor Potential (TRP) family of ion channels have been identified as physiological temperature sensors. These receptors respond to changes in temperatures, ranging from hot to cold, as well as chemical agonists such as menthol, capsaicin etc. Transient Receptor Potential Cation Channel, Melastatin subfamily, member 8 (TRPM8) has been shown to function as a cold sensing channel within the mammalian somatosensory system and acts by converting thermal stimuli into bio-chemical and electrical signals. Although much emphasis has been placed on the expression of TRPM8 in cold-sensitive sensory neurons, it is currently unknown whether functional TRPM8 receptors are expressed in the human respiratory tract. Preliminary studies in our lab have demonstrated the expression of TRPM8 mRNA in the bronchiolar epithelial (BEAS-2B), alveolar epithelial (A549), and normal human bronchiolar epithelial (NHBE) cells using RT-PCR. Additional studies aimed to assess the expression of functional TRPM8 channels in these cells demonstrated that menthol, the prototypical agonist of TRPM8, produced significant increases in the intracellular calcium concentrations that ranged from 1.4-fold for A549 cells to 5.6- and 5.8-fold for BEAS-2B and NHBE cells, respectively. The EC_{50} values for induction of calcium flux were 2 ± 1 mM, 0.02 ± 0.009 mM and 1.5 ± 0.8 mM in BEAS2B, A549 and NHBE cells, respectively. Furthermore, the influx of calcium was inhibited by capsazepine, an antagonist of TRPM8 receptors. These data were consistent with the expression of functional TRPM8 channels in respiratory epithelial cells. Expression of these receptors in the respiratory tract may represent a significant finding with respect to adverse respiratory responses to cold air (e.g. asthma, cough).Support was provided by Grant # HL069813.

938 AEROSOLIZED HYALURONAN MODIFIES ENDOTOXIN-INDUCED LUNG INJURY IN A TIME-DEPENDENT MANNER.

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Inhalation of an aerosolized preparation of low-molecular weight (150 kDa) hyaluronan (HA) was previously shown by this laboratory to prevent experimentally induced pulmonary emphysema without causing additional lung inflammation. As a result, aerosolized HA has been proposed as a potential treatment for human emphysema. However, other investigators have found that low-molecular weight HA may be proinflammatory, prompting us to determine whether aerosolized HA could possibly enhance inflammation in a model of pulmonary disease other than emphysema. Using endotoxin-induced lung injury, we tested the effects of aerosolized HA on the acute inflammatory response. Syrian hamsters were exposed via nebulization to a 0.1% solution of 150 kDa HA in water for 2 hrs, either immediately before or after intratracheal instillation of 0.1 mg E.coli endotoxin (controls received endotoxin alone). All animals revealed a rapid influx of inflammatory cells into the lung which peaked within the first 24 hrs. While microscopic examination did not show any significant differences between HA-treated and untreated lungs with regard to the magnitude of the inflammatory response, a marked elevation in the percentage of lavaged neutrophils (69% vs 5.4%; $p<0.05$) was seen at 4 hrs post-endotoxin in animals receiving HA after endotoxin administration. In contrast, administration of HA prior to endotoxin resulted in a decline in the proportion of lavaged neutrophils at 4 hrs (2.1% vs 5.0%; $p<0.05$). These results indicate that HA modifies endotoxin-induced lung injury in a time-dependent manner. The fact that HA stimulates a neutrophil influx only when given after induction of pulmonary inflammation suggests that it may specifically interact with activated inflammatory cells (e.g. macrophages) that recruit neutrophils to the lung. How these findings might affect the therapeutic potential of HA remains to be determined.

939 IMMUNE RESPONSE TO ZYMOSAN-INDUCED PULMONARY INFLAMMATION IN RATS.

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1→3-β-Glucans have been associated with increased pulmonary inflammation in mold-related indoor air problems. The objective of the present investigation was to determine the immune response to zymosan-induced pulmonary inflammation in SD rats. Rats received a single dose of zymosan A (2.5 mg/kg body weight) via intratracheal instillation (IT) and were euthanized on days 1, 4, 6, and 8 post IT. Inflammation and lung injury were assessed by measuring (1) neutrophil (PMN) infiltration into bronchoalveolar lavage fluid (BALF) and (2) albumin, total protein and lactate dehydrogenase levels in BALF. Alveolar macrophage activation was determined by chemiluminescence (CL). Immune response was investigated via immunophenotyping of lymphocytes and lymphokine production. Immunophenotyping was performed on BAL cells and lung-associated lymph node cells. Lymphokine production was measured from lymph node cells with or without concanavalin A stimulation by an enzyme-linked immunosorbent assay. Upon challenge with zymosan, rats exhibited increased inflammation and injury at the early time points post-IT exposure. Although elevations in PMN infiltration and CL had returned to control levels on day 4, lung-associated lymphocytes continued to proliferate and reached a maximum on day 6. The ratio of CD4 to CD8 T cells in the lymph node and BAL was lower in zymosan-treated rats than in control rats, indicating a greater increase in CD8 T cells as compare to CD4 T cells. Zymosan also increased the number of infiltrating NK cells, B cells, and T cells in BAL at all time points. B cells in BALF were found to be highest in number on day 1 for zymosan-treated rats. The ratio of T/B cells in BALF increased significantly on day 6 and 8. Zymosan treatment increased IL-2, IL-10 and IFNγ but not IL-4 production in lymphocytes. These data along with immunophenotyping of lymphocytes suggests that helper CD4 T and cytotoxic CD8 T cells are involved in the immune activation caused by zymosan treatment. In summary, rats exposed to zymosan had increased inflammation and altered lymphocyte profile, indicating an activation of innate and/or adaptive immune response in rats.

