166.1

NOVEL ASSOCIATION OF CONGENITAL ADENOVIRUS INFECTION IN PRETERM INFANTS AND BRONCHOPULMONARY DYSPLASIA DEMONSTRATED BY POLYMERASE CHAIN REACTION

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Bronchopulmonary dysplasia (BPD) is recognized as an important cause of morbidity and mortality in preterm infants. Since the role of congenital infections in BPD has been debated, the purpose of this study was to test the hypothesis that detection of infectious agents in tracheal aspirate samples was associated with the development of BPD. Tracheal aspirate samples were obtained within the first week of life and screened by polymerase chain reaction for adenovirus, cytomegalovirus, parvovirus, enteroviruses, *Ureaplasma urealyticum*, *Mycoplasma hominis*, *Mycoplasma pneumoniae* and *Chlamydia* species. BPD was defined as persistent oxygen dependence at 28 days of age and 36 weeks postconceptional age (PCA). Infants that expired prior to these time points were excluded from statistical analysis. Out of 89 infants studied, at 28 days of life, 13 had expired, 45 had BPD and 31 had no BPD (controls). At 36 weeks PCA, 15 infants expired, 39 still had BPD and 35 did not. A significant increase in the frequency of adenovirus genome was identified in BPD patients compared with controls, both at 28 days of life (12/45=27% versus 1/31=3%: $p<0.01$) and at 36 weeks PCA (10/39=29% versus 2/35=6%: $p=0.01$). Other microorganisms were rarely detected and not associated with the development of BPD. This is the first study that reveals both the frequency of congenital adenoviral infection and its significance in the development of BPD and may be important in the pathogenesis of other fetal and neonatal diseases.

166.2

INTERSTITIAL PNEUMONITIS ELICITED BY TRACHEAL INSTILLATION OF HELICOBACTER PYLORI UREASE IN RAT

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Helicobacter Pylori (HP) infection is widely studied in the GI tract, but less is known about its potential effect on the lung when gastric contents are regurgitated and aspirated. Recently, HP infection has been associated with Sudden Infant Death Syndrome and sarcoidosis. We report the effect of repeated aspiration of the HP-secreted product urease on the lung parenchyma of rats.

Halothane anesthetized male rats (200-250 grams weight) chronically received 3 weekly doses of 0.545 units of Jackbean urease (Sigma) dissolved in 0.1 ml normosaline. This dose is the equivalent of urease produced by instillation of 10^8HP. Controls received an equal volume of normosaline.

Extensive bronchial disepithelization with presence of necrotic material, neutrophils, and macrophages was evident, especially after the first week of treatment. Small peribronchial arteries showed periadventitial edema and medial thickening. This vasculitis progressed with the number of instillation and was severe at the end of the experiment (6 weeks). At that time, collagen and macrophages spread from the adventitia to the alveolar septa and interstitial pneumonitis was diffuse. Tumor necrosis factor was overexpressed in bronchial epithelium and in alveolar macrophages, especially in the phase preceding the onset of fibrosis. Saline controls showed a mild inflammatory response but no vasculitis or fibrosis.

This model demonstrated a risk for pulmonary damage consequent to repeated regurgitation of HP products. The model could also be useful for the study of pathogenesis of interstitial pneumonitis and fibrosis.

166.3

EXPERIMENTAL PROPIONIBACTERIOSIS INDUCED BY *P.acnes*

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It is believed that *Propionibacterium acnes* is responsible for pathogenesis of sarcoidosis in Japan. Sarcoidosis is one of granulomatous disease with unknown origin and continuous efforts are being made toward clarification of sarcoidosis. In order to investigate the role of *P.acnes* in sarcoidosis, we carried out the following experiments. Live *P.acnes* (10^{10}) was injected to IFN-gamma, TNF-alpha knockout mice subcutaneously. Two week later live, dead P.acnes or plasmid containing *P.acnes*-derived hyaluronidase gene was injected three times at two-week intervals. *P.acnes* was also introduced once in lungs of these knockout mice by air-borne route. Glanulomatous lesions were induced in the lungs of the knockout mice when live Propionibacterium was used. No granulomatous lesions were recognized in the lungs of wild-type mice and knockout mice injected with dead *P.acnes*. Plasmid containing *P.acnes*-derived hyaluronidase gene did not induce any granulomatous lesions. It is interesting that granulomatous lesion could be induced in mice with immunological abnormality such as IFN-gamma, TNF-alpha knockout mice. We are now examining severity granulomatous lesions in other knockout mice with immunological abnormalities.
(Collaborators: Prof. Y. Iwakura, Inst. Med. Sci., University of Tokyo. Dr. K. Sekikawa, Inst. Poultry Sci. Hygiene, Ministry of Agriculture, Japan. K. Watanabe, School of Medicine., University of Gifu.)

166.4

IL-15 Release in Interstitial Lung Disease.

