AIRWAYS INFLAMMATION

Organizer: Stephen Rennard

March 5-11, 1990

Plenary Sessions Page

Poster Sessions

Airways Inflammation

Keynote Address

J 001 BRONCHOALVEOLAR LAVAGE - EVOLUTION AND FUTURE OF THE PROCEDURE, Herbert Y. Reynolds, Department of Medicine, Pennsylvania State University College of Medicine, Milton S. Hershey Medical Center, Hershey, PA 17033.

Bronchoalveolar lavage (BAL) extends the usefulness of bronchoscopy by providing a sample of bronchial airway secretions and alveolar epithelial lining fluid and respiratory cells from both of these areas. This has opened the window into the living airways a bit wider than was anticipated. BAL has been performed for over 50 years through a variety of conduits placed in the trachea or major bronchi, but with development of the flexible fiberoptic bronchoscope in the late 1960's, the procedure became more widely used in normals and in those with a variety of lung diseases. Spurred by immunologic technology of the 1970's that could identify and manipulate respiratory cells and proteins, the cellular milieu of the airspaces was described for normals, cigarette smokers and patients with diffuse interstitial lung diseases of many kinds. Later in the 1980's cell secretory products, cytokines and mediators, were measured in BAL which gave additional insight into how cells might communicate and mutually stimulate or down regulate each other, as the process of fibrosis, inflammation or granuloma formation was studied. Presently, immunogenetic probes are revealing even more about intranuclear metabolism, forces that propel cell differentiation and mechanisms of protein secretion.

With this age of molecular biology, what can BAL cellular and fluid analyses reveal about normal host defenses and derangements that occur with disease? First, the responses of lung cells may be unique and not like counter parts in peripheral blood. Second, subpopulations of cells, based on special profiles of mediators or cellular activity, will permit subsets of patients with heretofore "single" diseases to be identified and selective therapy to be individualized. Third, molecular genetic manipulation of precursor cells will condition end-stage differentiated cells to have new functions or augmented capability. Fourth, more selective study of conducting airway versus the alveolar space will be possible with improved BAL technology. Fifth, more precise indications for use of BAL analysis will be formulated for clinical situations, as is underway now by groups of European and U.S. colleagues. It seems probable that BAL will continue to be an important research tool and will have expanded clinical uses.

General References: Reynolds HY: Bronchoalveolar Lavage - State of art. Am Rev Respir Dis 137:250-267, 1987. Reynolds HY: Pulmonary host defenses - State of the art. Chest 95:223-230S, 1989.

Techniques to Sample the Lung

J 002 THE POTENTIAL UTILITY OF NASAL LAVAGE (NAL) IN ASSESSING AN INFLAMMATORY RESPONSE IN THE LOWER AIRWAYS, Hillel S. Koren, Delores E. Graham, David A. Johnson and Robert B. Devlin, US EPA Clinical Research Branch, Research Triangle Park, N.C. 27711, and E. Tennessee State University, T.N. 37614

The nose is the primary portal of entry for inspired air, and therefore, the first region of the respiratory tract in contact with airborne irritants. Nasal lavage is easy to perform, is non-invasive and atraumatic, allowing collection of multiple sequential samples from the same person. The number of neutrophils (PMN) has been shown to increase by a factor of 10-100 during an upper respiratory tract viral infection. In this study we wished to determine the utility of NAL as an indicator of an acute inflammatory response occuring in the lower airways as also determined by bronchoalveolar lavage (BAL). Volunteers were exposed to 0.4 ppm ozone or air for 2h. NAL was performed pre-, immediately post-and 18h post-exposure, while BAL was performed at 18h post exposure only. The results indicated significant increases (6 to 7-fold) in the number of PMN in NAL immediately and 18h post-exposure and in BAL at the 18h time point. Tryptase, released by mast cells, was also increased in the NAL fluid immediately post exposure (2-fold). While the albumin level, which is an indicator of epithelial cell permeability, was elevated 18h post exposure (1.5-fold) in both BAL and NAL, tryptase level was no longer elevated at this time point in the NAL but was elevated in BAL. Other markers of acute inflammation (e.g.: PGE2, C3a, urokinase-type plasminogen activator) which were found to be elevated in the BAL, were not elevated in the NAL at any time point. The data suggest that NAL may serve as a sensitive measurement to detect inflammation in the upper respiratory tract, and there appears to be a relationship between the inflammatory response in the upper and lower airways, 18 hr after exposure to ozone, as measured by PMN and albumin levels.

This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

J 003 CULTURE OF NORMAL HUMAN AIRWAY EPITHELIAL CELLS, John F. Lechner and Curtis C. Harris, Laboratory of Human Carcinogenesis, NCI, NIH, Bethesda, MD 20892.
Because of frequent exposure to chemical and physical cytotoxic agents, hyperplasia and squamous metaplasia, phenomena for sequestering and detoxifying xenobiotics and mending injured airway epithelium, are commonly encountered. Clearly, these repair processes demand strict inter-regulation of cell replication and squamous and mucoepidermoid differentiation control mechanisms, and, not unexpectedly, aberrations in these pathways are positively correlated with neoplastic transformation of the involved cells. In order to efficaciously investigate these pathways for growth regulatory processes and eventually identify the structural and controlling genes, we have developed methods and media for culturing the main-stem airway epithelial cells obtained at autopsy from donors without cancer. These techniques have been described in detail (1); however, the model is continually being upgraded and improved. The presentation will initially, briefly, recount these published methodologies and their rationales and lastly, delineate newly perfected features of the model.

(1) Lechner, et al., Cancer Res. 41:2294 '81; Lechner, et al., In Vitro 18:633 '82; Lechner, et al., Cancer Res. 43:5915 '83; Lechner, et al., Differentiation 25:229 '84; Willey, et al., J. Cell. Physiol. 124:207 '85 and Lechner & LaVeck J. Tiss. Cult. Method. 9:43 '85.

J 004 NEW DEVELOPMENTS IN DIFFERENTIATION OF AIRWAY EPITHELIALCELLS IN CULTURE, Reen Wu, Cynthia B. Robinson, Walter R. Martin, and Tim H. Huang, California Primate Research Center, University of California, Davis California 95616.
Expression of differentiated function is an intrinsic property of most epithelial cells. The phenotypic expression of this intrinsic property is regulated by the interplay between growth factors and extracellular matrix. Conducting airway epithelial cells, like many other epithelial cells, tend to lose their differentiated functions in culture. However, this phenotypic dedifferentiation is transient and can be reversed in most cases by providing cells with appropriate conditions. This idea was first realized several years ago in the hamster tracheal epithelial cell culture system. Both ciliated and mucus-secreting cell populations increase with time in confluent culture. Vitamin A and collagen gel substrata are both important to the development of hamster cell differentiation. However, this result was not reproduced in human and other animal cells. To overcome this deficiency, several areas have been examined in detail. 1) A biphasic culture system has been developed, in which conducting airway epithelial cells are fed basally in the same way as in vivo. Overall cell morphology and polarity of cell differentiation are improved and, most of all, guinea pig tracheal cells are able to express new cilia and mucus secreting granules. 2) Optimiziation of medium calcium level has resulting in an increase of mucous cell differentiation in culture. 3) Interference with normal matrix formation by TGF-β results in an inhibition of the expression of mucous cell function. These new findings are all related to the integrity of the epithelial cell layer in culture. It is proposed that the proper maintenance of epithelial-epithelial cell interactions may play a critical role in the development of differentiated cells.

Mechanisms of Airways Hyperresponsiveness

J 005 ASTHMATIC REACTIONS, AIRWAY HYPERRESPONSIVENESS AND AIRWAY INFLAMMATION INDUCED BY TOLUENE DIISOCYANATE (TDI).

Leonardo M. Fabbri and Cristina E. Mapp, M.D., Laboratory of Respiratory Pathophysiology, University of Padova, 35127 Padova, Italy

To test the hypothesis that toluene diisocyanate (TDI) causes late asthmatic reactions and increases airway responsiveness through an acute inflammatory reaction of the airways, we examined sensitized subjects during asthmatic reactions induced by exposure to TDI in the laboratory. We observed that late and dual, but not early, asthmatic reactions induced by TDI are accompanied by a transient increase of airway responsiveness and, by a bronchoalveolar neutrophilia followed by eosinophilia and by an increase of LTB4 and albumin in bronchoalveolar lavage fluid. The late asthmatic reactions, the increase in airway responsiveness, and the increase of neutrophils, eosinophils, LTB4 and albumin concentration in bronchoalveolar lavage induced by exposure to TDI were all prevented by the pretreatment with prednisone but not with the nonsteroidal antiiflammatory agent indomethacin. Recently, we observed that TDI, in vitro, activates the "efferent" function of capsaicin-sensitive primary sensory nerves. TDI-induced contractions are partly due to the release of substance P or other tachykinins from sensory nerves. TDI-induced contractions are significantly inhibited by steroids and by the tachykinin antagonist (D-Arg1,D-Pro2,D-Trp7,9,Leu11)-substance P. These results suggest that TDI-induced asthmatic reactions may be due to both smooth muscle contraction and airway inflammation and that the release of neuropeptide(s) may be involved.

Fabbri LM, Boschetto P, Zocca E et al. Bronchoalveolar neutrophilia during late asthmatic reactions induced by toluene diisocyanate (TDI). Am Rev Respir Dis 1987; 136: 36-42.

Boschetto P, Fabbri LM, Zocca E et al. Prednisone inhibits late asthmatic reactions and airway inflammation induced by toluene diisocyanate in sensitized subjects. J Allergy Clin Immunol 1987, 80: 261-267

J 006 MECHANISMS OF AIRWAYS HYPERRESPONSIVENESS, Gary W. Hunninghake, Pulmonary Division, Department of Internal Medicine, University of Iowa College of Medicine, Iowa City, Iowa 52242.

A characteristic feature of allergic asthma is hyperresponsiveness of the airways. To evaluate the mechanisms of this hyperresponsiveness, we triggered an asthmatic response in the airways of allergic asthmatics by instilling the relevant allergen into a single airway by bronchoalveolar lavage. After instillation of the allergen, the airway was lavaged with saline. Other airways which were sham challenged with allergen and lavaged in the same manner were used as controls. After exposure to allergen, there was an immediate increase in histamine in allergen challenged but not sham challenged airways. A variety of nonspecific challenges did not result in an increased release of histamine. These findings were evidence of mast cell degranulation in response to allergen. The amounts of histamine in the airways at baseline (prior to challenge) correlated with the numbers of eosinophils in the airways and sensitivity of the airways to methcholine. These observations show that ongoing mast cell degranulation correlates with airways inflammation and airways hyperreactivity. Challenge of airways with allergen also resulted in an increase in airways permeability as measured by an influx of radiolabeled albumin into the airways. These observations demonstrate that this technique can be used to evaluate airways inflammation and hyperreactivity in patients with asthma.

J 007 INFLAMMATION, ASTHMA AND THE LATE ASTHMATIC RESPONSE. Sally E. Wenzel, Hunter R. Smith, Richard J. Martin, Robert A. Bethel, Norbert K. Voelkel, Jay Y. Westcott and Gary L. Larsen, Departments of Medicine and Pediatrics, National Jewish Center for Immunology and Respiratory Medicine, and University of Colorado Health Sceinces Center, Denver, CO 80206

The pathogenesis of asthma has been associated with an inflammatory process within the lung at both a cellular and biochemical level. To study the relationship of inflammation with airway obstruction and hyperresponsiveness, two clinical models have been employed: the antigen-induced late asthmatic response (LAR) produced within the laboratory and naturally occurring nocturnal asthma. In terms of the former model, when atopic asthmatics were divided into 3 groups based on their response to antigen challenge (immediate asthmatic response [IAR] alone, IAR with equivocal LAR, and IAR with a definite LAR), a markedly increased airway responsiveness prior to antigen challenge identified the group of subjects most likely to have an IAR followed by a definite LAR. Additional increases in the level of airway responsiveness to methacholine were not noted after antigen exposure in asthmatics with immediate responses alone. On the other hand, subjects with equivocal and definite LARs had significant increases in methacholine responsiveness. Evaluation of bronchoalveolar lavage (BAL) fluid 12 hours after antigen inhalation showed airway inflammation (as reflected by the percent of lavage neutrophils plus eosinophils) was related to the severity of the LAR. In contrast, no relationship was seen between the type of airway response and the pattern of eicosanoid production found in lavage fluid 12 hours after challenge. However, a variation of the airway challenge model utilizing localized allergen instillation with BAL 5 minutes following instillation detected differences in mediator levels between asthmatic subjects with and without LARs. Subjects with LARs actually had significantly smaller increases in a variety of mediators (histamine, prostaglandin D_2 and leukotriene C_4) than subjects without a LAR. No differences in cell counts or differentials were seen at this early time point. Late responders again had significantly greater airway responsiveness before antigen exposure. In the other clinical model used for study, subjects with nocturnal asthma had marked increases in airway responsiveness at 0400 versus 1600 hours. Significant increases in lavage neutrophils, eosinophils, lymphocytes, and epithelial cells were also seen at 0400 hour in asthmatics with nocturnal asthma but not in asthmatics without nocturnal problems. In studies examining both the late asthmatic response and nocturnal symptoms in stable asthmatics, it was noted that antigen challenge in the evening as opposed to the morning led to a greater number of LARs, more severe airway obstruction, and greater increases in airway responsiveness. This suggests that exposure to a phlogistic stimulus (antigen) when the airways may be more vulnerable to develop inflammation (night) may lead to enhanced physiologic responses. While much remains unknown regarding inflammation and asthma, these studies suggest that events are occurring in the airways at a cellular and biochemical level which may lead to altered airway function with the production of obstruction and/or increased responsiveness.

Bronchial Epithelial Cell Biology in Inflammation

J 008 ONCOGENES AND TUMOR SUPPRESSOR GENES INVOLVED IN HUMAN LUNG CARCINOGENESIS, Curtis C. Harris, Roger Reddel, Rama Modali, Teresa A. Lehman, Deborah Iman, Mary McMenamin, Haruhiko Sugimura, Ainsley Weston and Andrea Pfeifer. Laboratory of Human Carcinogenesis, National Cancer Institute, Bethesda, MD 20892.

Six families of activated proto-oncogenes, ras, raf, fur, jun, neu and myc have so far been associated with human lung cancer. Human bronchial epithelial cells in vitro are being used to investigate the functional role of these specific oncogenes and growth regulatory genes in carcinogenesis and tumor progression. When transferred into normal human bronchial epithelial cells by the highly efficient protoplast fusion method, the v-Ha-ras oncogene initiates a cascade of events in the cells leading to their decreased responsiveness to inducers of squamous differentiation, aneuploidy, and rarely, "immortality" and tumorigenicity with metastasis in athymic nude mice. Transfection of the SV-40 T antigen gene leads to nontumorigenic cell lines that have a nearly normal pathway of terminal squamous differentiation and can be transformed to malignant cells by transfected ras oncogenes. The combination of transfected c-myc and c-raf-1 will also cause transformation of human bronchial epithelial cells to neoplastic cells that exhibit some phenotypic traits found in small cell carcinomas. These and other results indicate that oncogenes dysregulate pathways of growth and differentiation in human bronchial epithelial cells and play an important role in human lung carcinogenesis.

