Meeting report

New York Academy pulmonary meeting – March 22, 1990

W. Kreutner and W. Selig

Schering-Plough Corp., 60 Orange Street, Bloomfield, NJ 07003/USA

There has been considerable clinical and experimental interest in recent years concerning the role of inflammatory processes in pulmonary diseases. To meet an important requirement for up to date information on this topic, a meeting was held on March 22, 1990 at the New York Academy of Sciences to review the current status of cells and mediators contributing to pulmonary inflammation in various disease states (asthma, adult respiratory distress syndrome, asbestosis, etc.). This meeting was jointly sponsored by the Inflammation Research Association and the Pulmonary Research Group.

Dr. Joe G. N. Garcia (Indiana University School of Medicine, Indianapolis) began the session by reviewing the pathogenesis of asbestos-induced lung inflammation and related effects on alveolar macrophage function. Asbestosis is a chronic, progressive lung disorder characterized by fibrosis, restricted lung volumes and abnormal lung-gas exchange. Early histopathology reveals alveolar wall thickening with the accumulation of asbestos fibres within the lung interstitium which eventually leads to alveolar destruction. Unlike emphysema, there is no evidence for the involvement of neutrophil elastase in asbestosis, although there is a marked increase in active collagenase I, which may be neutrophil derived. Alveolar macrophages, which normally constitute > 90% of recovered cells in bronchoalveolar lavage (BAL) fluid, are the initial cells to interact with and ingest asbestos fibers. Asbestosis-induced activation of macrophages releases chemoattractant mediators (i.e.

leukotriene B₄; LTB₄) for neutrophils and eosinophils whose numbers increase in BAL fluid following asbestos inhalation. Garcia et al. [Am. Rev. Respir. Dis. (1989) 139, 1494] have shown enhanced basal and stimulated *in vitro* release (A23187 or arachidonic acid) of LTB₄ by BAL macrophages isolated from asbestosis patients. Significant elevations in BAL fluid LTB₄ were noted following asbestos challenge in this group of patients. In addition, preliminary evidence was presented suggesting that asbestos interacts with monocytes/macrophages to induce the gene expression of the proinflammatory cytokine, interleukin-1. Dr. Garcia summarized his findings and proposed that asbestos-induced activation of alveolar macrophages causes the release of chemotactic factors such as LTB₄ which subsequently attract both neutrophils and eosinophils. These leukocytes are stimulated to release active collagenase and reactive oxygen species (i.e. O₂⁻) which ultimately cause parenchymal injury and interstitial fibrosis.

Dr. Thomas Martin, of the University of Washington at Seattle, delivered an informative talk concerning the regulation of inflammatory cell traffic in the lung. This work derived from an interest in lung diseases such as Adult Respiratory Distress Syndrome (ARDS) where there is striking evidence of airway neutrophil accumulation in association with diffuse lung injury. Dr. Martin designed studies to further explore and understand the chemotactic factors released from resident lung cells, such as alveolar macrophages, that may contribute

to neutrophil accumulation within the lung. While alveolar macrophages release LTB_4 (a major chemoattractant for neutrophils) upon stimulation with zymosan, there was experimental evidence presented suggesting that LTB_4 did not meet the criteria to implicate it as the major chemoattractant in inflammatory lung changes associated with such diseases as ARDS. When LTB_4 was delivered to the right lung lobe of human volunteers via bronchoscopy, large increases in total BAL cell counts and neutrophil differentials were noted. Despite the large increases in BAL neutrophils, only modest increases in total BAL protein concentrations (an indicator of permeability changes) were elicited in response to LTB_4 instillation suggesting that neutrophil recruitment and/or BAL protein accumulation may not be an adequate index of lung injury under these experimental conditions. Dr. Martin went on to discuss work performed in his laboratory to identify novel neutrophil chemotactic peptides released by alveolar macrophages. Alveolar macrophages isolated from porcine BAL fluid were exposed to LPS. Fractions of the macrophage supernatant exhibiting chemotactic activity were purified using SP-Sephadex, SP-HPLC and RP-HPLC. Two 10 kD proteins with chemotactic activity were found and designated AMCF-I and AMCF-II. AMCF-I exhibited sequence homology with NAP-1/IL-8. AMCF-II shares sequence homology with NAP-2. Both NAP-1 and NAP-2 are peptides produced by activated human monocytes. In contrast to LTB_4 , instillation of AMCF-I into pig lungs elicits an inflammatory response characterized by profound pulmonary neutrophil accumulation and protein exudation. Dr. Martin suggested that it may be appropriate to view the pulmonary inflammatory response as an acute phase involving chemoattraction elicited by LTB_4 and a prolonged phase involving chemoattraction elicited by proteins such as AMCF-I and AMCF-II. From these findings, Dr. Martin suggested that future drug strategies to treat lung inflammation should incorporate both AMCF-I and AMCF-II as a target molecules.

