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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 α1-proteinase inhibitor (α1PI). 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 α1PI is linked to a high frequency of this disease (2). By contrast, demonstration of elastase-α1PI 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-α1PI complexes (3, 4). We therefore decided to use one of these methods (3) to measure the amount of elastase-α1PI 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., 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 ± 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 ± 10.8 yr and 25 ± 2.9 yr, respectively. The smokers (smoking rate, 8.5 ± 4.9 pack-years) did not smoke for at least 24 h before lavage. The nonsmokers’ and the smokers’ fluids contained 12.1 ± 7.2 x 10^6 and 28.7 ± 18.5 x 10^6 alveolar macrophages and 0.28 ± 0.14 x 10^10 and 0.28 ± 0.51 x 10^10 polymorphonuclear neutrophils per lavage, respectively.

The elastase-α1PI concentration was determined on the concentrated fluids using the enzyme-linked immunosorbent assay described in detail 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-α1PI, 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-α1PI 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-α1PI 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 ± SD) were 0.36 ± 0.48 mmol elastase-α1PI/mol albumin in nonsmokers and 0.33 ± 0.29 mmol elastase α1PI/mol albumin in smokers. The relative concentrations of immunoreactive (total) α1PI were 88 ± 34 mmol α1PI/mol albumin in nonsmokers and 92 ± 27 mmol α1PI/mol albumin in smokers (5). Hence, the percentage of elastase-bound α1PI was 0.31 ± 0.31 in nonsmokers and 0.34 ± 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-α1PI complex are very scattered. Thus, smokers and nonsmokers have very similar levels of complex. In addition, the concentration of elastase-α1PI complex does not significantly correlate with the smoking rate (pack-years) or with the concentration of polymorphonuclear leukocytes present in the lavage fluids.

**Summary** Although α1-proteinase inhibitor (α1-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-α1-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 ± 0.48 mmol/mol albumin in nonsmokers and 0.33 ± 0.29 mmol/mol albumin in smokers. Less than 1% of lavage fluid α1-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 α1-proteinase inhibitor efficiently acts as an antielastase barrier in the lower respiratory tract.

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-α1PI complex than did the nonsmokers. This might in part be due to the short smoking history of the volunteers (8.5 ± 4.9 pack-years). It is also possible that some of the elastase-α1PI 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.

**Notes**

1. From INSERM Unité 237, Faculté de Pharmacie, Université Louis Pasteur, and Service de Pneumologie, Centre Hospitalier Universitaire, Strasbourg, France, and Abteilung für Klinische Chemie und Klinische Biochemie in der Chirurgischen Klinik der Universität München, D 8000, München, FRG.
2. Supported by grants from the INSERM (C.R.L. 81502), C.R.L. 815030, and CP 81021), from the Fondation pour la Recherche Médicale, and from the Deutsche Forschungsgemeinschaft München (Son­derforschungsbereich 0207, LP8).
3. Requests for reprints should be addressed to Dr. Joseph G. Bieth, INSERM Unité 237, Faculté de Pharmacie, B.P. 10, 67048 Strasbourg-Cédex, France.

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trophils recovered per lavage in our population of healthy subjects. The amount of elastase-α1PI complex must therefore be considered as high despite the fact that it represents only 0.3% of total α1PI. It must also be emphasized that in normal plasma only about 0.006% of α1PI 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 α1PI efficiently acts as an antielastase barrier.

The present data also show that in vivo complex formation between α1PI and neutrophil elastase does not account for the high proportion of functionally inactive α1PI present in lung lavage fluids (5). Other factors such as proteolytic degradation (9) might be responsible for the partial inactivity of α1PI.

Acknowledgment

The writers thank Dr. S. Neumann and H. Lang, Biochemical Research Institute, Merck, Darmstadt, FRG for supplying the reagents for the immunoassay of elastase-α1PI complex.

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