Allelic sequence deletion and somatic cell hybrid analyses are being used to identify the chromosomal localization of putative tumor suppressor genes. Among 23 squamous cell carcinomas (SQC), loss of heterozygosity was more frequent than among 23 adenocarcinomas or 8 large cell carcinomas. Loss of heterozygosity for chromosome 17p was found in 89% of SQC when compared to 18% of adenocarcinomas and mutations in the putative tumor suppressor gene, p53, on chromosome 17p have also been observed in some cases. Allelic deletion analysis of chromosome 11 revealed two commonly deleted regions (11p13-11q13 and 11p15.5). Somatic cell hybrids between normal human bronchial epithelial cells and Hut292DM, a lung carcinoma cell line, had a finite lifespan in vitro. Tumor suppressive effects of individual or combinations of specific human chromosomes on Hut292DM are being examined by formation of microcell-cell hybrids. Chromosome 11 has tumor suppressor activity in these hybrids. Both of these studies suggest that tumor suppressor genes may play a major role in lung carcinogenesis and provide in vitro model systems for isolating these genes by subtraction library and insertional mutagenesis technologies.

J 009 THE ROLE OF GROWTH FACTORS IN THE REGULATION OF CELL PROLIFERATION OF NORMAL AND TRANSFORMED TRACHEOBRONCHIAL CELLS. P. Nettesheim, P.C. Ferriola, A. Robertson, R. Steigerwalt, Laboratory of Pulmonary Pathobiology, N.I.E.H.S, Research Triangle Park, NC 27709

A large number of positive and negative growth factors have been discovered during the past decade which regulate cell proliferation with varying degrees of tissue specificity. Relatively little is known about the regulation of cell proliferation in different cell compartments of the respiratory tract and how it may be influenced by disease, e.g. inflammation and transformation. Our laboratory has engaged in studying critical differences in growth behaviour between normal and transformed tracheobronchial epithelial cells in an effort to determine the mechanisms underlying the enhanced and seemingly unlimited capacity for growth of preneoplastic and neoplastic cells, respectively. In this presentation I will discuss the following topics: **1)** Identification of some important growth factors relevant for tracheobronchial epithelial cells. Studies in our and other laboratories have identified EGF/TGFα and TGFβ as important positive and negative growth factors, respectively, for tracheobronchial epithelial cells. **2)** Changes occurring in growth factor dependence or responsiveness during transformation. Transformed tracheobronchial cells have a reduced requirement for EGF/TGFα to sustain growth in culture. Their responsiveness to growth inhibitory effects of TGFβ is often markedly reduced as well. **3)** Possible mechanisms for changes in TGFα and TGFβ responsiveness. These may involve changes in receptor number or function and changes in post receptor signal pathways. **4)** Sources of growth factors in normal and diseased tissues. These include macrophages and possibly other cells involved in the inflammatory process.as well as the tracheobronchial epithelial cells themselves. Thus, growth factors such as TGFα and TGFβ may fulfill paracrine as well as autocrine functions in the tracheobronchial mucosa, and may play a role in epithelial regeneration or during various stages of neoplastic transformation.

J 010 AIRWAY EPITHELIAL CELL DIFFERENTIATION IN RESPONSE TO INJURY.
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The tracheobronchial epithelium may be injured by physical factors, infectious agents, as well as inhaled irritants and toxic oxidants. The recruitment of mononuclear cells to sites of inflammation and injury is accompanied by the release of soluble factors, such as the inflammatory cell-derived peptide growth factors, which play a key role in the regulation of inflammatory events, as well as in the tissue injury and repair (1). All these factors contribute to tissue damage either by injuring the paracellular pathway and increasing the tight junctional permeability or by injuring directly the epithelial cells. The acute response, as well as the regeneration of the airway epithelium, vary according to the severity and duration of the injury. If the epithelial damage is minor, there is a limited epithelial cell loss, whereas in severe denuding injuries, cells at the wound margin divide and migrate to restore the injured epithelial structure. If the damage is more severe, with a denudation of the basal lamina, a marked mitogenic stimulation associated to basal and mainly to mucous cells is observed. The restoration of the barrier function exerted by tight junctions represents an early step in the process of regeneration and restoration of the tracheal epithelium. Recent studies focused on the regeneration of the airway epithelium following mechanical or chemical injury, in vivo and in vitro, have shown that the restoration of the mucociliary epithelium depends upon mucous cell proliferation which may produce a transient epidermoid metaplasia (2). The regeneration of the epithelium is mainly the result of a "transdifferentiation" mechanism, with mucous cells differentiating into ciliated cells. The development and regeneration of tracheal epithelium following injury in humans, as well as in animals, show striking similarities with modulations which normally occur in the maturation process of the tracheal epithelium. Studying the in vivo ciliogenesis during human tracheal epithelium maturation, we have demonstrated that, as in the epithelium repair, the secretory cells play a major role in the in vivo ciliogenesis (3). Fused or compound-like cilia also described in bronchial infections, represent a rapid ciliary differentiation rather than a sign of an acquired ciliary abnormality.

1. WAHL S.M., WONG H. and Mc CARTNEY-FRANCIS N. : J. Cell Biochem., 40, 193-199, 1989.
2. Mc DOWELL E.M., BEN T., COLEMAN B., CHANG S. and DE LUCA L.M. : Am. J. Pathol., 129, 511-522, 1987.
3. GAILLARD D., LALLEMENT A., PETIT A. and PUCHELLE E. : Am. J. Anat., 185, 415-428, 1989.

J 011 BRONCHIAL EPITHELIAL CELLS AS MODULATORS OF AIRWAYS INFLAMMATION, Stephen I. Rennard, Shunsuke Shoji, James Linder, Sekiya Koyama, Susanna Von Essen, Debra Romberger, Joe Beckmann, Ron Ertl, Austin B. Thompson, Richard A. Robbins, Department of Internal Medicine, University of Nebraska Medical Center, Omaha, NE 68105.

The epithelial cells which line the surface of the airways are the first parenchymal lung cells to encounter environmental agents. These cells participate in host defense through several well recognized mechanisms including providing a barrier and in helping to mediate the clearance of inhaled agents. In addition, the airway epithelial cells are capable of modulating diverse aspects of the immune and inflammatory response. In order to evaluate the capability of airway epithelial cells to participate in the recruitment and modulation of inflammatory cells, airway epithelial cells have been cultured using the technique of Wu to prepare cells from bovine bronchi. Airway epithelial cells can then be maintained in culture and exposed to a variety of stimuli. Supernatant medium from these cultures have been tested for chemotactic activity using the modified Boyden chamber technique. Utilizing these techniques, it has been demonstrated that airway epithelial cells are capable of mediating the recruitment of neutrophils, eosinophils, lymphocytes and monocytes. A variety of stimuli including endotoxin, cigarette smoke and grain dust extract are capable of upregulating the release of the chemotactic activities. While not completely characterized, both low molecular weight lipid and peptide chemotactic activities appear to be released. In addition to mediating the recruitment of lymphocytes, airway epithelial cells express class I and class II MHC antigens, and the expression of these antigens can also be stimulated suggesting that airway epithelial cells cannot only recruit lymphocytes, but participate in the regulation of their state of activation. Finally, airway epithelial cells are capable of releasing chemotactic activities for both fibroblasts and other epithelial cells suggesting a possible role in mediating repair following injury. This activity appears to be mediated, at least in part, by fibronectin, a multifunctional glycoprotein thought to function in many locations as a mediator of repair. Airway epithelial cells not only produce fibronectin, but production can be upregulated by TGF-beta. In summary, airway epithelial cells are capable of releasing a diverse array of chemotactic activities in response to a number of stimuli. The ability of these cells to mediate the recruitment of other cells may play an important role in regulating airway inflammation and, possibly, in airway repair following injury.

Low Molecular Weight Mediators of Inflammation

J 012 PAF-ACETHER (paf) IN AIRWAYS INFLAMMATION. B.Arnoux, A.Denjean*, J.Benveniste. INSERM U 200 and *Lab Physiol. Hôpital A. Béclère, 92140 Clamart, France.

Paf, a proinflammatory phospholipid mediator is released by various inflammatory cells under specific or unspecific stimulation. It is also synthetized by pneumocyte type II cells. Paf and its precursors were detected in bronchoalveolar lavage (BAL) from inflammatory occupational lung disease patients, and also in bronchial mucus from children with cystic fibrosis and chronic pulmonary diseases. The pathophysiological role of paf was particularly studied in man and in animal models in inflammatory bronchial hyperreactivity (BHR) associated to asthma. Paf is released by stimulated alveolar macrophages from atopic patients. It is well established that,in man as well as in baboons, rabbits and guinea pigs, intratracheal deposition of paf induces an immediate and reversible increase of lung resistance and a decrease of dynamic lung compliance associated with blood cells and gas modifications. In guinea pigs and baboons, transitory platelet accumulation was detected in the lung during paf-induced bronchoconstriction. One hour following tracheal paf deposition in baboons we observed by BAL an eosinophilia that was still present 14 days later. Eosinophils were also detected in guinea pig lung parenchyma after i.v. paf administration, but no information on paf-induced eosinophilia is available in man. The specific chemotactic activity of paf on human eosinophils has been reported in vitro, and was inhibited by anti-paf drugs or anti-asthma drugs. Moreover, in humans as well as in monkeys, dogs, sheeps and guinea pigs, paf induced a non specific BHR to methacholine, that was detectable within an hour following paf inhalation. It peaked at day-3 in humans, to return to baseline value on the 14th day. In some human volunteers, BHR could be detected up to 3 weeks. In our recent experiments in baboons, non specific BHR was detectable 1 hr after paf deposition, peaked at day +7, and was still present on day + 14. It was inhibited by the anti-asthma drug Nedocromil Na (but not the eosinophilia) and by the anti-paf drug WEB 2170. A correlation between the number of alveolar eosinophils and spontaneous non specific BHR was reported in cynomologus monkeys; yet we failed to observe such a correlation in baboons treated with paf. Since pharmacological means do not allow to definitely associate the presence of eosinophils with bronchial hyperreactivity, the relationship between the two latter parameters is still not properly understood. It remains that the bulk of these data makes paf a good candidate as (one of) the mediator(s) of airways hypersensitivity observed in human airway inflammatory diseases.

J 013 SULPHIDOPEPTIDE LEUKOTRIENES AND THE ASTHMATIC RESPONSE: MULTIPLE LINES OF EVIDENCE FOR A CRITICAL ROLE FOR THIS FAMILY OF INFLAMMATORY MEDIATORS. Jeffrey M. Drazen, Robert Dermarkarian, Larry Pliss, and Elliot Israel, Combined Pulmonary Program at Beth Israel Hospital, Brigham and Women's Hospital, Children's Hospital and Harvard Medical School, Boston, Massachusetts 02215.

The sulphidopeptide leukotrienes, LTC_4, LTD_4 and LTE_4, are lipoxygenase derivatives of arachidonic acid with a peptide conjugated via a thioether link at the C-6 position. Two cell types thought to be of importance in the asthmatic response, the mast cell and the eosinophil, have the enzymatic capacity to produce these mediators. When these mediators are administered by inhalation to normal and asthmatic human subjects they elicit potent and long lasting airway constriction. The inhalation of micromolar concentrations of LTD_4 results in airway narrowing of similar magnitude and of prolonged duration compared to that produced by millimolar concentrations of methacholine or histamine. The bronchoconstriction elicited by inhalation of LTD_4 can be blocked by LTD_4 receptor antagonists consistent with action at a specific receptor in the airways. Sulphidopeptide leukotrienes have been recovered in greater amounts in the blood, urine, and bronchoalveolar lavage fluid of subjects with asthma compared to normal subjects. The amount of LTE_4 recovered in the urine of subjects presenting to an emergency service with acute asthma decreased in concert with improvement of the airway obstruction. Two structurally distinct LTD_4 antagonists, when given at doses that are capable of shifting the LTD_4 dose-response curve approximately 4 fold, have been shown to have a small but salutary effect on laboratory induced asthmatic responses. In contrast an inhibitor of 5-lipoxygenase, an enzyme critical in the formation of leukotrienes, had a much stronger protective effect on asthmatic responses induced by the inhalation by isocapneic hyperpnea of cold-dry air. These 4 distinct lines of evidence are consistent with the hypothesis that sulphidopeptide leukotrienes play at least a partial role in the induction of asthmatic responses.

J 014 REGULATION OF ARACHIDONIC ACID OXYGENATION IN SURFACE EPITHELIAL CELLS FROM LUNG AND SKIN, Michael J. Holtzman, Department of Medicine, Washington University School of Medicine, Saint Louis, MO 63110

The surface epithelial cell serves as an active interface between environmental agents and the underlying cells. Several recent findings suggest that the epithelial cells carry out their sentinel role in part by releasing lipid mediators: (i) the cells contain abundant stores of potential fatty acid substrates (including arachidonic acid), (ii) they have the capacity to release these substrates through the action of specific phospholipases, (iii) they express enzymatic pathways for selective oxygenation of fatty acids at a high level relative to other cell types, and (iv) the oxygenated metabolites have potent effects on endorgans such as smooth muscle, nerves, and glands as well as on inflammatory cells and on epithelial cells themselves.

Studies of purified epithelial cells from epidermis and pulmonary airway demonstrate that all three of the enzymatic pathways capable of fatty acid oxygenation—cyclooxygenase, lipoxygenase, and monooxygenase are expressed selectively under different culture conditions and to a variable degree in different cell types. Examples which have been thoroughly characterized include: (i) a cyclooxygenase in sheep tracheal epithelial cells which is regulated by upward transcriptional control under conditions that stimulate cell growth and which exhibits a novel interaction with enzymatic inhibitors; (ii) a low level of 5-lipoxygenase activity in dog and sheep tracheal epithelial cells resulting in the release of the chemotaxin leukotriene B_4; (iii) an arachidonate 15-lipoxygenase in human tracheal epithelial cells which catalyzes the generation of a series of distinct metabolites following increases in endogenous or exogenous arachidonic acid availability; (iv) a 12-lipoxygenase in bovine tracheal epithelial cells which is distinct from leukocyte and platelet forms of the enzyme in that it has the capacity for a broader range of substrate acceptability despite other similar molecular characteristics; and (iv) a novel regiospecific NADPH-dependent monooxygenase in human epidermal cells which also causes predominant oxygenation of arachidonic acid at carbon-12 but employs a distinct enzymatic mechanism resulting in variable proportions of enantiomer (e.g., 12*S*- vs 12*R*-hydroxy acid) generation with differing biological activities. Taken together, the studies demonstrate considerable heterogeneity among species, cell type, and enzymatic mechanisms for mediator generation from surface epithelial cells. The significance of species diversity for airway lipoxygenases is uncertain, but it is possible that the heterogeneity represents molecular divergence within a family of closely related enzymes, implying that diverse enzymatic pathways and their products might serve the same function in different species. This hypothesis seems most likely in the comparison of 12- and 15-lipoxygenase pathways in view of their similar enzymatic characteristics and proposed functional properties.