The next three speakers focused on asthma as a lung disease with an important inflammatory component. Dr. Robert Schellenberg of the University of British Columbia delivered a presentation concerning putative mechanisms and mediators of airway hyperreactivity. His initial discussion dealt with mechanical factors such as altered smooth

muscle shortening and airway wall thickness which may affect airway resistance to airflow. Factors contributing to increased airway wall thickness include edema formation, inflammatory cell infiltration and plasma exudation. Dr. Schellenberg went on to describe an allergic guinea pig model in which animals exposed to aerosolized 1% ovalbumin + 4% Bordetella pertussis toxin twice a week for 4–6 weeks developed a prolonged airway hyperreactivity to acetylcholine. Histological examination of lungs taken from these animals revealed marked tissue eosinophilia in the absence of epithelial damage. A role for leukotrienes (LT) and platelet-activating factor (PAF) in this model of airway hyperreactivity was studied using selective inhibitors or enzyme antagonists of these mediators. L-663 536, a LT synthesis inhibitor, did inhibit the hypersensitivity but not the maximal pulmonary resistance changes due to acetylcholine, whereas SRI-64 412, a PAF receptor antagonist, markedly reduced airway hyperreactivity to acetylcholine without effecting antigen-induced tissue eosinophilia.

The regulation of BAL eosinophil accumulation in an actively sensitized, antigen-challenged guinea pig model was further described by Dr. Robert Egan of Schering-Plough Research in New Jersey. Unlike Dr. Schellenberg's model, this model utilizes only a single acute exposure to inhaled antigen to induce a marked eosinophilia in unanesthetized animals. Peak accumulation of BAL eosinophils (55% of total recovered cells) occurred 24 hrs following antigen exposure. Numbers of BAL eosinophils correlated well with BAL fluid eosinophil peroxidase concentrations. Histological examination of lung tissue removed from guinea pigs at 24 hrs post-antigen showed evidence of significant accumulation of eosinophils in the bronchi and bronchioles and lesser amounts in the alveoli. Based on measurement of BAL fluid guinea pig albumin (as an index of vascular permeability) out to 24 hrs post-antigen challenge, there was evidence of increased vasopermeability only immediately (5 min) following antigen challenge. The pharmacology of this model of lung eosinophilia was studied using various selective enzyme inhibitors and receptor antagonists. Betamethasone, phenidone (a 5-lipoxygenase inhibitor) and WEB 2086 (a PAF antagonist) all inhibited eosinophil accumulation whereas indomethacin was ineffective in this model. A new compound, Sch 37 224,

which inhibits leukotriene and thromboxane release from allergic lung *in vitro* and exhibits anti-anaphylactic activity *in vivo*, was equipotent with betamethasone in inhibiting antigen-induced eosinophil influx.

The final speaker was Dr. Robert Lemanske, Jr., from the University of Wisconsin, who reviewed our current understanding concerning the importance of viral infection as a causative or exacerbating agent in asthma and airway hyperreactivity. He presented evidence suggesting that allergic rhinitis patients infected with rhinovirus can develop an airway hyperreactivity to histamine similar to that seen in asthmatics. Furthermore, patients who routinely exhibit only acute pulmonary responses to allergen challenge appear to develop a dual phase pulmonary response (early and late phase bronchospasm) during episodes of respiratory infection. The type of viral infection, baseline airway resistance, and bronchoprovocative agent used to measure airway function are all factors which influence the degree of airway hyperreactivity noted in this patient population. Although the

precise mechanism(s) of how viral infections contribute to heightened airway responsiveness are not completely understood, various studies have revealed a number of possible explanations. Basophils that infiltrate lung tissue following antigen challenge exhibit an increased sensitivity (measured as histamine release) to antigen after rhinovirus exposure. Likewise, interferon production during upper respiratory infection appears to facilitate histamine release from basophils. Interestingly, the virus-induced enhanced responsiveness noted in basophils is not present in airway mast cells. Dr. Lemanske also alerted us to recent studies which suggest that viral infections may alter normal respiratory epithelial cell function by decreasing the neutral endopeptidase (an enzyme responsible for tachykinin degradation) activity associated with these cells. Removal of this mechanism results in a heightened airway response to tachykinins.

The meeting was closed with an active question and answer session from the audience. This was followed by a dinner to honor the speakers.