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Pulmonary Morphologist—Opportunity for Ph.D. or M.D. for appointment as Assistant or Associate Professor (research) in the Institute for Environmental Medicine and the Department of Pathology and Laboratory Medicine at the University of Pennsylvania School of Medicine. Successful candidate will collaborate with established investigators in respiratory physiology/experimental pathology and will develop an independent research program in respiratory cell biology. Experience with electron microscopy is essential. Ample space, funding opportunities, and state-of-the-art facilities are available. Deadline for applications is December 31, 1985. Interested applicants should send C.V., statement of research interests, and names of three references to Aron B. Fisher, M.D., Director, Institute for Environmental Medicine, Chairman, Pulmonary Morphology Search Committee, University of Pennsylvania School of Medicine/G2, Philadelphia, PA 19104. The University of Pennsylvania is an equal opportunity/affirmative action employer.

Pulmonologist: Board certified or eligible to join expanding 2-man group in midwest city with large university. Must be proficient in all aspects of pulmonary and critical care medicine and be willing to do some internal medicine. Please reply with C.V. to Box 575.

Pulmonary physician sought to join pulmonary division of a major Canadian university hospital. Candidate must be experienced and capable of conducting independent clinical or basic research. Reply Box 583.

Pulmonologist/Internist, require BE/BC—pulmonary. N.Y.C. suburb, active ICU, office consultation, 80% pulmonary, PVT prac-

tice, teaching affiliations. Proficient all procedures. Available now or July 1986. Reply Box 582.

Postdoctoral Research Fellowships in Lung Research for 1985–86 and 1986–87 available in the University of California, Davis (UCD) Interdisciplinary Pulmonary Disease Research Group. Ph.D., M.D., or DVM required. Ongoing research areas include nutritional aspects of lung disease, alveolar macrophage cytochemistry, respiratory glycoprotein biosynthesis and metabolism, lung collagen and elastin, lung liposomes and lipoprotein metabolism, airway nerves and blood supply, relationships of xenobiotic metabolism to pulmonary cytotoxicity, mechanism of respiratory epithelial differentiation, respiratory tract permeability, and inhalation toxicology. Eight UCD faculty with research activities in lung disease involved in fellowship training program. Send resume to: Dr. C. E. Cross, Division of Pulmonary Medicine, 4301 "X" St., University of California, Davis Medical Center, Sacramento, CA 95817. Equal Opportunity/Affirmative Action Institution.

Practice for sale: Pulmonary and Internal Medicine. Active practice for 11 years. Nice Chicago suburban location. Practice includes office building, fully equipped office. Will introduce. Reply to Box 584.

Pulmonologist—To join 7 internists in 20-man multispeciality group. Must be able to practice excellent general internal medicine as well. City of 32,000 with 2 hospitals, serving area of 200,000. No pulmonologist in area. Attractive family environment, nearby lake, convenient to Twin Cities. Send C.V. to Administrator, Park Clinic, 890 North Eisenhower, Mason City, IA 50401.

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The Concentration of Leukocyte Elastase- α 1-Proteinase Inhibitor Complex in Bronchoalveolar Lavage Fluids from Healthy Human Subjects¹⁻³

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The protease-antiprotease theory of pulmonary emphysema holds that alveolar structures may be destroyed by proteases such as neutrophil elastase but are normally protected from destruction by elastase inhibitors such as α 1-proteinase inhibitor (α 1PI). There is, however, only circumstantial evidence in favor of this theory. For instance, emphysema may be induced in animals with large doses of neutrophil elastase (1), and inherited deficiency of α 1PI is linked to a high frequency of this disease (2). By contrast, demonstration of elastase- α 1PI complex in the lower respiratory tract would provide direct evidence that elastase is released and inhibited at the alveolar level. Recently, 2 groups of investigators have developed enzyme-linked immunosorbent assays for the quantitation of human leukocyte elastase- α 1PI complexes (3, 4). We therefore decided to use one of these methods (3) to measure the amount of elastase- α 1PI complex in bronchoalveolar lavage fluids from healthy human smokers and nonsmokers.