J 015 MODULATION OF AIRWAY INFLAMMATORY RESPONSES BY EPITHELIAL CELLS
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The active participation of the airway epithelium in the modulation of inflammatory responses has gained initial acceptance. Increasing evidence suggests that the epithelium plays an active, rather than passive, role in airway inflammation. In response to irritant stimuli, airway epithelial cells release mediators that can initiate and direct leukocyte migration to sites of injury. Upon arrival at inflammatory sites, migratory cells can then develop cooperative cell responses that amplify the initial response. For example, leukotriene B_4 production can be increased by such interactions. Recent evidence suggest that human airway epithelial cells, possessing leukotriene A_4 hydrolase but little 5-lipoxygenase, may be capable of modulating local leukotriene B_4 biosynthesis through transcellular metabolism of leukocyte-produced leukotriene A_4. This increase in leukotriene B_4 release may enact further leukocyte chemotaxis and activation. Other physiological consequences of the resulting increased mediator production could include autocrine/paracrine activation of epithelial cellular processes controlling receptor-secretion coupling (e.g., ion transport). Following this immediate exudative phase, prolonged mediator release may also influence the subsequent proliferative and repair phases by providing a mitogenic microenvironment conducive to epithelial growth or hyperplasia. Thus, epithelial-derived mediators may have several transducer-selector-effector functions in airway inflammation.

Leukocytes in the Airways

J 016 EOSINOPHILS (EOS): THEIR ROLE AS INFLAMMATORY CELLS IN THE AIRWAYS, William W. Busse, University of Wisconsin, Madison, WI

EOS are prominent in the peripheral circulation, airway secretions and bronchial tissue in asthma. Recognition of the EOS's inflammatory properties suggests it is a major participant in airway injury in asthma. For example, EOS contain preformed basic proteins, e.g. major basic protein, which can directly injure airway epithelium, cause bronchial hyperresponsiveness, and promote further mediator release to accentuate EOS participation in the inflammatory process. In addition, EOS generate other mediators including leukotriene C_4, platelet activating factor, and toxic oxygen products to further compound bronchial obstruction and injury. To fully appreciate the role of EOS in airway disease, it is essential to know that EOS are a heterogeneous collection of cells when evaluated by density, function, and membrane receptors. Because low density EOS are felt to be metabolically more active, it is of interest, and importance, that this population increases in asthma and during late asthmatic reactions to antigen. Recent studies from our laboratory found inhaled antigen causes eosinophilia and release of EOS inflammatory constituents when evaluated by bronchoalveolar lavage. Moreover, our results indicate that antigen-associated recruitment of EOS to the lung is associated with the appearance of low density cells in the airway. Furthermore, the appearance of a large number of airway EOS following antigen exposure is associated with a significant increase in eosinophil-derived basic proteins. Thus, we hypothesize that antigen provoked asthma causes EOS recruitment to the airway with subsequent up-regulation to low density cells, enhanced release of EOS inflammatory mediators, and consequently the development of bronchial inflammation and obstruction.

J 017 T CELL/GRANULOCYTE INTERACTIONS IN AIRWAY INFLAMMATION, A.B. Kay, Department of Allergy and Clinical Immunology, National Heart & Lung Institute, London, U.K.

Evidence to support the general hypothesis that T cell, eosinophil, neutrophil interactions are of pathogenetic importance in chronic asthma has come from a number of studies. These include: (1) the substantial eosinophil, neutrophil and lymphocyte infiltration in bronchoalveolar lavage in dual (early + late-phase) responders after allergen inhalation induced asthma [1]; (2) the preferential local accumulation of CD8+ in single early responders [2]; (3) the identification, by electron microscopy, of activated "irregular" lymphocytes in the bronchial mucosa of mild atopic asthmatics as compared with controls [3]; (4) the substantial infiltration of CD3 (probably predominantly T helper) cells in the bronchial mucosa of a guinea pig model of late asthmatic reactions and its correlation with local eosinophilia [4]; (5) the infiltration of substantial numbers of CD4 IL-2R+ lymphocytes, and activated (EG2+) eosinophils in allergen-induced cutaneous late phase reactions (LPR) in atopic subjects and the correlation between CD4+ and EG2+ cells, and between EG2+ cells and the size of the LPR [5]; (6) the demonstration that in acute severe asthma ("status asthmaticus") activated (IL-2R, HLA-DR and VLA-1+ve) CD4+ cells were present in the circulating blood [6]; (7) that the elevated numbers of activated T cells in acute severe asthma were associated with increased serum concentrations of gamma-interferon and soluble IL-2 receptors and that the changes correlated with the severity of the disease and tended to return to baseline levels after treatment with corticosteroids [7]; (8) that blood mononuclear cells from patients with acute severe asthma, when cultured for 48 hr, spontaneously elaborated a 20 kD neutrophil chemotactic activity with a pI of approx. 6 [8], and (9) that patients with relative unresponsiveness to treatment with oral corticosteroids have chronically activated circulating T cells which proliferate in response to polyclonal T cell activators even in the presence of high concentrations of dexamethasone [9].

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J 018 ASSESSMENT AND CHARACTERIZATION OF INTRALUMINAL AIRWAYS NEUTROPHILIA ASSOCIATED WITH CHRONIC BRONCHITIS. **AB Thompson, J Metcalf, R Robbins, S Rennard, Dept. of Internal Medicine,UNMC, Omaha, NE.**

Investigations have suggested a pathogenetic role for inflammation in both large and small airways of subjects with chronic bronchitis. Furthermore, evidence has accrued that inflammation may contribute to airways hyperresponsiveness. In order to characterize the intraluminal inflammation associated with chronic bronchitis, bronchoalveolar lavage (BAL) was performed in 25 subjects with chronic bronchitis (CB), 15 asymptomatic smokers (AS), and 25 normal non-smoker (N) volunteers. BAL was performed by instilling 5, 20 ml aliquots of normal saline into 3 sites. The first aliquots (the bronchial sample) were analyzed separately from the final four (the alveolar sample). The bronchial sample lavage fluids from CB tended to contain more cells (6.1 $\pm$ 2.2 x 10^6 cells) than the bronchial sample fluids from the AS (3.6 $\pm$ 0.6 x 10^6 cells) or N (3.7 $\pm$ 0.5 x 10^5 cells). Furthermore, CB had a higher percentage of neutrophils in their bronchial lavage fluid (35.8 $\pm$ 5.6 percent) than either AS (20.7 $\pm$ 2.6 percent, p=0.0001) or N (10.3 $\pm$ 5.6 percent). In order to correlate airway inflammation and clinical parameters, the chronic bronchitics were divided into two groups, those with low (<20%) and high (>20%) bronchial sample neutrophils. Those with higher bronchial sample neutrophils had significantly more sputum production and lower FEV1, FEV1/FVC, and FEF_{25-75}. Thus, chronic bronchitis was found to be associated with airways neutrophilia. In order to investigate the possible role of compliment and the cochemoattractant GcGlobulin (GcG) in the recruitment of neutrophils into the airways, GcG was measured by ELISA in BAL fluid from CB (n=13), AS (n=5), and N (n=8). GcG levels estimated in alveolar epithelial lining fluid were higher in CB than in the non-bronchitic groups (2110 ± 346 vs. 848 ± 239 ng/ml, p<.01). Furthermore, BALF enhanced C5a des arg-mediated chemotaxis above BALF (55 ± 5.6 vs. 18.8 ± 1.5 cells/hpf, p<.01) alone. This effect was blocked by GcG antibody (44.9 ± 3.0 vs. 24.3 ± 1.8 cells/hpf, p<.01) Thus, BALF from chronic bronchitics reflects recruitment into the airways; increased levels of functionally active GcG suggests one possible mechanism for the neutrophilic influx into the lungs of patients with CB.

Neuropathways in Airways Inflammation

J 019 UPPER RESPIRATORY TRACT NEUROPEPTIDES, Michael A. Kaliner, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892.

The mucosa of the upper respiratory tract is innervated by parasympathetic, sympathetic, and peptide-containing sensory nerves. We have localized the following neuropeptides by immunohistologic analysis: sensory nerves = substance P, CGRP, neurokinin A, and GRP; parasympathetic nerves = VIP; and sympathetic nerves = NPY. SP, NKA, and CGRP were found associated with the epithelium, glands, and both arteriolar and venous vessels. GRP, on the other hand, was most closely associated with glands. VIP was found in nerves in glands and venous vessels, while NPY was exclusively associated with arterioles. The quantity of neuropeptides found in the nasal mucosa was estimated by RIA: NPY (3.1 pmol/g), VIP (2.2 pmol/g), SP (1.0 pmol/g), NKA (0.8 pmol/g), GRP (0.6 pmol/g), and CGRP (0.5 pmol/g). Receptors for the neuropeptides were localized by autoradiography: the sensory neuropeptide receptors were found in the epithelium, glands, and vessels, with the exception of GRP receptors which were rather exclusively and intensely found on glands and epithelium. VIP receptors were on the glands, while NPY was found on arterioles. The capacity of the neuropeptides to cause glandular secretion was examined in short-term cultures, and GRP was the most active secretagogue. VIP potentiated the actions of methacholine on glandular secretion. We conclude that nasal mucosal neuropeptides may be important factors controlling mucosal function, especially vascular tone and glandular secretion.

J 020 MODULATION OF NEUROGENIC INFLAMMATION BY NEUTRAL ENDOPEPTIDASE, J.A. Nadel, Cardiovascular Research Institute and Departments of Medicine and Physiology, University of California, San Francisco, CA 94143

Mechanical, chemical, or immunological stimulation of the airways results in the release from the sensory nerves of small peptide mediators called "tachykinins," exemplified by substance P. The released tachykinins produce an inflammatory response characterized by increased vascular permeability, neutrophil adhesion, gland secretion, smooth muscle contraction, and cough. This constellation of responses, known as "neurogenic inflammation," is normally modulated by an enzyme known as neutral endopeptidase (NEP), which cleaves and thus inactivates tachykinins. The selectivity of NEP is determined in part by the loci on peptide molecules where it causes cleavage. However, in addition to its cleavage of tachykinins, NEP also cleaves other peptides such as bradykinin and neurotensin. A novel characteristic of NEP is its location on the surfaces of cells in close association with tachykinin receptors, and it is thus in a strategic position to cleave these peptides close to their target receptors. Decreased NEP activity can be produced by selective enzyme inhibitor drugs (e.g., phosphoramidon, thiorphan), by respiratory infections (e.g., viruses), by occupational pollutants (e.g., toluene diisocyanate), or by oxidants (e.g., cigarette smoke). Decreased NEP activity results in exaggerated neurogenic inflammatory responses in all airway tissues affected by neurogenic inflammation. Human NEP has recently been cloned. Aerosolized recombinant human NEP inhibits cough produced by exogenously delivered and endogenously released tachykinins. It also inhibits increased vascular permeability induced by tachykinins in the skin.

Multiple stimuli that normally enter the airways stimulate the sensory nerves to release tachykinins and produce small, nonsymptomatic responses. When NEP activity in a tissue is decreased, the neurogenic inflammatory responses become exaggerated. These exaggerated responses may play a significant role in inflammatory diseases in airways and in other organs. Exogenously delivered recombinant NEP may provide a therapeutic strategy to inhibit pathologic responses to inflammatory peptides.

J 021 VASOACTIVE INTESTINAL PEPTIDE (VIP) IN ASTHMA, Sudhir Paul, Department of Pharmacology, University of Nebraska Medical Center, Omaha, NE 68105

VIP is a potent bronchodilator and may be a modulator of inflammatory mediator release in the airways. Although the blood levels of VIP in asthma subjects are not different from those in non-asthmatics, the content of VIP in nerves supplying the asthmatic airways is reduced. IgG type of antibodies directed against VIP are found in subpopulations of asthmatic individuals and healthy subjects. The autoantibodies found in asthmatics have a higher binding affinity for VIP than those present in healthy individuals. Binding of VIP by autoantibodies results in inhibition of VIP binding by airway receptors and VIP-responsive cyclic AMP synthesis by lung membranes. Some of the autoantibodies directed against VIP exhibit a novel biological function - the catalytic hydrolysis of peptide bonds in VIP. The catalytic antibodies, purified by affinity chromatography on immobilized VIP, exhibit a restricted clonotype distribution, judged by IEF. The K_m of catalytic VIP autoantibodies is relatively low and their k_{cat} values (turnover numbers) are relatively large, suggesting a high catalytic efficiency of these antibodies. Recognition and hydrolysis of VIP by these antibodies occurs in a sequence-specific fashion. Six peptide bonds in VIP hydrolyzed by the antibodies have been identified. These are Thr^{7}-Asp^{8}, Arg^{14}-Lys^{15}, Gln^{16}-Met^{17}, Met^{17}-Ala^{18}, Ala^{18}-Val^{19}, and Lys^{20}-Lys^{21}. A low molecular weight inhibitor of the catalytic antibodies is apparently present in healthy individuals but is absent in an individual with bronchitis. This inhibitor appears to be active site directed. The pathogenetic potential of catalytic antibodies is larger than that of antibodies that simply bind antigen. Humoral autoimmunity to VIP may be a factor in the pathogenesis of asthma.

Mucociliary Clearance and Airways Secretions

J 022 THE EFFECTS OF BRADYKININ (BK) ON HUMAN AIRWAY EPITHELIUM, Richard C. Boucher, Lane Clarke, Sarah Mason, and Anthony Paradiso, School of Medicine, Dept. of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7020.