940 HYDROGEN SULFIDE EXPOSURE CAUSES INTRACELLULAR ACIDIFICATION OF RAT NASAL RESPIRATORY EPITHELIAL CELLS.

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Hydrogen sulfide (H_2S) is a naturally occurring gas that is also generated by several industries. The potential for widespread human inhalation exposure to this toxic gas is recognized as a public health concern. Human epidemiological investigations and experimental laboratory animal studies have confirmed that the nasal epithelium is a sensitive site for H_2S-induced pathology. Cytochrome oxidase (Co) inhibition has been postulated as one mechanism of H_2S toxicity. Inhibition of Co disrupts the electron transport chain and impairs oxidative metabolism, leading to decreased ATP production and the build-up of lactic acid. While Co is a sensitive marker of H_2S exposure, there is an incomplete correlation between H_2S-induced nasal lesions and Co inhibition. Another mechanism by which H_2S (a weak organic acid) could cause nasal injury is intracellular acidification and cytotoxicity. Literature reports show that changes in intracellular pH due to exposure to organic acids such as vinyl acetate can lead to cell death. To further understand the mechanism by which H_2S damages the nasal epithelium, nasal respiratory epithelial cells were isolated from naive rats; loaded with the pH-sensitive dye, SNARF-1; and exposed to air or 400 ppm H_2S for 90 min. Vinyl acetate (1000 μM) was used as a positive control. Intracellular pH was measured by flow cytometry and cell lysates were used to quantify total protein and Co activity. Flow cytometric analysis showed that nasal respiratory epithelial cells exposed to air maintained a pH of 7.6 ± 0.05. A significant decrease in intracellular pH occurred following exposure to either vinyl acetate (15% decrease from control) or H_2S (5% decrease from control). Co activity in air-exposed cells was 1.39 ± 0.01 △abs/min/mg protein, while Co activity in cells exposed to H_2S was not detectable. The intracellular acidification of nasal respiratory epithelial cells by H_2S demonstrates a novel mechanism of H_2S-induced nasal respiratory cellular toxicity.

941 PERTURBATION OF COPPER HOMEOSTASIS AND EXPRESSION OF COPPER-BINDING PROTEINS IN CADMIUM-RESISTANT LUNG FIBROBLASTS.

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To probe molecular mechanisms of cadmium (Cd) damage to the lung extracellular matrix (ECM) we developed Cd-resistant (CdR) cells derived from rat fetal lung fibroblasts (RFL6) by incubation with graded doses of Cd. CdR cells have been shown to display upregulation of metallothionein (MT) and glutathione (GSH), two metal scavenging agents, coupled with downregulation of lysyl oxidase (LO), a copper (Cu)-dependent enzyme necessary for crosslinking collagen and elastin in the ECM. Here we examined cellular uptake and distribution of Cu and compared expression of LO with other Cu-binding proteins in CdR cells. A ^{64}Cu 1-h pulse assay revealed that the amount of radioactivity associated with CdR cells was 2.1-fold of the parental Cd-sensitive (CdS) control. Thus, LO deficiency in CdR cells is not due to a reduced cell uptake of Cu, a cofactor of LO. Antibody affinity chromatography assays indicated a low level of ^{64}Cu bound to the LO fraction amounting to 9% of the CdS control as compared to 1, 400% of the control of ^{64}Cu associated with the MT fraction in CdR cells pulsed with isotope for 4 h. This suggests that the high level of cellular Cu in CdR cells at least in part resulted from elevated levels of cellular MT. Steady-state protein levels as determined by Western blot

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New Orleans, Louisiana

TOXICOLOGICAL SCIENCES

The Official Journal of the Society of Toxicology
Supplement

OXFORD UNIVERSITY PRESS

ISSN 1096-6080
Volume 84, Number S-1, March 2005
www.toxsci.oupjournals.org