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Thickness of the basement membrane of bronchial epithelial cells in lung diseases as determined by transbronchial biopsy

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The thickness of the basement membranes of bronchial epithelial cells varies under various pathological conditions. It has been reported that this membrane is thickened in patients with bronchial asthma. By light microscopy, this parameter was measured in biopsy specimens of bronchial mucosa obtained by fibre-optic bronchoscopy. These specimens were obtained from 171 patients who had undergone bronchial biopsy between 1984 and 1994. It was demonstrated that the thickness of the basement membrane of bronchial epithelial cells was weakly correlated with the patient's age, when thickness was examined in patients with lung cancer ($r=0{\cdot}242$, $P=0{\cdot}0268$). The basement membranes in patients with bronchial asthma ($8{\cdot}193 \pm 1{\cdot}362\,\mu$, mean ± SEM) were significantly thicker than those without bronchial asthma ($5{\cdot}145 \pm 0{\cdot}233\,\mu$) ($P=0{\cdot}0180$, Mann–Whitney's *U*-test). In addition, it is noteworthy that the basement membranes in patients with diabetes mellitus ($7{\cdot}217 \pm 0{\cdot}753\,\mu$) were also significantly thicker than those without diabetes mellitus ($4{\cdot}968 \pm 0{\cdot}235\,\mu$) ($P=0{\cdot}0038$, Mann–Whitney's *U*-test). The background or underlying pathophysiology in such patients should be studied further, with attention directed towards the thickness of the bronchial basement membrane in bronchial biopsy specimens.

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Introduction

The basement membrane is a specialized form of extracellular matrix seen as a thin sheet surrounding epithelial tissue, peripheral nerves and muscle, as well as lining all blood vessels (1). It is composed of basal lamina and subjacent reticular lamina in which collagen fibrils are embedded (2,3).

Thickening of the basement membrane is seen under various conditions. Asthmatic patients are known to have thickened basement membranes of bronchial epithelial cells (4–8). The aetiology of the basement membrane thickening in asthma is not known. Probably, it is a consequence of the inflammatory process injuring bronchial epithelial cells, resulting in basement membrane hypersecretion (4,9), or enhancing fibrogenesis by inflammatory cells such as mast cells or eosinophils infiltrating the bronchial mucosa (6). There also have been reports of thickened bronchial basement membranes in various lung diseases such as lung cancer, chronic obstructive pulmonary disease (COPD), bronchiectasis and tubeculosis (8).

Not only glomerular basement membranes but also alveolar basement membranes are thicker in patients with diabetes, and alveolar capillary and epithelial basement membranes are thicker in rats with streptozocin-induced diabetes (10,11). Thus, hyperglycaemia may directly cause endothelial and other cells to increase their production of basement membrane components (7).

Biopsies of lung parenchyma and bronchial mucosa using fibre-optic bronchoscopy are performed frequently for diagnosing various lung diseases. This method has greatly facilitated the diagnosis of diffuse lung diseases as well as lung cancer. However, few investigators have closely examined the basement membrane of bronchial epithelial cells. This paper reports differences in the thickness of basement membranes of bronchial epithelial cells in patients with various lung diseases as determined by bronchial biopsy via fibre-optic

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bronchoscopy. Since the thickness of the basement membranes of bronchial epithelial cells in patients with diabetes mellitus was expected to be of significance, this parameter was also assessed.

Materials and Methods

Specimens from biopsies performed in the Pulmonary Division of the Second Department of Internal Medicine at Fukuoka University Hospital from 1984 to 1994 were used in this study. Since fibre-optic bronchoscopy was conducted for large numbers of cases with lung cancer, only those samples obtained between 1988 and 1993 were used.

The specimens were fixed in 10% buffered formalin, embedded in paraffin, and stained with haematoxylin-eosin by routine procedures. Since the trachea and proximal bronchus have thick basement membranes (12), only specimens which fulfilled the following criteria were selected for this study:

(1) The bronchial epithelial cells and lamina propria were well preserved;
(2) Samples obtained from trachea and main or lobar bronchus by bronchoscopists were excluded;
(3) Samples with pseudostratified, ciliated columnar epithelial cells were included, and samples with simple columnar or cuboidal epithelial cells were excluded, since epithelial cells lining the airway become thinner as proximal airway goes distal to respiratory bronchiole (i.e. pseudostratified to simple cuboidal cells);
(4) Samples in which mucosal glands or pieces of cartilage were found were included;
(5) Sections apparently oblique to the epithelial layers were excluded.

Thus, samples were confined roughly to those obtained from segmental bronchi to the smallest intrapulmonary bronchi or large bronchioles.