Airway epithelia are exposed to a variety of inflammatory stimuli including BK. To explore the effects of bradykinin on human airway epithelia dose-effect studies measuring the effectiveness of mucosal (M) or submucosal(S) BK on the short circuit current of amiloride pretreated cultured human nasal epithelial (HNE) cells were performed. These studies revealed that the potency of BK (ED_{50} = 10^{-7}M) was similar for M and S additions whereas the effectiveness was greater (2 fold) when BK was applied to the S surface membrane. Studies with BK analogs revealed that the BK receptors were BK_{II}. To test for one mode of transmembrane signalling, HNE were loaded with Fura-2 and the intracellular calcium ($CA^{2+}{}_i$) response to BK measured. BK initiated a rapid increase in $CA^{2+}{}_i$ levels that peaked at 600 nM (baseline 100 nM) and rapidly (45 seconds) relaxed to a plateau of approximately 200 nM. Removal extracellular of CA^{2+} revealed that the BK induced $CA^{2+}{}_i$ spike persisted after BK stimulation but the $CA^{2+}{}_i$ levels relaxed to below baseline. No changes in cAMP levels were measured following BK exposure. The responses of the individual apical and basolateral membrane ionic conductances (G) to BK was measured utilizing the double barrelled intercellular ion selective microelectrode technique. These studies revealed that the major effect of BK was to activate the basolateral membrane K^+ conductance. A smaller (20%) effect on the apical membrane chloride conductance was also detected. Like the $CA^{2+}{}_i$ responses, the membrane effects rapidly relaxed from peak values over one to two minute intervals. We conclude that BK activates airway epithelial cells via a specific receptor gated mechanism that involves calcium and leads to activation of the basolateral membrane. This response, which may trigger both an increase in sodium absorption as well as induce chloride secretion, would perturb the ion and water content of the airway surface microenvironment.

J 023 FATE OF PARTICLES, PATHOGENS, AND ALLERGENS IN AIRWAYS. Joseph D. Brain, Respiratory Biology Program, Department of Environmental Science and Physiology, Harvard School of Public Health, Boston, MA 02115.

Inspired air contains many particles which can elicit airway inflammation and injury. Examples are irritant gases, urban and occupational dusts, microbes, and tobacco smoke. Studies with radioactive or fluorescent particles demonstrate aerosol retention in large and small airways. What is their fate?

Soluble particles can dissolve and be absorbed into the blood. Although coughing can displace retained particles along airways when there is excess mucus, in normal humans mucociliary transport is far more important. The airway mucous blanket containing particles is moved mouthward by cilia. Factors affecting the speed of mucous flow may be divided into two categories: those affecting the cilia themselves and those affecting the properties of the mucus.

But other possible fates of injurious particles should also be considered. Some microbes may bind to cells in the airway lumen and be subjected to phagocytosis and killing. Neutrophils, eosinophils, and lymphocytes are present in airways during inflammation. Even in normal airways, macrophages are present in both large and small airways. When lungs are carefully fixed, many macrophages can be seen. Some are visible as cells suspended in the mucous blanket; they are being transported to the pharynx where they will be swallowed. However, macrophages can also be seen beneath the airway fluid where they are adherent to the bronchial epithelium. Such macrophages may release mediators that attract lymphocytes, neutrophils, or mast cells into the airways and regulate their activity there.

A third possible fate of particles is transepithelial transport. Particles may be taken up by airway epithelial cells and gain access to the underlying connective tissue. Transepithelial transport may be enhanced by injury produced by such diverse agents as sulfur dioxide and tobacco smoke. To better understand models of airway injury and inflammation produced by inhaled particles, we need to quantify their deposition and fate in airways. (Supported by NIH grants HL-31029 and HL-19170.)

J 024 REGULATION OF CILIARY BEAT FREQUENCY (CBF) *IN VIVO*, Donovan B. Yeates and Lid B. Wong, Section of Environmental and Occupational Medicine, Department of Medicine, University of Illinois at Chicago, Chicago, IL 60612

Mucociliary clearance, an integral part of the respiratory system, removes secretions, irritants and inflammatory debris from the airways. The response of the mucociliary system to either irritants or the initiation of an inflammatory response could be either the cleansing of the airways to maintain patent airways and facilitate gas transport, or the impairment of mucociliary transport with resultant abnormalities of airway function. The airway secretion removal rate is dependent on the rate at which the cilia beat. We have investigated the response of CBF to irritants and inflammatory mediators to determine the neural and humoral pathways mediating these responses in a minimally traumatized canine trachea. Capsaicin was used as a surrogate irritant to stimulate C-fibers; substance P (SP) as a neuropeptide purportedly released from afferent C-fibers, and bradykinin as a mediator of inflammation. Paratracheal surgery was performed to investigate the responses of CBF to surgically induced inflammation. To determine the pathways involved, hexamethonium was used to inhibit cholino-nicotinic receptors; ipratropium bromide to inhibit cholino-muscarinic receptors and indomethacin to inhibit the metabolism of arachidonic acid via the cyclooxygenase pathway. Aerosolized SP, delivered to the isolated tracheal lumen, stimulated CBF via cyclooxygenase dependent, parasympathetic pathways. Aerosolized capsaicin delivered to both airways and alveoli demonstrated an initial response mediated by these pathways lasting 15-20 minutes. In addition, capsaicin produced a prolonged parasympathetic dependent stimulation that did not involve cyclooxygenase pathways. CBF was stimulated by aerosolized bradykinin delivered to the lungs by pathways consistent with a cyclooxygenase dependent parasympathetic reflex. Paratracheal surgery, which presumably released inflammatory mediators, caused a prolonged stimulation of CBF that reached a maximum after one hour. None of the inhibitors used decreased CBF below baseline values, indicating that the mechanisms whereby CBF was stimulated differ from those that maintain basal CBF. The initial capsaicin induced stimulation of CBF is consistent with the stimulation of CBF by SP. Neither capsaicin, SP nor bradykinin appear to stimulate CBF directly, but rather act through cyclooxygenase and/or cellular dependent neural pathways. The delayed CBF stimulation by capsaicin suggest that an additional cellular pathway located in the lung periphery activates a parasympathetic reflex. The pathways mediating the stimulation of CBF depend on both the specific irritant and the site of irritation. Such stimulatory pathways can be proposed on a teleological basis to maintain a sustained stimulation of ciliary beat, removing secretions and debris from the airways and maintaining airway patency and effective gas transport.

Infections and Clinical Topics

J 025 INFLAMMATION CONTRIBUTES TO LUNG DESTRUCTION IN CYSTIC FIBROSIS, Pamela B. Davis, Department of Pediatrics, Case Western Reserve University, School of Medicine, Cleveland, OH 44106

It is generally accepted that infection is an important cause of pulmonary decline in cystic fibrosis (CF); antibiotics are a mainstay of therapy. The inflammatory response may also contribute in major ways to lung destruction in CF. Young CF patients often have low serum IgG when their pulmonary function is good. As pulmonary disease advances, IgG levels increase. Antiinflammatory treatment with alternate-day corticosteroids in CF results in slower decline of pulmonary function and increase in IgG. Data from our laboratories indicate that a neutrophil exoproduct, elastase, contributes in major ways to the persistence of bacterial infection and the alterations of homeostasis in the airway. Neutrophil elastase from CF bronchoalveolar lavage fluid is sufficient to destroy CR1 receptor on neutrophils and C3bi on the opsonized bacteria, producing receptor-opsonin mismatch and contributing to impaired bacterial clearance. Neutrophil elastase (10 ug/ml) reduces beta-adrenergic receptor density in human airway epithelial cells by 50%, which may impair homeostatic mechanisms. In a rat model of chronic Pseudomonas bronchitis, lung beta-adrenergic receptor density is reduced by over 50%. In this rat model, treatment with the antiinflammatory agent, ibuprofen (35 mg/kg), results in improved weight gain ($p<0.01$) and reduced area of lung inflammation (34% vs. 50% placebo control, $p<0.05$) without increase in the body burden of Pseudomonas. Experiments in humans provide additional rationale for clinical trial of ibuprofen for therapeutic purposes in CF. At concentrations >25 ug/ml, ibuprofen inhibits neutrophil production of LTB_4 as well as PGE_2; LTB_4 is a potent neutrophil chemotaxin. In vivo, high-dose (>20 mg/kg/dose) ibuprofen inhibits recruitment of neutrophils to an inflammatory site, the gingiva. Since high blood levels of ibuprofen are achieved in CF with minimal toxicity, clinical trial seems warranted. Data from animal and human studies indicate that the persistent massive neutrophilic infiltration characteristic of the CF lung disease impairs bacterial clearance and homeostatic mechanisms in the airway, and produces systemic consequences, but can be limited by antiinflammatory treatment strategies.

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J 026 CLINICAL ASSESSMENT OF THE PATIENT WITH CHRONIC COUGH, Richard S. Irwin, Frederick J. Curley, Cynthia L. French, Division of Pulmonary and Critical Care Medicine, University of Massachusetts Medical School, Worcester, MA 01655.

A successful, systematic, anatomic, diagnostic protocol for evaluating patients with chronic cough was presented in 1981 (1). To determine whether it was still valid, we prospectively evaluated, over a 22 month interval, 102 consecutive and unselected immunocompetent patients complaining of cough an average of 53 $\pm$ 97 months (range, 3 weeks to 50 years). Utilizing the anatomic, diagnostic protocol modified to include prolonged esophageal pH monitoring (EPM), the cause(s) of cough was determined in 101/102 (99%) of patients leading to specific therapy that was successful in 98%. Cough was due to one condition in 73%, two in 23%, and three in 3%. Postnasal drip syndrome was a cause 41% of the time, asthma 24%, gastroesophageal reflux (GER) 21%, chronic bronchitis 5%, bronchiectasis 4%, and miscellaneous conditions 5%. Cough was the sole presenting manifestation of asthma and GER 28% and 43% of the time respectively. While history, physical examination, methacholine inhalational challenge (MIC) and EPM yielded the most frequent true positive results, MIC was falsely positive 22% of the time in predicting that asthma was the cause of cough. Laboratory testing was particularly useful in ruling out suspected possibilities. We conclude that the anatomic diagnostic protocol was still valid and that it has well defined strengths and limitations (2).

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J 027 NEDOCROMIL SODIUM: A NOVEL ANTI-INFLAMMATORY DRUG FOR THE TREATMENT OF ASTHMA,
Dr. D. K. Rainey, Ph.D., Fisons R&D Labs., Loughborough, U.K.

Asthma has classically been defined clinically in terms of a series of symptoms with varying explanations of aetiology. It is only within the past five years that the underlying pathology of the disease has been clearly identified as one of inflammation; more importantly, that treatment of this inflammation leads to reversal of symptoms and to treatment of the disease itself.
Nedocromil sodium (TILADE) is a novel anti-inflammatory drug which was specifically designed to treat inflammation in the lung and which has subsequently demonstrated unequivocal efficacy in the treatment of bronchial asthma of a wide range of chronicity, severity and aetiology.

Some 4½ years ago when the first reports of efficacy in pilot therapeutic trials were received, an extensive programme of pre-clinical research studies was set up at centres of excellence around the world, in parallel with the main clinical programme. The objective of this programme, carried out in models of inflammation in the airways, was to establish the anti-inflammatory profile of the drug and to explore its breadth of activity in order to provide guidance to the clinical evaluation programme.

Thus in in vitro studies nedocromil sodium inhibits activation of and mediator release from a range of inflammatory cells from animals and man, including eosinophils, neutrophils, monocytes, macrophages, mast cells and platelets, whether the stimuli used are specific or non-specific; the drug inhibits both pre-formed or newly generated mediators.

Nedocromil sodium has also shown excellent activity in animal in vivo models of individual facets of asthmatic inflammation, including bronchospasm, vascular permeability, the late reaction (and associated cellular influx) and bronchial hyperreactivity, and in models which attempt to incorporate all these facets.

J 028 THE ROLE OF INFECTION IN NON-CYSTIC FIBROSIS PATIENTS WITH BRONCHITIS.
R. A. Stockley, The Lung Immunobiochemical Research Laboratory, The Clinical Teaching Block, The General Hospital, Steelhouse Lane, Birmingham B4 6NH, U.K.

Bacterial infection in the lower respiratory tract is associated with recruitment of the lung secondary defence system. This includes an inflammatory response resulting in an increase in the secretion concentration of plasma proteins such as immunoglobulins and complement. In addition neutrophil recruitment and activation results in a change of sputum from mucoid to purulent and the release of excess neutrophil products including elastase. This enzyme is released in quantities which overcome the inhibitory capacity of the natural inhibitors and enzyme activity persists (1).

Neutrophil elastase (NE) is known to damage ciliated epithelium, digest lung connective tissues and other lung proteins, including immunoglobulins. Thus its presence will have a major effect on the airways and lung defences of patients with chronic lung disease and is likely to affect the longterm progression of disease, particularly in the presence of chronic infection (2).

Patients with bronchiectasis often have persistently purulent sputum, even when a clear infection (temperature, chest pain) is absent, due to neutrophil recruitment as a result of bacterial colonisation (3). This is associated with the continued presence of NE which is toxic to the ciliated epithelium (4). Successful reduction of the colonising bacteria results in an increase in well being and improvement in sputum purulence, NE content and a reduction in the toxic effect on the ciliated epithelium.

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Proteases and Antiproteases in Airways Disease

J 029 PROTEASES AND ANTIPROTEASES IN AIRWAY INFLAMMATION. Chapman, H.A., Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston MA, 02115.

All major classes of proteases have been described within the bronchoalveolar compartment of the human lung. These enzymes are highly regulated since they not only have important homeostatic functions but also have the potential to contribute to airway inflammatory processes. In terms of regulation of bronchoalveolar proteolytic activity, proteases can be divided into those that are cell-associated and those that are freely soluble. It is proposed that the regulation of cell-associated enzymes is primarily due to concomitant synthesis by the cells of both specific receptors and specific inhibitors for each protease. The receptor and inhibitor cooperate in highly compartmentalizing proteolytic activity. In contrast, freely soluble enzymes are regulated by determinants of protease/protease inhibitor balance in the bronchoalveolar lining layer. Diffusion of inhibitors from plasma plays a major role in this balance, as the pathobiology of neutrophil elastase in plasma alpha-1-antitrypsin deficiency illustrates. For other soluble proteases, such as plasminogen activator (uPA), an imbalance in favor of excess enzyme seems to be important to maintenance of the normal airway lining layer. Recent studies of patients with the Adult Respiratory Distress Syndrome indicate a deficiency of the normal bronchoalveolar uPA activity contributes to the organization of hyaline membranes. This acquired deficiency is due in part to an influx of plasma uPA inhibitors. These and other examples illustrate the diversity of proteolytic processes and mechanisms for their regulation in human lung biology.

J 030 ANTILEUKOPROTEASE, A NEUTROPHIL ELASTASE INHIBITOR IN THE AIRWAYS. Joop H. Dijkman and Johannes A. Kramps, Department of Pulmonology, University Hospital (C3-P), NL-2333 AA Leiden, The Netherlands.

