ceramides, a second messenger involved in cell apoptosis, and transient activation of the transcription factor NFkB, in lung and alveolar macrophages. These signal transduction events were accompanied with accumulation of free radicals and lung damage, including edema, excessive release of type II cells ls were given N-acetyl cysteine rd gas exposure, there was more about 50-70% inhibition of the mustard gas induced signal transduction events. Thus, N-acetylcysteine, an antioxidant is a potent antidote for mustard gas induced lung injury. [Supported by grants from NIH (2S06-GM 08037) and Army (DAMD 17-99-1-9550)]

861.3

Comparison of the Responsiveness of Primary Alveolar Macrophages(AM's), Primary Alveolar Type II Cells(TII), and a Rat Alveolar Type II Cell Line (RLE-6TN) to Lipopolysaccharide (LPS) and Silica Exposure.

Rania Kanj[1], Jihee Kang[2], Vincent Castranova[1]. [1]NIOSH and West Virginia University, 1095 Willowdale Road, Morgantown, WV 26505, [2]Ewha Womans University, Seoul, Korea

The goal of this study was to compare the responses of AM's and TII cells to LPS or silica and eventually determine their mutual interactions. AM's were collected by bronchoalveolar lavage, TII cells were isolated by enzymatic digestion and purified by panning (~20 million cells/rat, 95% pure), and RLE-6TN were commercially obtained. Cells were exposed in vitro to LPS or silica for 18 hours, after which nitric oxide (NO) and tumor necrosis factor-alpha (TNF-α) release were measured in the supernate using a modified Greiss reaction and ELISA, respectively. Cytotoxicity was measured by trypan blue exclusion and lactate dehydrogenase (LDH) activity. Upon LPS exposure, AM's released more NO and TNF-α than TII cells, which in turn were more responsive than RLE-6TN. Peak release of NO and TNF-α occurred at LPS doses of 1-5 μg/ml, 5-10 μg/ml, and 50-100 μg/ml for AM's, TII cells and RLE-6TN cells, respectively. Silica doses below 50 μg/ml failed to stimulate NO or TNF-α production in any of these cell types. These data indicate that LPS is a potent inducer of NO and TNF-α release with AM's being the most responsive and RLE-6TN the least responsive alveolar cell type tested.

861.4

IL-1β, IL-6 and TNFα mRNA expression in mild pulmonary interstitial edema

Marta Sabbadini, Donatella Barisani, Elena Conforti, Giuseppe Miserocchi, Raffaella Meneveri. Dip. Med. Sper. Amb. e Biotecn. Mediche, Università Milano-Bicocca, Via Cadore 48, MONZA, MILANO 20052 Italy

In mild pulmonary interstitial edema, a small increase in extravascular lung water occurs with a marked change in parenchymal stresses and transcapillary pressure gradient due to low tissue compliance. Interstitial edema was induced in anaesthetized rabbits on varying intravenous saline infusion rate (0.5 to 6 ml/kg×min) and time (45 to 180 min), causing an increase of the lung wet-to-dry weight ratio (W/D) from 5.4±0.4 to 6.5-6.8. Cytokine mRNA response was evaluated on lung tissue samples by northern blot hybridisation and normalization with β-actin control probe. Specific probes were generated by PCR amplification of rabbit cDNAs and confirmed by automatic sequencing. Proinflammatory IL-1β mRNA markedly increased (~ 7 fold) with increasing W/D, flow rate and infusion time. TNFα and IL-6 (proinflammatory and anti-inflammatory, respectively) did not correlate with flow rate, but increased with increasing W/D; furthermore, both cytokines peaked within 45 min, remaining thereafter steady. Despite a relatively small change in extravascular water, mechanical stresses acting at lung tissue level may trigger cytokine activation that in turn may affect protein turnover.

Study supported by MIUR, Italy.

861.5

Interferon-γ Enhances the Pulmonary CXC Chemokine Response in Rats Challenged with Intratracheal Lipopolysacharide

Ping Zhang[1], Gregory J Bagby[2], Lee J Quinton[2], Warren R Summer[1], Steve Nelson[1]. [1]Medicine, LSU Health Sciences Center, 533 Bolivar St., New Orleans, Louisiana 70112, [2]Physiology, LSU Health Sciences Center, New Orleans, Louisiana