The 17 lavage fluids used in this study are part of the 20 samples collected and described previously (5). Briefly, a B3 fibroscope (Olympus Corp. of America, New Hyde Park, NY) was wedged into the distal branch of the lingula, and five 60-ml portions of saline were infused into the lung and recovered in a vacuum trap (mean recovery, 50 \pm 10%). The 5 samples were pooled, centrifuged, and frozen until used. After thawing, they were concentrated 5-fold by Amicon UM2 ultrafiltration (Amicon Corp., Lexington, MA).

The mean age of the 8 nonsmokers and of the 9 smokers was 27.8 \pm 10.8 yr and 25 \pm 2.9 yr, respectively. The smokers (smoking rate, 8.5 \pm 4.9 pack-years) did not smoke for at least 24 h before lavage. The nonsmokers' and the smokers' fluids contained 12.1 \pm 7.2 \times 10⁶ and 28.7 \pm 18.5 \times 10⁶ alveolar macrophages and 0.28 \pm 0.14 \times 10⁶ and 0.28 \pm 0.51 \times 10⁶ polymorphonuclear neutrophils per lavage, respectively.

The elastase- α 1PI concentration was determined on the concentrated fluids using the enzyme-linked immunosorbent assay described in detail by Neumann and coworkers (6). Briefly, the samples were added to microtitration plates coated with sheep antielastase IgG. This antibody does not cross-react with cathepsin G and other neutrophil proteinases. After incubation and washing, the solid phase-bound elastase- α 1PI complexes were reacted with alkaline phosphatase-labeled rabbit anti- α 1PI IgG. After further washings, *p*-nitrophenylphosphate was added to measure the amount of solid phase-bound elastase- α 1PI complexes. The assay was calibrated using a standard solution of known

SUMMARY Although α 1-proteinase inhibitor (α 1-antitrypsin) is widely thought to protect lung elastin against the elastolytic action of leukocyte elastase, there is only circumstantial evidence for such a protective role. We have demonstrated and quantified elastase- α 1-proteinase inhibitor complex in bronchoalveolar lavage fluids from healthy smokers and nonsmokers using a new enzyme-linked immunosorbent assay. The relative concentration of complex is 0.36 \pm 0.48 mmol/mol albumin in nonsmokers and 0.33 \pm 0.29 mmol/mol albumin in smokers. Less than 1% of lavage fluid α 1-proteinase inhibitor is complexed with elastase (0.31% in nonsmokers and 0.34% in smokers). This proportion is, however, much higher than in normal plasma where only approximately 0.006% of inhibitor is bound to elastase. Our data confirm that α 1-proteinase inhibitor efficiently acts as an antielastase barrier in the lower respiratory tract.

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elastase- α 1PI concentration. The preparation of this solution is described in detail in the report by Neumann and coworkers (6). Calibration curves identical to those reported by these investigators were always obtained. To determine whether ultrafiltration impairs the measurement of elastase- α 1PI, we used 3 lavage fluids collected from polytraumatized patients, and we measured the concentration of complex before and after 5-fold fluid concentration by ultrafiltration (in these fluids the elastase- α 1PI concentration was sufficiently high to allow the assay to be performed on unconcentrated samples). The 3 concentrations of complex expressed as microgram of elastase per milliliter of unconcentrated fluid, were 0.81, 1.04, 6.38, and 0.86, 1.01, 6.14 before and after ultrafiltration, respectively. These data show that the concentration process does not significantly distort the data.