Antileukoprotease (ALP), a low molecular weight inhibitor of neutrophil elastase, was isolated from bronchial secretions in 1972, and is identical to the inhibitors found in nasal secretion, saliva, tears, cervical mucous and semen. In the airways, ALP has been demonstrated in the serous cells of bronchial glands and in non-ciliated cells in bronchioles (Clara cells and goblet cells) [1]. The molar ALP/α_1AT ratio in alveolar lavage fluid is low (0.08), whereas in bronchial secretions it is much higher (up to 10). ALP is able to penetrate into the subepithelial connective tissue and into the alveolar interstitium, where it has been demonstrated in association with elastic fibers [2,3]. In human lungs, a positive correlation has been found between the number of ALP-secreting cells in bronchiolar epithelium and parameters of "small airways disease", which is a smoking-related type of inflammation in peripheral airways. Moreover, a negative correlation has been found between the number of bronchiolar ALP-secreting cells and the number of normal alveolar attachments surrounding these bronchioles [4].

These findings suggest that ALP is part of the anti-inflammatory tissue reaction on inhaled noxes, by which proteases are set free. However, the increased supply of ALP to inflamed structures appears to be insufficient to retain normal architecture of the tissue. As the pathologic process associated with increased supply of ALP is linked to cigarette smoking and inflammation, part of this ALP may be oxidized by components of smoke [5,6] or by oxygen species from neutrophils [7].

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J 031 BRONCHIAL SECRETORY CELL METAPLASIA. Gordon L.Snider, Edgar C.Lucey and Thomas G. Christensen. Boston VA Medical Center, Boston University Pulmonary Center, and Mallory Institute of Pathology, Boston MA.

Secretory cell metaplasia (SCM) and submucosal gland enlargement are commonly seen in human chronic bronchitis. SCM is defined as the development of secretory granules in nongranulated secretory epithelial cells and an increase in the number of granules in granulated secretory cells. SCM is induced in the central intrapulmonary bronchi of the hamster by a single intratracheal instillation of a serine protease, which must be enzymatically active, but which need not have elastolytic activity. SCM does not occur in the trachea or bronchioles. The phenotypic expression of the bronchial secretory cell is permanently changed, since the lesion persists indefinitely. Mild, transient SCM is induced by elastase in rat intrapulmonary bronchi, suggesting that the lesion is not species specific. Enzymatic treatment does not cause cell death, and ciliated cells are not affected. Secretory granule discharge occurs within minutes after enzyme instillation followed by restoration of granule density to normal by day 3 and the development of SCM by day 8 with progression of the lesion to day 21. Secretory granule discharge and restoration to normal granule density occurs in the trachea. More N-acetyl galactosamine binding sites, revealed by Helix pomatia lectin, are present on the surface of tracheal than bronchial Clara cells; elastase treatment increases binding in bronchus but decreases it in trachea. This change may be related to the signal that induces SCM, but further study is needed to elucidate the precise mechanism of secretory granule accumulation. SCM is also induced by H^+, and by endotoxin- or ozone-induced pulmonary neutrophilia but not by a brief exposure to 0.8ppm ozone. Enzyme-induced SCM is prevented by prior treatment with antiproteases such as alpha-1-antiprotease or secretory leukocyte protease inhibitor, but not by administration of flurbiprofen. Secretory leukocyte protease inhibitor, which is secreted by human airways epithelial cells, may serve to protect the epithelium from enzymatic injury.

Inflammation and Airways Hyperresponsiveness

J 100 VIRUS-INDUCED AIRWAY HYPERREACTIVITY & INFLUX OF INFLAMMATORY CELLS IN THE GUINEA PIG RESPIRATORY TRACT. G. Folkerts, M.P.W. Janssen & F.P. Nijkamp. Department of Pharmacology, Faculty of Pharmacy, Catharijnesingel 60, 3511 GH Utrecht, The Netherlands. The relation of viral respiratory infections to either the development or enhancement of airway hyperreactivity poses an important clinical problem and may also provide insight into the pathogenesis of bronchial hyperresponsiveness and, possibly asthma. Therefore, we developed an animal model to investigate the cause of hyperresponsiveness due to viral infections. Guinea pigs were inoculated intra-tracheally with parainfluenza-3 virus (PI-3) and airway responsiveness to histamine was measured in vivo and in vitro. Lung lavages were performed to investigate whether broncho-alveolar cell numbers were correlated with airway responsiveness. The recovered cells were counted and subsequently discriminated.
Histamine (0.2-2.0 µg/100 g b.w., i.v.) induced a dose-dependent increase in pulmonary resistance (RL) in spontaneously breathing anaesthetized guinea pigs. The increase in RL was significantly enhanced 4 and 8 days after PI-3 infection as compared to controls. Histamine concentration-response curves were made on isolated guinea pig tracheal spirals, bronchi and parenchymal strips. A significantly increased responsiveness to histamine was recorded in all parts of the infected guinea pig airways on 4, 8, and 16 days after PI-3 inoculation. The total number of inflammatory cells in the lung lavage fluid was increased in the PI-3 treated animals 4, 8, and 16 days after infection. This was predominantly due to an enhanced number of alveolar macrophages and lymphocytes.
This animal model will be used to investigate whether inflammatory cell numbers and/or products contribute to virus-induced respiratory airway hyperreactivity.
The financial support of the Dutch Asthma Foundation is greatfully acknowledged.

J 101 DIFFERENTIATION OF THE PULMONARY RESPONSE TO INHALED PARTICLES BY BRONCHOALVEOLAR LAVAGE FLUID ANALYSIS, Rogene F. Henderson, Jack R. Harkema, Edward B. Barr and A. Frank Eidson, Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM 87185
The purpose of this study was to determine if changes in bronchoalveolar lavage fluid (BALF) content could be used to distinguish between the response of the lung to particles likely to cause some form of pneumoconiosis (such as α-quartz) versus a relatively nontoxic particle (TiO_2). Similar studies in which intratracheal instillation has been used to administer particles have shown some inflammatory response even to relatively nontoxic particles. We wished to determine if inhalation exposures would more clearly distinguish between the lung's response to toxic versus relatively nontoxic particles. Female F344/N rats were exposed to 0, 0.1, 1.0, 10 or 35 mg/m^3 of quartz or TiO_2 for 6 hr/day, 5 days/wk for 4 wk. Animals were observed at 1, 8 and 26 weeks after the end of the exposures. Dose-dependent increases in BALF content of neutrophils, protein, fibronectin and lactate dehydrogenase and β-glucuronidase activities were observed and increased with time post exposure in the quartz-exposed rats. There were no statistically significant increases in any of the parameters at any time in the TiO_2-exposed rats. Multiple microgranulomas were observed by six months in the quartz-exposed rats. Only mild macrophage hyperplasia was observed in the TiO_2-exposed rats. The data suggest that BALF analysis of rats exposed by inhalation can distinguish between toxic and nontoxic particles more clearly than in animals exposed by instillation. (Research supported by P & G under Funds-in-Agreement DE-FI04-87AL44004 and by OHER of DOE under Contract No. DE-AC04-76EV01013.)

J 102 ACTIVATION OF PLATELETS BY THE HUMAN EOSINOPHIL GRANULE BASIC PROTEINS, MAJOR BASIC PROTEIN AND EOSINOPHIL PEROXIDASE, Teresa J. Kreofsky, Christine L. Wheatley, Gerald J. Gleich and Michael S. Rohrbach, Departments of Medicine and Immunology, Mayo Clinic, Rochester MN 55905
Increased numbers of platelets and eosinophils are found in the airways of allergin-challenged patients with asthma. Often these cells are in close approximation and show evidence of activation. In this study, we have demonstrated that two of the eosinophil granule basic proteins, major basic protein (MBP) and eosinophil peroxidase (EPO) are strong platelet agonists. Both proteins cause the dose-dependent, nonlytic secretion of 5-hydroxytryptamine (5-HT) from unstirred suspensions of washed human platelets and this secretion is not inhibited by the cycloxygenase inhibitor, indomethacin. Further studies on MBP indicated that it also promoted secretion of ß-thromboglobulin from the platelet alpha granules and ß-N-acetylglucosaminidase from the lysosomes. Comparison of the effect of PGE_1 on 5-HT secretion mediated by MBP or thrombin revealed significant differences. While PGE_1 caused the expected rightward shift in the dose response curve for thrombin, it reduced the maximal secretion of 5-HT by MBP without altering the concentration dependence suggesting that MBP acted by a mechanism distinct from thrombin. Examination of the production of diacylglycerol (DAG) during platelet activation confirmed the difference in the mechanisms of platelet activation by thrombin and MBP. While thrombin caused a > 3-fold increase in DAG 10 seconds after stimulation, MBP evoked no increase at this time. Thus, the mechanism of platelet activation by MBP is less dependent on phospholipase C activation than that of thrombin.

J 103 A ROLE FOR THE SUBMANDIBULAR GLAND IN MODULATING PULMONARY INFLAMMATION Ronald Mathison, Debrah Helmer, David Kirk J.S. Davison and Dean Befus. Faculty of Medicine, University of Calgary, Calgary, Alberta, Canada, T2N 4N1

Male rats sensitized 30 to 35 days previously to the nematode Nippostrongylus brasiliensis (Nb) exhibit a marked influx of macrophages and neutrophils into broncho-alveolar lavage (BAL) fluid 8 h following intravenous injection of Nb antigen. We have found that bilateral decentralization of the superior cervical ganglia (SCG; sympathectomy), 1 week prior to allergen challenge reduces cellular influx into BAL by 65%. In contrast to these results in male rats, sympathectomy did not protect against anaphylaxis induced pulmonary inflammation in female rats. Since the submandibular glands, which are innervated by the SCG, exhibit sexual dimorphism in several biological functions, we suspected that they may mediate the anti-inflammatory effects of sympathectomy. Simple removal of the submandibular glands, however, did not modify the extent of antigen induced pulmonary inflammation. The submandibular glands, nevertheless, play a role in modulating anaphylaxis induced pulmonary inflammation because the protective effect of sympathectomy was abolished if the submandibular glands were simultaneously excised. Thus, the central nervous system may be involved in regulating the production and/or release of a factor(s) from the submandibular gland that exert anti-inflammatory actions.

The financial assistance of the Council for Tobacco Research, New York, and the Alberta Heritage Foundation for Medical Research is acknowledged.

J 104 BRONCHIAL HYPERRESPONSIVENESS IN ASTHMA IS ASSOCIATED WITH INCREASED PERMEABILITY OF THE RESPIRATORY MEMBRANE, Theo A. Out, Ed A. van de Graaf, Carel M. Roos and Henk M. Jansen, Clinical Immunology Laboratory and Department of Pulmonology, AMC, and Lab. for Exp. and Clin. Immunology, CLB, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands.

We have investigated the relation between inflammatory reactions in the lungs of asthma patients and bronchial hyperreactivity, by measuring histamine responsiveness and the permeability of the respiratory membrane. Fourteen non-smoking patients with asthma and eight non-smoking healthy volunteers participated in the study. The permeability of the respiratory membrane was assessed in the following way: alpha-2-macroglobulin(A2M) was measured in broncho-alveolar lavage fluid and serum, and the concentrations in the epithelial lining fluid were calculated. We used (A2M in ELF)/(A2M in serum), cQA2M, as a measure for the permeability of the respiratory membrane. The cQA2M in controls ranged from 1.12 to 11.4 and in the patients from 1.07 to 132. Six patients had cQA2M above the highest control. The inhaled histamine concentrations causing a 15% drop in FEV1 showed a significant correlation with cQA2M in asthma patients: Spearman Rank correlation, $r = 0.79$, $p < 0.01$. The increased permeability of the respiratory membrane may be the result of local inflammation which contributes to bronchial hyperresponsiveness.

J 105 DISCREPANT MORPHOLOGICAL AND PHYSIOLOGICAL FINDINGS AFTER CAPSAICIN (CAP) EXPOSURE. Jeffrey S. Tepper, Shelley Fitzgerald and Daniel L. Costa*, NSI-Technology Services and *US EPA, Research Triangle Park, NC, 27709

Guinea pigs were protected from a lethal challenge of 80 mg/kg CAP i.p. 3 weeks after treatment with repeated injections of CAP (10 progressively increasing i.p. doses over 2 d, total= 125 mg/kg). This protection is thought to be mediated by a long lasting depletion of tachykinins in the lung. Immunohistochemical evaluation of Substance P immuno-reactive (SP-IR) fibers 2 wk after repeated CAP administration revealed a substantial depletion of SP-IR containing neurons in the lung, but prominent SP-IR containing fibers remained in the trachea and larynx. Since tracheal and laryngeal fibers were not depleted of SP, this would suggest that the site of action should be primarily in the bronchi and lower airways. In agreement with this mode of action, injections of less than 5 mg/kg CAP resulted in large and small airway changes as demonstrated by increased resistance, gas trapping, decreased oxygen saturation, as measured by pulse oximetry and finally, death by asphyxation. However, insertion of a transoral tracheal tube prevented death in naive guinea pigs when a 25 mg/kg dose of CAP was injected, indicating that the primary site of restriction was the larynx or proximal trachea rather than the bronchi or lower airways. Thus, if repeated CAP pretreatment does not deplete SP-IR fibers in the trachea and larynx, then the mechanism of protection after such pretreatments on subsequent acute CAP exposures remains unclear. (This abstract does not reflect EPA policy.)

J 106 MECHANISMS OF PAF-INDUCED HYPERRESPONSIVENESS OF FERRET TRACHEAL SMOOTH MUSCLE AND MUCUS SECRETION IN-VITRO, Stephen E. Webber, Tsuguo Morikawa and John G. Widdicombe, St George's Hospital Medical School, London SW17 ORE, U.K. Treatment of the ferret in-vitro trachea with platelet-activating factor (PAF) induces hyperresponsiveness of smooth muscle and submucosal gland secretion (including lysozyme secretion from serous cells) to methacholine (Mch) (J.Physiol. In Press). PAF also reduces potential difference (PD) across the trachea and Mch-induced epithelial albumin transport, suggesting loss of epithelial function and integrity. PAF relaxed the tracheal smooth muscle. We have investigated the mechanisms of these effects. PAF-induced (1μM) smooth muscle relaxation (-27±1mmH_2O) was abolished by indomethacin (-6±3mmH_2O), suggesting release of a bronchodilator cyclo-oxygenase product, possibly PGE_1. The PAF-induced hyperresponsiveness of tracheal smooth muscle (+96±4% increase in response to Mch, 1μM, n=18), mucus volume (+316±27%) and lysozyme (+350±41%) outputs to Mch were unaffected by indomethacin, the leukotriene antagonist FPL55712, or a combination of H_1 and H_2-antagonists (mepyramine and cimetidine); but they were significantly reduced by a combination of catalase (inhibitor of H_2O_2) and superoxide dismutase (SOD, inhibitor of superoxide anion) (+39±11, +105±20 and +11±10% increases respectively in smooth muscle, mucus volume and lysozyme responses to Mch, n=5) and also by the PAF receptor-antagonist WEB 2086 (-21±9, +22±31 and -8±6% responses to Mch, n=4). Similarly, PAF-induced reductions in PD (+3.8±0.5mV from control of -7.6±0.5mV) and Mch-induced albumin output (-64±6% decrease in response to Mch) were also unaffected by indomethacin, FPL55712 or the histamine antagonists, but were reduced by catalase and SOD (+0.9±0.2mV for PD and -21±9% for albumin) and by WEB 2086 (-0.3±0.2mV; -8±6%). Thus, PAF in-vitro induces hyperresponsiveness of ferret tracheal smooth muscle and submucosal gland secretion (including secretion from serous cells) and inhibits tracheal epithelial function, at least in part, by acting on PAF-receptors to release oxygen free-radicals. The cellular source of these free-radicals remains to be established.

