Localisation of a low-molecular-weight bronchial protease inhibitor in the peripheral human lung

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ABSTRACT The localisation of a low-molecular-weight bronchial protease inhibitor (LMI) in human peripheral lung tissue was studied by an immunohistochemical method. This inhibitor was found exclusively in a part of the non-ciliated cell population of the bronchiolar epithelium. An inverse relationship was observed between the number of LMI-positive non-ciliated cells per millimetre of basement membrane and the bronchiolar diameter. These findings may have a bearing on the protease-antiprotease theory of the development of pulmonary emphysema, LMI possibly playing a part in the protection of peripheral lung tissue against proteolytic destruction by enzymes liberated from granulocytes or migrating alveolar macrophages.

According to the protease-antiprotease theory, pulmonary emphysema may result from a disturbed balance between proteases and antiproteases—for example, neutrophil elastase and α_1 -antitrypsin.¹ Because a small proportion of cases of pulmonary emphysema are associated with a severe genetic α_1 -antitrypsin deficiency it seems probable that other elastase inhibitors play a part in preventing emphysema.

The low-molecular-weight bronchial protease inhibitor (LMI) isolated from sputum has been proved to be a potent elastase inhibitor.^{2,3} Immunohistochemical studies using a monospecific antiserum against LMI have shown this inhibitor to be present in serous cells, but not in mucous cells of the submucosal glands lining the larger airways.^{4,5} The function of LMI in the larger airways might well be the prevention of protease-induced epithelial damage.

Extensive studies on the localisation of LMI in lower airways have not yet been reported, although the presence of the inhibitor in non-ciliated bronchiolar epithelial cells has been suggested.⁶ We studied the localisation of LMI in the lower airways using an immunohistochemical method and also obtained quantitative data on the number of LMI-positive cells in lower airways. We discuss the results in relation to the protease-antiprotease theory of the

development of emphysema. The occurrence of lysozyme in upper airways, which has been found to have the same localisation as LMI,^{4,5,7} is also described.

Methods

Lungs (or lung lobes) were obtained by thoracotomy from 15 patients with limited bronchial carcinoma. The tissue was fixed in Bouin's fixative for four hours, inflated at a constant pressure of 20 cm H₂O. After fixation samples of macroscopically unaffected segments of peripheral tissue were routinely processed and embedded in paraplast. Rabbit antisera directed against LMI or human lysozyme were prepared and characterised as we have described.⁴ Immunohistochemical detection of LMI and lysozyme was performed by a peroxidase-antiperoxidase technique.⁸ Control experiments were performed by substituting normal rabbit serum or anti-LMI antiserum blocked by purified LMI for the specific antiserum.⁴ Morphometric measurements were performed on histologically normal tissue samples. Sections showing emphysematous lesions (parenchyma with a mean linear intercept exceeding 200 μ^2) or signs of small airways disease (goblet-cell metaplasia) were excluded from this study.

In LMI-stained sections bronchiolar diameters were determined by measuring the length of the basement membrane surrounding a bronchiole on a graphic tablet (MOP-AM 03, Kontron). The length

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of the basement membrane divided by π was used as diameter of the bronchiole.

In bronchiolar epithelium LMI-positive and LMI-negative non-ciliated cells were counted. Occasional goblet cells were not counted. From these data we calculated (a) the number of LMI-positive non-ciliated cells per millimetre of basement membrane surrounding the bronchiole and (b) the total number of non-ciliated cells (LMI-positive and LMI-negative cells) per millimetre of basement membrane. Both indices were interpreted as cell densities.

Fig 1 (a) Bronchial epithelium stained for low-molecular-weight protease inhibitor (LMI), showing LMI-positive non-ciliated cell (▲). (b) LMI-stained bronchiolar epithelium: LMI-positive (▲) and LMI-negative (★) cells. ($\times 950$.)