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IL-15 shares several biological activities with IL-2, i.e. it stimulates T cell proliferation and activates B cells, NK- and LAK-cells. Both cytokines use the β and γ-chain of the IL-2 receptor. IL-15 as well exhibits regulatory properties on macrophage pro-inflammatory cytokine release. IL-15 is found to be released by non-lymphoid cells, e.g. monocytes and macrophages. In many lung diseases alveolar macrophages (AM) are activated and release proinflammatory cytokines. We asked whether IL-15 is released in vitro by AM and peripheral blood mononuclear cells (PBMC) from patients with sarcoidosis (SAR), tuberculosis (TB), hypersensitivity pneumonitis (HSP), cryptogenic fibrosing alveolitis (CFA), and pneumonia (PN). Additionally, we looked for the kinetics of the IL-15 release of these cells. During 24h of culture AM from controls (CO) released 5.2±2.3 pg/ml (mean±SD) which was not significantly different from the amount released by AM from patients with inactive SAR (6.9±1.8 pg/ml), CFA (5.7±1.5), PN (7.8±2.6) and HSP (9.3±9.5). IL-15 release by AM was significantly increased in patients with active SAR (8.7±3.9, $p<0.02$) and TB (8.4±1.9, $p<0.005$). PBMC from patients with inactive and active SAR released significantly more IL-15 than PBMC from CO (9.8±2.8 and 10.8±8.9 versus 5.4±0.8, $p<0.05$) whereas the PBMC IL-15 of the other groups did not differ from CO (TB: 5.7±1.4; CFA: 4.6±1.6; HSP: 4.9±3.8). Kinetic studies revealed for AM and PBMC a first peak after 5h and a main peak from 12h to 35h. In summary, AM from patients with sarcoidosis and tuberculosis release increased levels of IL-15 although the total amount of this cytokine is very low.

166.5

HEPARAN SULFATE PROTEOGLYCANS REGULATE ACCUMULATION AND ELASTASE-MEDIATED RELEASE OF bFGF IN PULMONARY FIBROBLASTS. J. Buczek-Thomas and M. A. Nugent, Boston University School of Medicine, Boston, MA 02118

Basic fibroblast growth factor (bFGF) is a potent regulator of elastase-mediated injury in the lung. bFGF elicits its effects by binding to high affinity cell surface receptors and to heparan sulfate proteoglycans (HSPG) which are also targets for elastase *in vitro*. While HSPG facilitate the interaction of bFGF with its receptor, the role of HSPG in modulating bFGF release by elastase within the extracellular environment is not well characterized. In the present study, we have investigated the release of bFGF and glycosaminoglycan (GAG) in native and HSPG-deficient pulmonary fibroblasts. HSPG-deficient fibroblasts were produced by treating the cells with 50mM sodium chlorate to inhibit (>97%) sulfation of cellular proteoglycans without affecting cellular viability. Native and HSPG-deficient fibroblasts were exposed to porcine pancreatic elastase at 2-4 day intervals and elastase-mediated release of bFGF and GAG were determined. Treatment of HSPG-deficient fibroblasts with elastase resulted in significantly less bFGF release relative to native cells at all times in culture as determined by ELISA. Quantitation of cellular glycosaminoglycans using the dimethylmethylene blue colorimetric assay indicated that GAG released by elastase increased as a function of time in culture in native cells whereas HSPG-deficient cells released significantly less GAG (0.5-8 fold) during the same culture period. These results indicate that HSPG play a central role in modulating bFGF accumulation and release from the ECM and suggest that HSPG have dramatic effects on the biological function of bFGF under conditions of elastase-induced lung injury.

166.6

Hemoglobin potentiates the generation of reactive oxygen species by alveolar macrophages. L.J. Huffman, X. Shi, L. Bowman and P.R. Miles, NIOSH, Morgantown, WV 26505.

The objectives of the present investigation were to study the effects of hemoglobin on free radical production by alveolar macrophages. Alveolar macrophages were harvested from anesthetized male rats. Free radical production by alveolar macrophages was assessed by measuring integrated luminol-enhanced chemiluminescence responses (cpm·10^3/0.5·10^6 cells) and electron-spin resonance profiles under basal and activated conditions. A soluble phorbol ester (PMA; 12-O- tetradecanoyl-phorbol-13-acetate) was used to initiate free radical production by alveolar macrophages. Hemoglobin (0.1-1.00 µM) significantly enhanced PMA-stimulated chemiluminescence from alveolar macrophages in a dose-related manner. The possibility that hemoglobin may act as a biological Fenton reagent to promote enhanced free radical responses by alveolar macrophages was explored next. Addition of either an iron chelator, the removal of hydrogen peroxide or superoxide anions, or the presence of hydroxyl radical scavengers significantly decreased the effect of hemoglobin. These findings suggest that hemoglobin enhances free radical production by alveolar macrophages by acting as a biological catalyst and that the pronounced increase in reactive species generation is the overall result of an iron-catalyzed Haber-Weiss reaction. Thus, alveolar macrophages may be a cell type which contributes to the potentiation of lung damage when hemoglobin is present.

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ABSTRACTS

FRIDAY MORNING
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American Society for Investigative Pathology

American Association of Anatomists

SATURDAY AFTERNOON
April 17, 1999

American Society for Investigative Pathology

American Society for Nutritional Sciences

American Association of Anatomists

SUNDAY MORNING
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The American Physiological Society