J 107 CELL-CELL ADHESION CONTRIBUTES TO THE PATHOGENESIS OF AIRWAY INFLAMMATION AND HYPERRESPONSIVENESS, Craig D. Wegner, Robert H. Gundel, Patty Reilly-DeLucia, Nancy Haynes, L. Gordon Letts and Robert Rothlein, Departments of Pharmacology and Immunology, Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, CT 06877
Airway eosinophilia, epithelium desquamation and hyperresponsiveness are characteristic features of the airway inflammation associated with asthma. Previously we have reported that eosinophil adhesion to vascular endothelium is partially mediated by intercellular adhesion molecule-1 (ICAM-1) and that ICAM-1 is expressed on an airway epithelial cell line *in vitro*. Using an anti-ICAM-1 monoclonal antibody (R6.5) we have evaluated the contribution of ICAM-1 to the airway eosinophilia and hyperresponsiveness induced by repeated antigen inhalation in monkeys. Five cynomolgus monkeys with a naturally occurring sensitivity to *Ascaris suum* were studied. Airway cell composition (assayed by bronchoalveolar lavage, BAL) and responsiveness (inhaled methacholine PC_{100}) were determined three days prior to (Day 0) and three days after (Day 10) three alternate day (Day 3, 5 and 7) inhalations of *Ascaris* extract. R6.5 was administered intravenously at 1.76 mg/kg daily on Days 2 - 9. Treatment with R6.5 was compared to bracketing control studies in each animal. R6.5 attenuated the eosinophil infiltration in all five animals (mean ± S.E. 337 ± 60 in control versus 165 ± 35 x10^3/ml of BAL in R6.5 treated). The increase in airway responsiveness was also inhibited in all five monkeys, markedly in four (mean ± S.E. change in log. PC_{100} was -1.00 ± 0.23 in control versus -0.17 ± 0.33 in R6.5). These results show that blocking ICAM-1 dependent cell-cell interactions inhibits the airway eosinophilia and hyperresponsiveness induced by repeated antigen inhalation in monkeys.

Airways Epithelial Cell Cultures

J 200 HUMAN AIRWAY EPITHELIAL CELL LEUKOTRIENE A_4 HYDROLASE. T.D. Bigby, D. M. Lee, M.J. Banda, T. Shimizu, N. Ohishi, M. Minami and D.C. Gruenert. Department of Medicine, University of California, San Diego 92092, the University of California, San Francisco 94143 and the University of Tokyo. We have previously shown that human airway epithelial cells contain LTA_4 hydrolase activity. The purpose of this investigation was to begin molecular characterization of this activity. Transformed human airway epithelial cells (9HTEo$^-$) were cultured to confluence in MEM with 10% fetal calf serum, were disrupted by sonication, and were fractionated by centrifugation at 15,000 x and 100,000 x g. Enzymatic activity was assessed by incubating fractions with 3 μM LTA_4 at 37° C for 15 min. LTA_4 hydrolase activity was present exclusively in the 100,000 x g supernatant (cytosolic fraction), was inactivated by heating at 56° C for 30 min and was inactivated by pronase, as is the case for the neutrophil LTA_4 hydrolase. The specific activity was 0.6 ± 0.04 pmol of LTB_4 per mg cytosolic protein. In contrast to the neutrophil hydrolase, time-dependence curves revealed peak activity at 15 min compared to 1 min for the neutrophil and inactivation of enzymatic activity could not be demonstrated when the cytosolic fraction was incubated with 10 μM LTA_4 three times. Western blot and immunoprecipitation of [^{35}S]-methionine labeled proteins followed by SDS-PAGE and autoradiography, using a polyclonal antibody to the recombinant human neutrophil LTA_4 hydrolase, revealed a band at 69 kDa corresponding to the neutrophil hydrolase. An additional band was present at approximately 115 kDa. When the cytosolic fraction was separated by gel filtration chromatography (Sephacryl 200-HR), enzymatic activity was present in fractions which were positive by Western blot for the 69kDa protein. We conclude that human airway epithelial cells appear to contain the LTA_4 hydrolase enzyme previously purified from neutrophils.

J 201 INTERACTION OF PLATELET ACTIVATING FACTOR WITH CULTURED RESPIRATORY EPITHELIAL CELLS, Laurie Churchill, Floyd H. Chilton and David Proud, Department of Medicine, Division of Clinical Immunology, The Johns Hopkins University School of Medicine, Baltimore, MD 21239.
The biological actions of platelet activating factor (PAF) have led to the suggestion that it may be an important mediator of airway inflammation. To further examine the properties of PAF in the airway we have examined the interaction of this lipid mediator with cultured tracheal epithelial cells from guinea pigs (GTE) and humans (HTE). We have demonstrated that both GTE and HTE metabolize endogenous arachidonic acid to produce PGE_2 and $PGF_{2\alpha}$ but no other prostanoids. In GTE, PAF was a potent stimulus for prostanoid generation. PAF-induced prostanoid production by GTE was dose-dependent (10^{-10} to 10^{-6} M) and was maximal within 5 minutes. Production of PGE_2 was increased from 0.43 ± 0.08 ng/10^6 cells upon exposure to buffer to 3.67 ± 0.79 ng/10^6 cells after exposure to 10^{-8} M PAF, while $PGF_{2\alpha}$ production was 0.49 ± 0.08 ng/10^6 cells after buffer and 2.91 ± 0.41 ng/10^6 cells upon exposure to 10^{-8} M PAF. PAF was also a stimulus for prostanoid generation by HTE. The ability of GTE to synthesize and/or catabolize PAF was also examined. GTE readily incorporated [^{3}H] acetate into a product which co-migrated with PAF on thin layer chromatography. However, further characterization of this product revealed that it was a sphingomyelin and not PAF. In contrast, GTE did demonstrate a marked ability to catabolize PAF. When [^{3}H] PAF was incubated with GTE, 60% of the total [^{3}H] PAF added was catabolized by an acetyl hydrolase to yield 1-O-[^{3}H]alkyl-2-acyl-sn-glycero-phosphocholine within 15 min. Determination of the nature of the long chain acyl group incorporated in the sn-2 position revealed that oleic and linoleic acids were the major fatty acids present, while smaller amounts of arachidonic and stearic acids were detected. We conclude that respiratory epithelial cells not only respond to PAF stimulation with enhanced production of PGE_2 and $PGF_{2\alpha}$ but may also have the capacity to modulate inflammatory reactions in the airways via their ability to degrade this potent inflammatory mediator.

J 202 THE USE OF A CELL LINE AS A MODEL SYSTEM TO STUDY THE INTERACTION OF ENVIRONMENTAL TOXICANTS WITH HUMAN AIRWAY EPITHELIAL CELLS. Robert B. Devlin[1], Terry Noah[2], Karen McKinnon[3], Michael Van Scott[2] and Hillel S. Koren[1]. [1]US Environmental Protection Agency, [2]University of North Carolina School of Medicine, and [3]CE Environmental, Inc. Chapel Hill, NC 27599.
The major difficulty in studying the effects of environmental toxicants on human airway cells is obtaining tissue from healthy subjects. We have therefore investigated the possibility of using a human cell line, BEAS-2B (Reddei et al., Cancer Res 48:1904-09, 1988), derived from normal human bronchial epithelial cells as a model system for studying pollutant/airway cell interactions. We have defined conditions for growing these cells on a rigid collagen-impregnated support and maintaining them with medium below the support and only a thin film of medium on top. This facilitaties cellular interaction with gaseous pollutants such as ozone. Using ozone as a model compound, we have defined exposure conditions so that the cells are damaged by ozone, but recover from the insult within 24 hours. Preliminary results indicate that exposure to 1 ppm ozone for 2 hours results in an increase in 15-HETE as well as other eicosanoids, and possibly an increase in mucin mRNA production. These results are comparable to those reported using animal primary airway epithelial cells. Thus it appears that the BEAS-2B cell line is a useful model with which to study the interaction of environmental agents and human airway epithelial cells.
This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

J 203 GENOTOXICITY, CYTOTOXICITY AND PROSTAGLANDIN E_2 RELEASE IN CULTURES OF HUMAN BRONCHIAL EPITHELIAL CELLS EXPOSED TO HYDROGEN PEROXIDE, Edward W. Gabrielson and E. William Spannhake, Johns Hopkins Medical Institutions, Baltimore, MD 21205.
We have studied the relationship of genotoxicity and cytotoxicity to PGE_2 release in cultures of normal human bronchial epithelial cells exposed to hydrogen peroxide, an important product of the inflammatory process. Sub-confluent cultures (10^4 cells/cm^2) were exposed to H_2O_2 in iron-free HBSS containing 100 uM Ca^{++}/Mg^{++}. H_2O_2 exposure resulted in DNA single-strand breaks, detected by alkaline elution technique, with a 100 uM exposure producing 300 rad equivalents DNA damage at 1 hr. In contrast, acute cytotoxicity, as assessed by trypan blue dye uptake or the release of LDH or ^{51}Cr, required approximately 10-fold higher concentrations of H_2O_2. Chelating intracellular iron with deferoxamine prior to exposure protected cells from these genotoxic and cytotoxic effects, implicating the formation of hydroxyl radical (OH^-) by the Fenton reaction as an important step in H_2O_2-initiated toxicity. Exposure of these cultures to H_2O_2 at concentrations as low as 30 uM also resulted in significant release of PGE_2 above baseline, as measured by enzyme immunoassay, which was not accompanied by release of LDH into the medium. These results indicate that hydrogen peroxide-induced release of PGE_2 occurs independent of measurable damage to membrane integrity, but at levels which do produce measurable damage to DNA. Although OH^- appears important in H_2O_2-induced genotoxicity, its relationship to the stimulation of PGE_2 synthesis by these cells is not yet known.

J 204 LINOLEIC ACID METABOLITES ARE PRODUCED BY GUINEA PIG TRACHEAL EPITHELIAL CELLS AND INFLUENCE THE RESPONSIVENESS OF TRACHEAL SMOOTH MUSCLE, P.A.J. Henricks, F. Engels, M.J. Oosthuizen, B. van Esch and F.P. Nijkamp, Department of Pharmacology, Faculty of Pharmacy, University of Utrecht, Catharijnesingel 60, 3511 GH Utrecht, The Netherlands.
Airway epithelial cells are often damaged, absent or show morphologic abnormalities in patients suffering from chronic obstructive lung diseases. Epithelial damage is associated with bronchial hyperreactivity. The production of fatty acid derived mediators may be one of the mechanisms by which epithelial cells modulate airway function. In the present study it was investigated which fatty acid metabolites are produced by guinea pig tracheal epithelial cells. Epithelial cells were isolated, cultured and incubated with exogenous ^{14}C-arachidonic acid or ^{14}C-linoleic acid. The metabolites were analyzed by reverse phase-high performance liquid chromatography. It was found that the 5- and 15-lipoxygenase pathways were predominantly operative, as evidenced by formation of 5- and 15-hydroxy-arachidonic acid. Interestingly, epithelial cells were able to convert linoleic acid to 9- and 13-hydroxy-metabolites (9- and 13-HODE), which has not yet been reported before. These linoleic acid metabolites may play a role in airway smooth muscle function. Therefore, histamine dose-response curves were made on tracheal smooth muscle preparations incubated with 10 μM 9- or 13-HODE. 13-HODE induced a hyporeactivity for histamine while 9-HODE was without effect on the responsiveness of the tracheal preparation. Further investigations will be carried out to elucidate the precise role of metabolites derived from linoleic acid and arachidonic acid on airway smooth muscle reactivity.
(Supported by the Dutch Asthma Foundation; Grant 86.17).

J 205 GLYCOPROTEINS FROM CULTURED AIRWAY EPITHELIAL CELLS, Scanlin, T.F., Xu, J., Glick, M.C. Department of Pediatrics, University of Pennsylvania, School of Medicine and The Children's Hospital of Philadelphia, Philadelphia, PA 19104.
In airway epithelial cells (AEC) the presence of certain cell glycoproteins has been correlated with the expression of differentiated functions such as mucin secretion (1). A limitation to characterizing the glycoproteins of AEC has been the inability to culture these cells in sufficient quantities to permit isolation of glycoproteins following *in vitro* metabolic labelling. The development of defined culture systems (2) provides an opportunity to study the expression of glycoproteins in development and differentiation as well as in pulmonary disorders such as cystic fibrosis (CF) (5). Tracheal AEC were isolated from male Golden Syrian hamsters and cultured on collagen gels as described (2). For the studies of human disorders, cells were isolated primarily from nasal polyps obtained from patients with CF and non-CF controls. Cells were seeded at a density of 1.0×10^5 cells/35 mm dish. *L*-[^{3}H]Fucose or *D*-[^{3}H]glucosamine was added for 48 h prior to harvest of the cells and removal of the surface glycopeptides by controlled trypsinization. Cell and surface glycopeptides were lyophilized, desalted on Biogel P-2, sized on Biogel P-10, and characterized according to binding to a series of immobilized lectin affinity columns selected to give information on the oligosaccharide structure. The AEC were found to contain fucosylated glycopeptides with unusual characteristics when compared to those previously described from cultured fibroblasts (4). 1) Wasano, K., et al. (1988) Histochem. Cytochem. *36*, 167. 2) Wu, R., et al. (1985) Am. Rev. Resp. Dis. *132*, 311. 3) Scanlin, T.F. (1988) in *Pulmonary Diseases and Disorders*, (A.P. Fishman, Ed.) McGraw-Hill, pp 1273. 4) Scanlin, T.F., et al. (1985) Pediatr. Res. *19*, 368.
(Supported by NIH grant R01 DK 16859 and the CF Foundation)

Vascular Responses; and Miscellaneous Topics

J 300 CAPSAICIN AND OZONE CAUSE PROTEIN ACCUMULATION IN THE TRACHEA AND LUNG BY FUNCTIONALLY INDEPENDENT MECHANISMS. Daniel L. Costa, Jeffrey S. Tepper* and Darrell W. Winsett*. US EPA and *NSI-ES, Research Triangle Park, NC, 27711

Groups of treated rats were evaluated for increased bronchoalveolar lavage fluid protein (LFP) or for Evan's Blue dye extravasation (EBDE) in the trachea. Both techniques measure primarily albumin, with the LFP measurement considered to reflect lung injury and/or inflammation while the EBDE technique is considered a measurement of neurokinin mediated neurogenic inflammation. Treatment consisted of either A) ozone (O_3) exposure for 2 hr to 0, 0.5, 1 or 2 ppm which is known to cause an inflammatory response and produce edema in the lung, or B) i.v. capsaisin (CAP) injection at 0.0, 0.05, 0.1 or 0.5 mg/kg. CAP is known to release neurokinins that induce a neurogenically mediated extravasation of protein into the trachea. In rats, O_3 caused a concentration related increase in LFP without a change in EBDE. In contrast, CAP caused a dose-related increase in EBDE without causing an increase in LFP accumulation. These data suggest that the irritant gas O_3 does not directly nor indirectly, via mediators, stimulate neurokinin release. Likewise, i.v. CAP does not seem to produce lung injury or inflammation. Preliminary data indicate that guinea pigs do not show a similar independence between LFP and EBDE. (This abstract does not reflect EPA policy.)