Results

The presence of LMI in peripheral lung tissue

In sections of peripheral lung tissue non-ciliated cells in the bronchiolar epithelium were seen to be stained for LMI (fig 1). Ciliated cells of the bronchiolar epithelium were invariably negative for LMI, as were the alveolar type I and type II cells and alveolar macrophages. Sections incubated with normal rabbit serum or with anti-LMI previously blocked with purified LMI were negative in all cell types. Lysozyme was not detected in any of these cells.

Frequency of LMI positive cells in the bronchiolar epithelium

Only a part of the non-ciliated cell population stained for LMI. In addition, the number of LMI-positive non-ciliated cells tended to increase with decreasing bronchiolar diameters. This is shown in fig 2, in which the density of the LMI-positive part of the non-ciliated cell population is plotted against the bronchiolar diameter. An inverse relationship is also observed between the density of the LMI-negative non-ciliated cells and the bronchiolar diameter. In fig 3 the relationship between the total number of non-ciliated cells per millimetre of basement membrane and the bronchiolar diameter is depicted. No non-ciliated cells were found in bron-

Fig 2 Number of LMI-positive non-ciliated cells per mm of basement membrane as a function of bronchiolar diameters (regression equation: $y = -4.2x + 11.0$, $n = 117$, $r = -0.32$, $p < 0.001$).

Fig 3 Total number of non-ciliated cells per mm of basement membrane as a function of bronchiolar diameters (regression equation: $\ln y = -1.1x + 3.2$, $n = 110$, $r = -0.84$, $p < 0.001$).

chioles with diameters exceeding 3 mm, indicating that these cells are exclusively present in small airways.

At the level of the respiratory bronchioles LMI was infrequently found in cylindrical, but not in cuboidal, epithelial cells.

Discussion

We have previously described the localisation of LMI in seromucous glands of nasal, tracheal, and bronchial submucosa.^{4,5} In this study we found that in peripheral lung LMI is exclusively located in non-ciliated (non-goblet) cells of the bronchiolar epithelium, confirming our preliminary report.⁶ Intracellularly, LMI appears to be localised in granules (unpublished observation).

The function of the non-ciliated cells in the bronchiolar epithelium is not known. In studies using rat lungs it was found that Clara cells, which are non-ciliated epithelial cells,¹⁰ synthesise phospholipases¹¹ as well as other lysosomal enzymes. Other authors reported the presence of P450 cytochrome in Clara

cells of rabbit lungs,¹² suggesting that these cells play a role in the metabolism of xenobiotics (for a review on the various functions of Clara cells see ref 13).

We did not detect lysozyme in any of the cells present in peripheral lung tissue (including alveolar macrophages), which is in agreement with the findings of other immunoperoxidase studies.^{14,15} Lysozyme synthesis has, however, been described in cultured alveolar macrophages as well as in bronchiolar epithelium.¹⁶

The results presented in this paper show that LMI is one of the proteins produced by human non-ciliated cells. The observation that not all non-ciliated cells stain for LMI may suggest the existence of at least two types of non-ciliated cells. Alternatively, the LMI-negative cells may represent a different state of LMI-positive cells—for example, a precursor or degranulated cell. It will be interesting to see whether these LMI-negative cells produce another elastase inhibitor, discovered recently.¹⁷

Our quantitative data suggest that there is an inverse relationship between the density of LMI-positive cells and the bronchiolar diameter. Although the lung tissue used for the localisation of LMI was not affected by bronchial carcinoma and was not emphysematous, possibly results will be different in healthy non-smokers. Preliminary findings, however, in two non-smoking patients confirmed with our results.

The production of LMI, known to be a potent elastase inhibitor, at the level of small bronchioles is interesting from the point of view of the protease-antiprotease theory of emphysema. LMI might play a part in the protection of peripheral lung tissue against proteolytic destruction by enzymes liberated from granulocytes or migrating alveolar macrophages. The amounts of both cell types are greatly increased in smokers and may be associated with the presence of small airways disease or eventually the development of centrilobular emphysema.¹⁸⁻²¹ The relation between the number of LMI-positive cells in bronchioles and the presence of anatomical emphysema is subject of our future study.

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