All lavage fluids except 1 contained detectable amounts of elastase- α 1PI complex. The absolute concentrations ranged from 0 to 15.6 ng elastase/ml in nonsmokers and from 0.6 to 7 ng elastase/ml in smokers. The relative concentrations (mean \pm SD) were 0.36 \pm 0.48 mmol elastase- α 1PI/mol albumin in nonsmokers and 0.33 \pm 0.29 mmol elastase α 1PI/mol albumin in smokers. The relative concentrations of immunoreactive (total) α 1PI were 88 \pm 34 mmol α 1PI/mol albumin in nonsmokers and 92 \pm 27 mmol α 1PI/mol albumin in smokers (5). Hence, the percentage of elastase-bound α 1PI was 0.31 \pm 0.31 in nonsmokers and 0.34 \pm 0.24 in smokers. The individual values obtained for the 2 groups of subjects are shown in figure 1; this confirms that both relative concentrations of elastase- α 1PI complex are very scattered. Thus, smokers and nonsmokers have very similar levels of complex. In addition, the concentration of elastase- α 1PI complex does not significantly correlate with the smoking rate (pack-years) or with the concentration of polymorphonuclear leukocytes present in the lavage fluids.

In vitro studies have shown that cigarette smoke condensate releases elastase from neutrophils (7). It is therefore surprising that the smokers did not have higher amounts of elastase- α 1PI complex than did the nonsmokers. This might in part be due to the short smoking history of the volunteers (8.5 \pm 4.9 pack-years). It is also possible that some of the elastase- α 1PI complexes formed during smoking disappeared from the lung surface during the 24-h nonsmoking period that preceded the smokers' lavages. Further experiments are required to clarify this point.

* * *

The mean absolute concentration of complexed elastase in the 17 samples is 4.2 ng/ml. Taking into account the mean volume of lavage fluids (150 ml), we get an average of approximately 600 ng of α 1PI-bound elastase per lavage. Because neutrophils contain about 3.5 μ g elastase per 10⁶ cells (8), the aforementioned amount of elastase corresponds to 0.17 \times 10⁶ neutrophils, i.e., to 60% of the neu-

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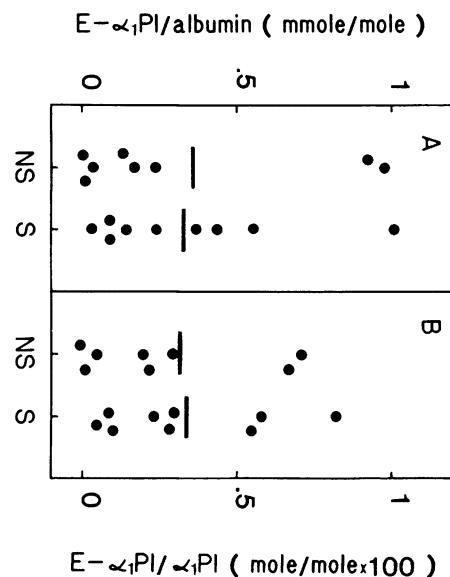


Fig. 1 Relative concentrations of elastase- α 1PI complex (E- α 1PI) in bronchoalveolar lavage fluids from 8 non-smokers (NS) and 9 smokers (S). Horizontal bars indicate mean values. A. The data are expressed relative to the concentration of albumin (5). Mean \pm SD = 0.36 ± 0.48 mmol elastase- α 1PI/mol albumin in nonsmokers and 0.33 ± 0.29 mmol elastase- α 1PI/mol albumin in smokers. B. The data are expressed relative to the concentration of immunoreactive (total) α 1PI (5). Mean \pm SD = $0.31 \pm 0.31\%$ elastase-bound α 1PI in non-smokers and $0.34 \pm 0.24\%$ elastase-bound α 1PI in smokers.

trophils recovered per lavage in our population of healthy subjects. The amount of elastase- α 1PI complex must therefore be considered as high despite the fact that it represents only 0.3% of total α 1PI. It must also be emphasized that in normal plasma only about 0.006% of α 1PI is complexed to neutrophil elastase (4, 6). Our data therefore suggest that even in healthy persons there is massive liberation of neutrophil elastase in the lower respiratory tract and that α 1PI efficiently acts as an antielastase barrier.

The present data also show that *in vivo* complex formation between α 1PI and neutrophil elastase does not account for the high proportion of functionally inactive α 1PI present in lung lavage fluids (5). Other factors such as proteolytic degradation (9) might be responsible for the partial inactivity of α 1PI.

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