J 301 MODIFICATION OF TRACHEAL SECRETION BY PROSTAGLANDINS E_2, D_2, AND $F_{2\alpha}$, Mark E. Deffebach, S.E. Webber, H. Islami, A. Price, and J.G. Widdicombe, Department of Physiology, St. George's Hospital Medical School, London, England, and Dept. of Medicine, Univ. of Washington, Seattle 98195
Airway lining fluid is very complex and made up of proteins, glycoproteins, water and electrolyes, as well as other components. In asthma and bronchitis the albumin content of airway secretions is increased. Active albumin transport into the tracheal lumen has recently been demonstrated, and this transport is increased by autonomic agonists. The control of tracheal secretion and albumin transport was further examined in these studies by evaluating the modification by prostaglandins of the secretion volume, lysozyme release, and albumin transport in methacholine stimulated ferret tracheal secretions. The trachea was mounted in an organ bath containing Krebs-Henseleit solution with additional bovine serum albumin (BSA). Undiluted tracheal secretions were collected and analyzed for albumin and lysozyme. The total secretion volume and output and concentrations of albumin and lysozyme were calculated. Secretion was stimulated with methacholine (20μM) (Mch). All responses were dose dependent. PG-$F_{2\alpha}$ at 10^{-5}M increased the volume of Mch stimulated secretion 2-fold, the lysozyme output 6-fold and concentration over 3-fold, while decreasing the albumin transport by half. PG-D_2 at 10^{-5}M reduced Mch-induced secretion volume to 75% control, increased albumin transport to 135%, without effecting lysozyme secretion. PG-E_1 at 10^{-5}M increased Mch stimulated albumin transport and concentration over 2-fold, decreased lysozyme release to less than 1/3 control, and had no effect on secretion volume. PG-E_1 caused the albumin concentration to exceed that of the outer bath, indicating active transport. We conclude that prostaglandins selectively alter tracheal secretion induced by cholinergic stimuli.

J 302 EFFECTS OF REPEATED ENDOTOXIN INSTILLATIONS ON NASAL INTRAEPITHELIAL MUCUS IN NEUTROPHIL-DEPLETED AND NON-DEPLETED RATS, Jack R. Harkema and Jon A. Hotchkiss, Lovelace Inhalation Toxicology Research Institute, Albuquerque, NM 87185
Bacterial-induced airway inflammation is often characterized by an influx of neutrophils (PMN) and hypersecretion of mucus. The purpose of our study was to determine how endotoxin (E), a component of gram-negative bacteria and chemotaxinogen for PMN, affects the amount of stored intraepithelial mucus (IM) in the nasal airway. Rats were intranasally instilled, once a day for 3 days, with E or saline (controls). Half of the rats were depleted of blood PMN, by intraperitoneally injecting a rabbit anti-rat PMN antiserum, prior to instillations. Animals were sacrificed 6 or 24 hr after the last instillation (AI). Nasal tissues were processed for light microscopy and histochemical detection of IM. Number of intraepithelial PMN and amount of IM were determined by image analysis. Six- and 24-hr AI, PMN-depleted and E-treated rats had no PMN-influx, but a significant increase in IM, compared to controls. In contrast, non-depleted, E-treated, rats had marked PMN-influx and depletion of IM. These results indicate that 1) E induces an increase in IM only when intraepithelial PMN are few or absent, and 2) PMN-influx induces hypersecretion of mucus. [Supported by the Department of Energy's Office of Health and Environmental Research under Contract No. DE-AC04-76EV01013.]

J 303 PHOSPHOLIPASE A_2 AND PROTEIN PATTERNS IN BRONCHO ALVEOLAR LAVAGE FLUID: CHARACTERIZATION WITH TWO-DIMENSIONAL GEL ELECTROPHORESIS. Mats Lindahl, Henning von Schenk, Jan Sörensen and Christer Tagesson. Departments of Occupational Medicine, Clinical Chemistry and Anesthesiology, University of Linköping, Linköping, Sweden.
Phospholipase A_2 (PLA_2), an enzyme important for the formation of inflammatory mediators, was purified from rat lung with affinity chromatography, analyzed with two-dimensional gel electrophoresis (2-DGE) and identified with western immunoblots.
PLA_2 activity was found to have a pH optimum at pH 9.5-10.0 and requirement for calcium. The molecular weight was estimated to 12 kDa, the enzyme could be identified with antisera raised against pancreatic PLA_2 and 2-DGE analysis revealed two isoforms with pI 7.8 and 9.5. Furthermore PLA_2 was with 2-DGE identified as a dominant protein in broncho alveolar lavage fluid (BAL) from rat. PLA_2 activity in human BAL was found to have a pH optimum at pH 7.4. 2-DGE analysis of healthy individuals revealed a complex protein pattern with over 50 different entities with albumin and transferrin as dominant proteins. A consistent pattern of about 30 proteins were registered in non-smokers and in comparison BAL from smokers contained an increased number of highly basic proteins. In different patient groups (septic shock, severe trauma and asbestosis) were found different patterns compared to healthy individuals.
In conclusion the 2-DGE technique may be useful for detection of early or specific changes in human BAL protein pattern and to study and identify PLA_2 and other proteins of importance in airway physiology and patophysiology.

J 304 **PHOSPHOLIPASE A2 AND PROTEASE INHIBITION BY CLARA CELL 10 KDa PROTEIN.** Gurmukh Singh, S.L. Katyal, W.E. Brown, A.L. Kennedy, Ushasi Singh. Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261 and the Department of Biological Sciences, Carnegie-Mellon University, Pittsburgh, PA 15213.

A Clara cell specific 10 kDa protein was isolated from human and rat lung lavages. The human protein consists of two identical polypeptides of 70 amino acids each whereas the rat monomer consists of 77 amino acids. The primary amino acid sequences of the rat and human 10 kDa proteins have marked similarity with rabbit uteroglobin. The predicted secondary structures of the rat and human Clara cell proteins are also similar to that of uteroglobin. However, there are marked differences in the tissue distribution between uteroglobin and the Clara cell 10 kDa proteins. Clara cell 10 kDa protein is present only in the non-ciliated epithelial cells of the airways where as uteroglobin is present in Clara cells and genital organs. Rat 10 kDa protein and rabbit uteroglobin inhibit papain whereas the human 10 kDa protein does not. All three proteins inhibit pancreatic Phospholipase A2 in an in vitro assay using labelled phosphatidylcholine. Human 10 kDa protein (100 µg/ml) inhibited 78% of the phospholipase A2 activity. Preliminary investigation suggests that the 10 kDa proteins compete for the substrate rather than directly inhibiting the enzyme. Synthetic peptides of the three proteins, corresponding to the region of similarity to Lipocortin I, failed to inhibit phospholipase A2 and carrageenan induced foot pad swelling in the rat. Phospholipase A2 inhibitory property of the Clara cell 10 kDa protein may be important in regulating the inflammatory activity in the lung.

J 305 CHARACTERIZATION OF INTERLEUKIN 5 (IL5) RECEPTORS

Tavernier, J., Devos, R., Plaetinck, G., Van der Heyden, J., Fiers, W. and Fischkoff, S. Roche Research Gent, Plateaustraat 22, B-9000 Gent, Belgium.

Interleukin 5 (IL5) induces the proliferation/differentiation of eosinophils, and hence might be involved in airways inflammation. Recombinant human and murine IL5 (rhIL5, rmIL5) have been efficiently produced in eukaryotic systems (Tavernier, J. et al. (1989), DNA 8, 491). Both were used to explore the presence and the structure of IL5 receptors. Murine IL5 was internally labeled using ^{35}S-methionine to high specific activity (100-300 µCi/µg) by injection of SP6 RNA polymerase transcripts in Xenopus laevis oocytes. Cross-linking of radiolabeled rmIL5 to B13 cells revealed two proteins (45 Kd : α - chain ; 130 Kd : β -chain) as candidates for the mIL5 receptor. A mIL5 binding protein could be detected on the surface of individual X. laevis oocytes by injection of the 25S mRNA fraction from B13 cells. The number of binding sites was estimated to be appr. $1,2 \times 10^7$ per oocyte (Devos, R. et al. in preparation). This specific binding could not be blocked by a monoclonal antibody R52 directed against the mIL5 receptor on B13 cells (Rolink, A. et al. (1989), J. Exp. Med. 169, 1693), suggesting that a different receptor type or -configuration is expressed on the surface of the X. laevis oocytes.
Human IL5 was affinity purified using a monoclonal antibody directed against rhIL5 and was ^{125}I-labeled to high specific activity (10 µCi/ug). Binding studies on subclones of the human HL60 promyelocytic cell line, committed to eosinophilic differentiation, revealed the specific induction of IL5 receptors after butyrate treatment. Scatchard plot analysis indicated the presence of up to 2000 receptors per cell, with a dissociation constant Kd 2.5×10^{-10}M. (Plaetinck, G., in preparation).

J 306 THE CONTRIBUTION OF LIPOXYGENASE MEDIATORS IN A RAT MODEL OF THE ADULT RESPIRATORY DISTRESS SYNDROME (ARDS), Claudia R. Turner, Lester W. Schwartz, Eric B. Wheeldon, Department of Experimental Pathology, Smith Kline & French Laboratories, King of Prussia, PA 19406

Rats received intratracheally aerosolized endotoxin (LPS, 7 mg/kg) after pretreatment with either 5 lipoxygenase (5LO) inhibitors, a specific LTB4 or LTD4 receptor antagonist (SK&F 106203), to assess the contribution of lipoxygenase metabolites in this model of ARDS. Twenty-four hours later, blood was sampled and rats were killed for measurements of wet/dry lung weight (W/D), and bronchoalveolar lavage (BAL). By 24 hours, survival of LPS-treated rats was decreased 35%, whereas no deaths were observed 24 h after 5LO inhibition or LTD4 receptor antagonism. Although no compound was able to prevent the LPS-induced increase in BAL inflammatory cells, pretreatment with the LTD4 receptor antagonist attenuated the LPS-induced increase in W/D. LPS-induced increases in BAL protein content (TP) were significantly reduced by 5LO inhibition. LPS-induced increases in BAL erythrocytes were significantly reduced by 5LO inhibition or LTD4 receptor antagonism. 5LO inhibition significantly reduced LPS-induced decreases in circulating platelets. Similarly, LPS-induced decreases in circulating leukocytes were significantly reduced by 5LO inhibition and the LTD4 receptor antagonist.

We conclude that the inflammatory cell influx was not decreased by pretreatment with the compounds tested, suggesting that leukotriene mediators do not contribute to the influx of leukocytes in this lung model. However, W/D and TP results suggest an association between increased microvascular permeability and 5LO metabolites. The beneficial effects of pretreatment with a LTD4 receptor antagonist suggest that LTD4 is important in mediating the pathogenesis of ARDS.

J 307 **CLEARANCE OF ^{99m}Tc-DTPA FROM THE SHEEP TRACHEAL LUMEN IS INVERSELY RELATED TO ARTERIAL INFLOW.** J. G. Widdicombe, D. R. Corfield, Z. Hanafi and S. E. Webber. Department of Physiology, St George's Hospital Medical School, London SW17 ORE, United Kingdom.

Vasoactive drugs infused into the sheep tracheal circulation that increase tracheal blood flow decrease clearance of ^{99m}technetium-labelled diethylenetriamine penta-acetate (^{99m}Tc-DTPA) from the tracheal lumen and vice versa (Corfield, D. R., Hanafi, Z., Webber, S. E. & Widdicombe, J. G. 1989, J. Physiol. 415, 85P).

Clearance of ^{99m}Tc-DTPA has now been studied by perfusing the tracheal arteries in ten anaesthetised (pentobarbitone sodium) paralysed (gallamine) and ventilated sheep. The tracheal arteries were isolated and perfused with the sheep's own blood at constant flow and perfusion pressure measured. The tracheal lumen was filled with Krebs-Henseleit solution containing ^{99m}Tc-DTPA. Clearance of ^{99m}Tc-DTPA was determined by measuring radioactivity in blood collected from a cannulated tracheal vein. Perfusion flow rate was changed for 15min periods (+50%, n=7; -50%, n=5; -100%, n=12); this changed perfusion pressure (+35.5±2.7**, -32.8±2.8**, -79.6±3.7**) venous outflow (+12.1±3.1**, -9.5±4.1, -26.6±6.8**) and ^{99m}Tc-DTPA output (-28.4±7.0**, +46.1±34.1, +151.1±59.4*). Methacholine (5μg.min^{-1} for 15 min) decreased (-22.4±5.2**, n=6) and phenylephrine (5μg.min^{-1} for 15 min) increased (+20.1±7.2*, n=5) perfusion pressure but neither significantly changed venous outflow (+6.6±3.6, -0.7±11.2 respectively) or ^{99m}Tc-DTPA output (+11.8±14.5, +5.9±21.9).

