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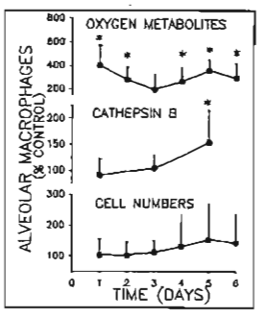
SEPSIS INDUCES INCREASED MONONUCLEAR PHAGOCYTE MIGRATION INTO ALVEOLI. Anil Mishra*, Sangeeta V. Karve* and Gary D. Nlehaus. Dept. of Physiology, NE Ohio Universities College of Medicine, Rootstown, OH 44272.

Peritoneal sepsis stimulates a delayed, transient increase in the number and cytotoxicity of pulmonary intravascular mononuclear phagocytes (PMMP). The current study tested our hypothesis that the hyperactivated PMMP mature and then migrate into the alveoli. The mononuclear phagocyte system (MPS) of male Sprague Dawley rats was stimulated by intraperitoneal injection of E. coli ($2x10^7$/100g) or saline (control). The rats were reanesthetized at defined times following MPS stimulation and alveolar macrophages (AΦ) were purified. The AΦ's capacity to produce cathepsin B and oxygen metabolites was measured. We previously demonstrated the PMMP numbers and oxygen metabolite production peaked 2 days after sepsis and then decreased. The current data (Figure) showed that a new population of AΦ (exhibiting increased oxygen metabolite and cathepsin B production as well as a tendency towards increased numbers) appeared 4 days post sepsis. We conclude that PMMP migrate into the alveoli 3-4 days after sequestering in the lung microvasculature.



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LUNG MACROPHAGE INTERACTION WITH INERT PARTICLES (TiO_2): FLOW CYTOMETRIC ANALYSIS. B.K. Stringer and L. Kobzik. Env. Health, Harvard Schl. of Public Health, Boston, MA 02115

Alveolar lining fluid (ALF) components may modulate AM uptake and response to inhaled inert particles. We sought 1) to establish a flow cytometric method to quantify the interaction of AMs with inert particulate titanium dioxide (TiO_2) and 2) to test the effect of ALF components on this interaction. After in vitro incubation of hamster AMs with TiO_2, flow cytometry showed a dose-dependent relationship between increasing AM light scattering (RAS) and concentration of TiO_2 (mean fold increase of RAS ± SE, n=6, for 2, 8, and 32 μg/ml TiO_2:1.71 ± 0.2, 4.0 ± 0.5, 9.1 ± 2.4). Similar results were obtained using a rat AM cell line (NR8383)(mean fold increase of RAS for 10, 20, and 60 μg/ml TiO_2: 1.71 ± 0.08, 2.18 ± 0.16, 3.66 ± 0.49; n=5). Consistent with the AMs unique avidity for particle uptake, a number of less differentiated myelomonocytic cell lines tested showed little or no uptake at these concentrations (THP1, HL60). To test whether ALF components can act as opsonins, TiO_2 was precoated with either human surfactant protein A (SpA) or concentrated ALF (both 0.5 mg/ml). While NR8383 AMs showed some uptake of coated and uncoated TiO_2, opsonization actually diminished uptake as measured by RAS changes (% decrease vs. unopsonized, @ 50 ug/ml TiO_2:SpA, 21.47 % ± 2.58 (n= 4); ALF, 27.23 % ±7.7, (n=2). Opsonization altered RAS values of free TiO_2 (mean channel number, n=3, control, ALF, or SpA coated TiO_2, respectively: 124 ± 2.4, 137 ± 9.4, and 190 ± 4.7). We conclude: 1) flow cytometry can be used to measure AM interaction with TiO_2; 2) In vitro, ALF components alter TiO_2 properties and modulate uptake by AMs.

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EFFECT OF SILICA ON NITRIC OXIDE (NO) PRODUCTION BY RAT ALVEOLAR MACROPHAGES. L.J. Huffman, D.J. Judy, and V. Castranova. Div. of Resp. Dis. Studies, NIOSH, Morgantown, WV.

In vivo exposure of rats to silica increases NO production by bronchoalveolar lavageable cells (BALC), a population of cells which includes alveolar macrophages (AMs). In the present study, we examined the in vitro effects of silica on NO production by rat AMs. BALC were obtained from normal male rats and cultured for 2 hrs. Non-adherent cells were then removed and the remaining AMs were exposed to test agents for 18-20 hrs. Media nitrate and nitrite concentrations were used to index NO production. Lipopolysaccharide (100 ng/ml) and rat interferon γ (IFN; 10 U/ml) increased NO production and enhanced inducible NO synthase mRNA levels in cultured AMs. Treatment with silica (1-100 μg/ml) had no effect on basal NO levels. Furthermore, IFN-induced increases in NO were not altered by the simultaneous addition of silica (100 μg/ml) or by a 2 hr pretreatment with silica (1 μg/ml). To evaluate whether cell-to-cell interactions might be required for the induction of NO production during in vivo silica challenge, AMs (1×10^6) and non-adherent splenic lymphocytes (20×10^6) were cultured separately or together, with or without silica (100 μg/ml). The combination of these cell types resulted in a synergistic increase in NO above levels produced by each of these cell types alone. Furthermore, silica appeared to further enhance this effect. These data indicate that silica alone is not a sufficient stimulus to increase NO production by AMs and suggest that cell-to-cell interactions are important in NO generation by BALC during in vivo silica exposure.