Rapid recruitment of neutrophils (PMNs) into the alveolar space is an important component of pulmonary host defense against invading bacteria. Locally produced CXC chemokines are major chemoattractants for tissue recruitment of PMNs. This study investigated the effects of interl (IFN-γ) on the pulmonary chemokine response to intrat lipopolysaccharide (LPS). Rats were treated with intratracheal recom rat IFN-γ ($1x10^5$ U/rat). Twenty four hours later, the animals challenged with intratracheal LPS (100 μg/rat). PMNs recover bronchoalveolar lavage (BAL) were significantly increased at 4 h foll LPS challenge (22.39±8.35 vs. 0.09±0.02 $x10^6$, $p<0.05$). significantly enhanced LPS-induced pulmonary recruitment of (71.06±6.54 $x10^6$, $p<0.05$). Intratracheal LPS caused significant inc in CINC and MIP-2 in BAL fluid at 90 min and 4 h post LPS chal IFN-γ did not induce pulmonary chemokine production in control an but significantly enhanced pulmonary CINC (14.4±1.2 vs. 7.5±1.1 and 45.8±5.1 vs.13.5±2.4 ng/ml, $p<0.05$, at 90 min and 4 h respect and MIP-2 (71.5±1.4 vs.33.4±5.8 ng/ml, $p<0.05$, at 4 h) responses to These data suggest that locally delivered IFN-γ may be a immunomodulator in enhancing pulmonary host defenses against ba pathogens.

NIH grant AA09803; Louisiana State HEF grant 2000-05-06

861.6

Neutrophil Migration into the Intratracheal LPS Challenged Alters Pulmonary CXC Chemokine Concentrations

Michael C Holmes[1], Ping Zhang[2], Steve Nelson[2], Warren R. Sum Gregory J Bagby[1]. [1]Physiology, LSU Health Sciences Center, Perdido, New Orleans, Louisiana 70112, [2]Medicine, LSU Health Sci Center, 533 Bolivar St., New Orleans, Louisiana 70112

When the lung is exposed to LPS, CXC chemokines are produced to a neutrophils into the lung. However, neutrophil migration can poten injure the host. In this study the intratracheal LPS-induced intrapulmc and systemic chemokine responses were determined for cytokine ind neutrophil chemoattractant (CINC) and macrophage inflammatory pro 2 (MIP-2) in rats under neutropenic (cytoxan,CPA), or neutrophilic CSF) conditions. By 4 hours after LPS, CPA treatment decreased I recruitment 88% and G-CSF increased PMN recruitment 164% v compared to vehicle animals. Neutropenic rats had increased CINC MIP-2 concentrations in BAL fluid at 4 hours after LPS. PMN chemo toward this BAL fluid was increased whereas chemotaxis toward l fluid from LPS challenged neutrophilic rats was decreased. In vitro I stimulated chemokine production by alveolar macrophages did not d with treatment. These data show that as neutrophils are recruited reduce chemokine levels and the chemotactic activity of the lung. modulation likely serves to regulate PMN recruitment, thereby decrea the risk for developing lung injury. NIH:AA09803

861.7

TNF Stimulates Na Absorption in CF Airway Cells Thro Translation and MAPK-dependent Pathways.

Frank A. Gesek, Bonita A. Coutermarsh, Keith J. DiPetr Pharmacology, Dartmouth Medical School, 7650 Remsen, Hanover, 03755

TNF is a pro-inflammatory cytokine significantly elevated in serum sputa of CF patients. CF airway epithelia exhibit enhanced reabsorption. A direct effect of TNF on ion transport in CF air epithelia is unknown. To test the hypothesis that TNF increases absorption in CF airway cells and examine signaling of TNF receptors used JME cells, a human airway CF line. To assess TNF effects, measured ^{22}Na uptake in suspended cells and amiloride-inhibitable sh circuit current (I_{sc}) in monolayers. Cells pretreated with TNF (48 hrs) TNF acutely added (10 ng/ml; 15 min), increase Na uptake by 43% 293±9 from a basal rate of 205 ±12 compared to 208±7 nmols/min protein for acute TNF alone. TNF increases I_{sc} by 44% to 72 from a b level of 50 $\mu A/cm^2$. To examine TNF signaling in airway cells, the MA inhibitor PD98059 was acutely added to block TNF-stimulated Na transp by 80%. Further, the ceramide analog C8 mimics the acute signaling TNF to increase Na transport in cells pretreated with TNF. Fina cycloheximide blocks the chronic effect of TNF on Na entry. We concl that TNF directly stimulates Na absorption in CF airway cells and requires actions on protein synthesis and acute signaling. TNF recept couple to pathways involving ceramide generation and activation of MA to regulate Na channels.

THE FASEB JOURNAL
A MULTIDISCIPLINARY RESOURCE FOR THE LIFE SCIENCES

Experimental Biology 2002®
New Orleans, Louisiana
April 20–24, 2002

ABSTRACTS
PART II

Abstracts 524.1–940.2

Official Publication of the Federation of American Societies for Experimental Biology
Volume 16, Number 5, March 22, 2002