Thus ^{99m}Tc-DTPA output was inversely related to perfusion flow rate; perfusion pressure changes produced with vasoactive drugs did not change ^{99m}Tc-DTPA output. ^{99m}Tc-DTPA clearance is related more closely to perfusion blood flow than perfusion pressure and is not dependent on other actions of the vasoactive agents.
(Results: mean±s.e.m. percentage change from control; *p<0.05 and **p<0.01, Student's paired t-test)

Therapeutic Strategies and Clinical Aspects

J 400 PURIFICATION AND CHARACTERIZATION OF NEUROPEPTIDES FROM HUMAN BRONCHOALVEOLAR LAVAGE FLUID, Samuel M. Aguayo, Talmadge E. King, Jr., Karen M. Sherritt, York E. Miller, Pulmonary Sciences Division, University of Colorado Health Sciences Center, Veterans Administration Medical Center, and National Jewish Center for Immunology and Respiratory Medicine, Denver, Colorado.
Pulmonary neuroendocrine (NE) cells produce neuropeptide growth factors thought to be important in lung development, inflammation, and tissue repair. Using monoclonal antibodies (MAbs) against the bioactive fragments of bombesin-like peptides (BLP) and neurotensin, we have measured increased levels (above 1 nM) of these in the bronchoalveolar lavage (BAL) fluid of smokers and some patients with pulmonary fibrosis. Interestingly, a transient increase in these neuropeptides was also detected in the heterologous lung BAL fluid of a patient who underwent single lung transplant while the homologous lung did not exhibit these changes. Immunohistochemical studies using BLP MAb support that the NE cells are the source of these neuropeptides. Biochemical characterization of the BAL fluid BLP and neurotensin was performed after purification using sequential reverse phase and gel filtration HPLC. Amino acid composition analyses of BLP and neurotensin purified from BAL fluid are consistent with the known sequences for these neuropeptides, and with the specificity of our MAbs. Amino acid sequence analyses have been complicated by blocking groups at the amino termini. We conclude that neuropeptide growth factors are increased in a variety of lung conditions characterized by inflammation and tissue repair. Considering that BLP and neurotensin have relevant pharmacologic effects, a role for the NE cells is suggested in the pathophysiology of these phenomena.

J 401 **AIRWAY HYPERRESPONSIVENESS IN PRETERM INFANTS WITH INCIPIENT CHRONIC LUNG DISEASE, Vinod K. Bhutani, S. Jay Mirmanesh, William W. Fox, and Soraya Abbasi, Newborn Pediatrics, Pennsylvania Hospital, Children's Hospital of Philadelphia, and University of Pennsylvania School of Medicine, Philadelphia, PA 19107.** Preterm neonates sustain airway injury following mechanical ventilation and high inspired oxygen which may result in reactive airway disease. This study evaluates the airway hyperresponsiveness at the incipient stages of chronic lung disease(CLD), and the role of α-receptor stimulation in mediating this response. Brief, noninvasive, α-adrenoceptor stimulation was studied in CLD infants at term postconceptional age. Fourteen infants (BW:0.8±0.1kg, GA:27±2wks, SW:1.9±0.4kg) with and without CLD were studied pre and 30 minutes post of either ophthalmic phenylephrine Hcl(2.5% 1gtt q15x3),or normal saline. Airflow and esophageal pressure were determined to calculate following data. Results are mean±SEM.

		MV ml/min/kg	CL ml/cm$H_2$0/kg	Rt cm$H_2$0/L/sec	WOB gm-cm/kg	Pes cm$H_2$0	$\dot{V}$exp L/min
CLD &	Pre	431±42	0.8±0.1	88±15	27±5	10±3	2±0.3
α-Stim	Post	371±34	0.5±0.2*	128±14*	47±10*	14±2*	1±0.1*
CLD &	Pre	431±43	0.8±0.2	89±15	27±5	10±4	2±0.3
Placebo	Post	401±54	0.8±0.1	81±13	24±4	9±3	2±0.3
Non-CLD	Pre	458±88	1.2±0.3	42±15	15±8	6±1	2±0.5
α-Stim	Post	442±56	1.3±0.3	37±8	12±4	6±1	2±0.4

Significant (*p <0.01) changes in airflow and pulmonary mechanics were observed as compared to placebo and non-CLD infants. This study indicates that incipient chronic lung disease is associated with reactive airway disease.

J 402 ANAPHYLACTIC CHALLENGE CAUSES EOSINOPHIL ACCUMULATION IN BRONCHOALVEOLAR LAVAGE FLUID OF GUINEA PIGS: MODULATION BY BETAMETHASONE, PHENIDONE, INDOMETHACIN, WEB 2086 AND A NOVEL ANTI-ALLERGY AGENT, SCH 37224, Robert W. Egan, Xiomara Fernandez, William Kreutner, Michael Minnicozzi and Arax R. Gulbenkian, Department of Allergy and Inflammation, Schering-Plough Research, Bloomfield, NJ 07003.
Eosinophil infiltration into bronchoalveolar areas of the lung has been assessed in guinea pigs sensitized to ovalbumin (OA) then challenged with the aerosolized antigen. Cell content, histamine and guinea pig albumin (GPA) have been measured in bronchoalveolar lavage (BAL) fluid from these animals. Extensive eosinophil accumulation resulted from sensitization followed by OA challenge, while monocytes that initially accounted for greater than 80% of the BAL cells remained essentially constant and neutrophils comprised < 3% of the population throughout. Eosinophils were elevated at 3 hours, peaked with a 5-fold increase at 24 hours and remained elevated for at least 7 days. Increased histamine and GPA were detected only at 5 minutes. Oral treatment with betamethasone (ED_{50}=0.4 mg/kg), phenidone (ED_{50}=15 mg/kg), Sch 37224 (ED_{50}=0.5 mg/kg) and WEB 2086 (ED_{50}=4 mg/kg) decreased eosinophil accumulation in the BAL fluid, indicating roles for 5-lipoxygenase products and PAF in this multimediator-dependent model of allergic inflammation. On the other hand, 4 mg/kg of indomethacin increased total cells with no effect on eosinophils, precluding a major role for cyclooxygenase products. Sch 37224, an anti-leukotriene agent and an orally active novel anti-allergy agent in sheep, guinea pigs and man, was as potent as betamethasone at blocking eosinophil infiltration, suggesting that it may also suppress human pulmonary inflammation.

J 404 **INHIBITION BY NEDOCROMIL SODIUM OF IMMUNIZATION-INDUCED LUNG HYPERRESPONSIVENESS AND EOSINOPHIL ACCUMULATION IN THE GUINEA-PIG.** Marina Pretolani, Jean Lefort, Patricia Silva, Claude Ruffié, Claude Dumarey and B.Boris Vargaftig, Unité de Pharmacologie Cellulaire, Unité Associée Institut Pasteur/INSERM n° 285, 25 rue du Dr. Roux, 75015 Paris, France.
The effect of a single and of multiple administrations of the anti-allergic drug nedocromil sodium (Tilade®, Fisons) on PAF-induced bronchoconstriction (BC) and release of leukotriene-like material and histamine from lungs from actively sensitized guinea-pigs and on cell composition of BAL were compared. The drug was either dissolved at 10 μM in the buffer solution for lung perfusion or it was administered s.c. for two days (i.e. the day of the booster injection of the antigen and the day after) or for one week, from the day of the booster injection to the day of the experiment, at a dose of 30 mg/kg/day. No inhibition of PAF-induced BC or mediator release from sensitized lungs was observed when nedocromil sodium was added directly to the perfusion medium. The two days' treatment only affected PAF-induced histamine secretion. By contrast, when guinea-pigs were treated for one week with nedocromil sodium, BC to 1 ng PAF-acether was reduced by around 50% ($P< 0.05$) and release of leukotriene-like material and of histamine were significantly inhibited by more than 60%. The week's treatment with nedocromil sodium also reduced markedly the number of eosinophils in the BAL of sensitized guinea-pigs. Indeed, 25.0 ± 5.5 and 12.2 ± 4.0 % eosinophils ($P< 0.05$) were found in BAL obtained from vehicle- and nedocromil sodium-treated guinea-pigs, respectively. Our results indicate that nedocromil sodium does not interfere directly with the enhanced effect of PAF-acether on lungs from actively sensitized guinea-pigs, but it prevents hyperresponsiveness (increased mediator release and BC) and eosinophil migration into the airways, when administered after the booster injection of antigen.

J 405 CHOLESTEROL SULFATE LIPOSOMES AS NOVEL SUSTAINED RELEASE DRUG CARRIERS FOR ANTI-INFLAMMATORY STEROIDS IN LUNG, R. Radhakrishnan, D. Levy, R.M. Fielding and M.J. Durrani, Liposome Technology, Inc., 1050 Hamilton Court, Menlo Park, CA 95025
We have investigated the utility of liposome incorporated beclomethasone dipropionate (BDP) administered via the lungs to provide prolonged local therapeutic activity with decreased systemic side effects. Several conventional multilamellar liposomes containing egg or soy phospholipids, cholesterol and drug were prepared and screened in an in vitro membrane interchange assay with excess small unilamellar vesicles as acceptor liposomes and an in vivo rat lung absorption model. Conventional liposomes immediately released BDP in both models. However, a novel multilamellar liposome (Cholesterol Sulfate/Cholesterol/BDP 5:4:1 molar ratio, ~0.5 μ size) which incorporated ten times as much drug as phospholipid vesicles, did not membrane exchange the drug up to 24 hours at 37°C. In addition, this formulation released BDP more slowly in vivo with an absorption half-life of 1-2 hours from rat lungs. Up to 25% of the intratracheally administered BDP could be detected in rat lungs after 3 hours. Free BDP administered IV or BDP in conventional liposomes administered IT as controls produced identical plasma levels after administration. The novel liposomal formulation altered the absorption kinetics of BDP and appears promising for prolonged delivery of corticosteroids to treat inflammation associated with respiratory conditions such as bronchial asthma.

J 406 IL-1ß INDUCED ALVEOLAR MACROPHAGE PRODUCTION OF LTB_4, Lester W. Schwartz, Alex W. Yem and Paul J. Marshall, Departments of Experimental Pathology and Immunology, SmithKline Beecham, King of Prussia, PA 19406.

We have reported previously that intratrachael instillation of human recombinant IL-1ß to the rat induced pulmonary neutrophilia in a dose-related manner. Maximal neutrophil influx occurred 24h post-administration of the rIL-1ß, evaluated by bronchoalveolar lavage. Since IL-1 has been demonstrated to be only chemokinetic, secondary to induction of prostanoid synthesis, and not chemotactic, the objective of this study was to further define the mechanism of IL-1ß induced pulmonary neutrophilia. Rat alveolar macrophages were harvested and exposed invitro to rIL-1ß for up to 3h. Supernatant was analyzed for LTB4, the time course for production indicated a plateau within 1h and constant production through 3h. rIL-1ß induced LTB_4 production in a dose related fashion with the peak responsed occurring between 10^3 and 10^4 units/ml. A comparison of LTB_4 production after IL-1 or A23187 (5µM) located A23187 to be the most potent stimulus. Pretreatment of alveolar macrophages with phenidone, an inhibitor of 5-lipoxygenase and cyclooxygenase, inhibited the increased LTB_4 production after rIL-1ß or A23187 treatment. These data suggest that IL-1 induce pulmonary neutrophilia is associated with enhanced LTB_4 production.

J 407 THE EFFECT OF PHOSPHODIESTERASE INHIBITORS ON PULMONARY INFLAMMATORY CELL INFLUX IN OVALBUMIN SENSITIZED GUINEA PIGS. Robert J. Sturm, Melville C. Osborne, and Richard J. Heaslip. Division of Immunopharmacology Wyeth-Ayerst Research, Princeton, NJ 08543.

Inflammatory cell influx into the alveolar spaces of ovalbumin (OA) sensitized guinea pigs (GPs) was induced by aerosol challenge with OA. Cell recovery via bronchoalveolar lavage revealed that this leukocyte influx reached peak levels at 18h-24h and remained elevated for greater than 72h post-aerosol challenge. At 24 h this resulted in a 3-fold increase in recoverable cells from 1.5 x 10^7 cells per control GP to 4.6 x 10^7 cells per aerosol challenged GP. The histologic profile of the recoverable inflammatory cells changed from 95% macrophage (MØ), 4% eosinophil (EO), and 1% polymorphonuclear leukocyte (PMN) in control GPs to 60% MØ, 33% EO, and 7% PMN in OA-challenged GPs 24 h post aerosol. The effects of both selective and non-selective phosphodiesterase (PDE) inhibitors on this pulmonary inflammatory response were quantified using a b.i.d., p.o. dosing regimen (-1h and +4h relative to aerosol challenge) in which cellular influx was determined at 24 h. The non-selective PDE inhibitor, theophylline, inhibited cell influx by 20% at 25 mpk (b.i.d.) and was toxic at higher doses (b.i.d.). The "PDE-IV" selective inhibitors, rolipram, nitraquazone and Ro 20-1724 inhibited leukocyte influx by >80%at 10 mpk (b.i.d.). In contrast, the "PDE-III" selective inhibitor, milrinone inhibited cell influx by only 27% at 10 mpk (b.i.d.). When single doses of theophylline (50 mpk) and rolipram (10 mpk) were administered at various times pre- and post-aerosol challenge, both compounds inhibited leukocyte influx >60% when administered at -1h. However, whereas rolipram exhibited >50% inhibitory activity when administered as late as +12h post-aerosol, theophylline had no effect when administered post-aerosol challenge. These results suggest that PDE-IV is an important regulator of antigen-induced inflammatory cell influx in this allergic pulmonary model.

J 408 **ASSOCIATION OF EOSINOPHILIA WITH RESPIRATORY SYMPTOMS OF COUGH AND PHLEGM, INDEPENDENT OF IgE LEVEL AND SKIN TEST POSITIVITY.** David J. Tollerud George T. O'Connor, David Sparrow, Scott T. Weiss. **Channing Laboratory, Brigham & Women's and Beth Israel Hospitals; Harvard Medical School; and VA Outpatient Clinic, Boston, MA.**

We investigated the relationship of peripheral blood eosinophil count, immediate skin test hypersensitivity to aeroallergens (ST) and serum IgE level to respiratory symptoms among 1071 men ages 41-86 years, including 158 current cigarette smokers and 913 nonsmokers. Study subjects were participants in the Normative Aging Study, a longitudinal study of adult men which began in the 1960's with enrollment of over 2000 men free of chronic respiratory disease. Serum IgE level was closely associated with symptoms of wheezing, while ST positivity was most strongly associated with hay fever syptoms. Logistic regression analysis was used to analyze the relationship of eosinophilia (>275 cells/mm^3) to respiratory symptoms, adjusting for age, cigarette smoking, IgE level and ST positivity. In this model, eosinophilia was not associated with asthma or hay fever symptoms. However, eosinophilia was significantly associated with symptoms of phlegm production (OR = 2.1; 95% CI 1.3-3.2), and self-reported diagnosis of chronic bronchitis (OR = 2.2; 95% CI 1.1-4.4), independent of IgE, ST, and cigarette smoking, even when current and past asthmatics were excluded. These results support the concept that the eosinophil plays an important role in the airways inflammation that characterizes chronic bronchitis, independent of atopy or IgE-mediated allergic phenomena.

(Supported by NHLBI HL34654 and VA Medical Research Service)