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EFFECT OF DEPLETION OF ALVEOLAR MACROPHAGES (AMs) ON THE PATHOGENESIS OF RICIN-INDUCED ACUTE PULMONARY TOXICITY IN RATS. A. Assaad, C. Wilhelmsen, G. Parker, R. LeClaire, J. Kokes, V. Telada, L. Smith, L. Pitt, J. Estep (SPON.: R. Wannemacher). USAMRIID, Ft. Detrick, MD 21702

Ricin, a potent plant toxin and protein synthesis inhibitor, causes acute lung injury (ALI) by inhalation. Stimulated AMs release a large array of inflammatory mediators that may damage the integrity of the air-blood barrier and cause ALI. We tested the hypothesis whether AMs, and their secretory cytokine TNF-α play a role in the pathogenesis of ricin-induced ALI. We depleted AMs in rats by intratracheal insufflation of liposome-encapsulated dichloromethylene diphosphonate (L+MDP), a calcium-chelating agent (80 μl containing 1.34 μmol of Cl_2MDP). We treated 3 groups of rats (n=16/group) with L+MDP, L+PBS, or HBSS. Forty-eight hrs later, half of each group was exposed to either a lethal dose of inhaled ricin (52.6 mg/min/m^3 of air, nose only), or PBS. Twenty-four hr postexposure, rats were killed, and ALI parameters in bronchoalveolar lavage (BAL), hematology, lung water and histopathology were evaluated. L+MDP reduced AMs population by ~ 80%. Liposome+MPD and L+PBS treated, ricin-exposed animals developed less edema, compared to ricin controls (BAL total protein 50±10, 41.5±13 vs. 97±13, BAL albumin 19±3, 14±5 vs. 48±7 mg/dl, respectively, p<0.05). Neutrophilic response was similarly attenuated (73±5, 72±6, vs. 89±1% segmented neutrophils, respectively, p<0.05). Lung water was lower in L+PBS, ricin-exposed than in ricin controls (3.73±0.11 vs. 4.25±0.1 ml/gm dry lung, p<0.05). There were no significant changes in BAL TNF-α levels. High molecular weight proteins were not detected in BAL of liposome-treated, ricin-exposed animals. Histopathologically, there was evidence of significant attenuation of ALI in the same groups. We conclude that neither AMs, nor TNF-α play a significant role in the pathogenesis of ricin-induced ALI. The mechanism by which liposomes, attenuated ALI is unknown, but might be attributed to increasing the anti-oxidant capacity of lung cells.

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MECHANISMS OF VANADIUM TOXICITY IN RAT LUNG MACROPHAGES. Gregory M. Grabowski, Joseph D. Paulauskis, & John J. Godleski, Dept of Environmental Health, Harvard School of Public Health, Boston, MA 02115.

Vanadium compounds are known to elicit an inflammatory response in the respiratory tract. This study determines intracellular transport of vanadium ions, and relates these mechanisms to the cellular responses of rat lung macrophages (LMs). Transport processes responsible for the cellular uptake of metavanadate in LMs appear to be sodium dependent. Rat LMs obtained via bronchoalveolar lavage demonstrate a 36%±6.4 (mean ± SE, n=4) decrease in the uptake of ^{48}V label in the absence of sodium. Initial studies using isolated plasma membrane preparations from LMs, indicate that both metavanadate and phosphate enter LMs via a sodium dependent cotransporter. LMs exposed in vitro to concentrations of metavanadate greater than 5μg/ml exhibit an oxidative burst within 15 minutes, determined using a microplate based DCF-DCFHDA fluorescence assay. One mechanism implicated in this increased oxidative burst is the inhibition of tyrosine phosphatase. LMs preincubated with ^{32}P, were exposed to metavanadate (5μg/ml), lysed, subjected to SDS-PAGE, and autoradiographed. A single 15kDa protein was strongly labeled, which also stained positively with HRP-conjugated anti-phosphotyrosine antibody after Western blotting. Consistant with our hypothesis, entry of vanadium via a sodium dependent cotransporter inhibits tyrosine phosphatase, resulting in the enhanced phosphorylation of proteins, and the observed increased oxidative burst associated with vanadium exposure. Supported by NIH EHS 00002, ES 05947, HL 0718, and a Parker B. Francis Fellowship to J.P.

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AMRINONE DOWN REGULATES MULTIPLE INFLAMMATORY RESPONSES OF ALVEOLAR MACROPHAGES IN VITRO. R.J. Helmke and H.A. Hidalgo (SPON.: V.F. German). Dept. of Pediatrics, The University of Texas Health Science Center, San Antonio, TX 78284-7815.

Amrinone, an inotropic agent used for the management of cardiogenic shock, has recently been shown to inhibit macrophage TNF production (Giroir and Beutler, Circ Shock 36:200, 1992). Using an alveolar macrophage cell line (NR8383) exposed to clinically relevant concentrations of amrinone we have shown a down-regulation of a variety of macrophage inflammatory responses. Overnight exposure of 10^6 NR8383 cells to 0-40 mg/ml levels of amrinone inhibited, in a dose-dependent manner, the Zymosan (Zym) or lipopolysaccharide (LPS)-stimulated release of interleukin 6 (IL-6), and tumor necrosis factor (TNF); the LPS and interferon γ (IFN)-stimulated release of nitrite (NO_2^-), and the zymosan-stimulated release of hydrogen peroxide (H_2O_2) as shown:

Function	IL-6		TNF		H_2O_2	NO_2^-
Stimulus	LPS	ZYM	LPS	ZYM	ZYM	LPS + IFN
% Inhibition	51-98	21-99	21-83	41-95	30-63	69-88
Amrinone μg/ml	20-40		20-40		10-40	20-40

These data suggest that amrinone affects a spectrum of macrophage inflammatory responses at concentrations that are found in the serum of patients treated with this drug.

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ABSTRACTS

PART I

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