Specimens from171 patients with various respiratory diseases including lung cancer, bacterial pneumonia, pulmonary tuberculosis and bronchial asthma were used in this study. The thickness of the basement membrane in each biopsy specimen was measured at the site of perpendicular transection by a light microscope using an ocular micrometer with a magnification of $\times 400$. The thickness of the basement membrane was expressed as the mean of five measurements in each case.

The diagnosis of lung cancer was based on pathological findings in biopsy specimens, and cytologic findings in bronchial brushing and washing or sputum. Bacterial pneumonia was diagnosed by the growth of bacteria in a sputum culture, the peripheral blood leukocyte count, the C-reactive protein, and chest radiograph findings before and after treatment with antibiotics. Only cases in which Niacin test-positive acid-fast bacilli from a sputum culture or epithelioid granuloma(s) with necrosis in the biopsy specimen was demonstrated were counted as cases of pulmonary tuberculosis. Diabetes mellitus was diagnosed according to fasting blood sugar and urine sugar, or the results of a 75 g oral glucose tolerance test (OGTT). All patients in this study were identified as non-insulin-dependent diabetes mellitus. Bronchial asthma was diagnosed according to the definition of bronchial asthma by the American Thoracic Society (13). Non-caseous epithelioid granulomas were demonstrated in biopsy specimens of all patients with sarcoidosis in addition to a negative tuberculin reaction. Bilateral hilar lymphadenopathy and/or diffuse nodular shadows on chest radiograph were also observed in these patients.

Some patients were diagnosed with more than one disease. In those with diabetes mellitus, in particular, there were other major pulmonary diseases.

To determine if the proximal airway has a thickened basement membrane, the calibre of the airway lumen and thickness of the mucosal basement membrane were assessed in nine autopsy cases at the authors' hospital (eight men and one woman who had died in 1994 from conditions unrelated to respiratory disease and diabetes mellitus). The calibre of four to five airway lumens and the thicknesses of their basement membranes were counted in each case.

Statistical Analysis

Statistical analysis was conducted using Mann–Whitney's *U*-test to assess differences in the thickness of basement membranes in various diseases. A P value $<0{\cdot}05$ was considered statistically significant. Linear regression analysis was used to assess the relationships between age and the thickness of the basement membrane, and the calibre of the airway lumen and the thickness of the basement membrane.

Results

Table 1 shows the clinical diagnoses, number of cases, gender and mean age ± SEM in all cases (171 cases), and Table 2 shows demographic and pulmonary function characteristics in patients with asthma. Figure 1 shows the thickness of the basement membrane for patients with various lung diseases and diabetes mellitus. The basement membrane thickness in patients with diabetes mellitus or bronchial asthma appeared thicker than in patients with lung cancer,

TABLE 1. Clinical diagnoses and number of cases

Clinical diagnosis	No. of cases (male/female)	Mean age (SEM)
Lung cancer	84 (63/21)	67·0 (1·2)
Bacterial pneumonia	41 (32/9)	62·5 (1·8)
Diabetes mellitus	23 (15/8)	69·6 (2·0)
Idiopathic pulmonary fibrosis	12 (9/3)	66·8 (3·8)
Pulmonary tuberculosis	12 (8/4)	63·5 (4·3)
Bronchial asthma	7 (6/1)	64·4 (5·0)
Sarcoidosis	4 (1/3)	38·5 (8·6)
Others	18 (14/4)	63·3 (3·6)

TABLE 2. Demographic and pulmonary function characteristics in patients with asthma (n=7)

Age (years)	64·4 ± 5·0	
Sex (F:M)	1:6	
IgE (IU ml^{-1})	1144 ± 407	(n=5)
FEV_1 (ml)	2110 ± 325	
FVC (ml)	3385 ± 396	
FEV_1/FVC (%)	62 ± 6	

FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; data represent mean values ± SEM.

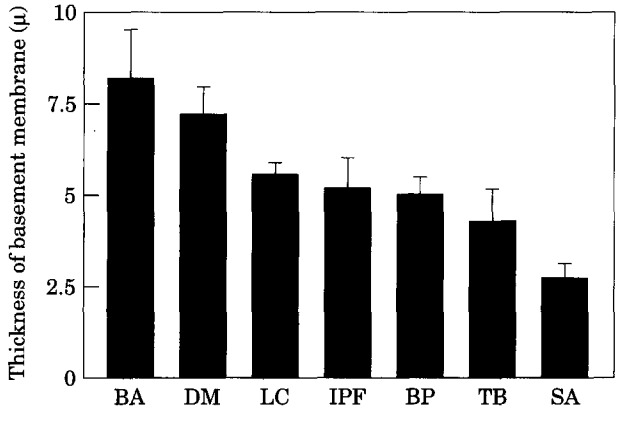

FIG. 1. Thickness of basement membranes of bronchial epithelial cells in various lung diseases. Data are expressed as mean ± SEM. BA, bronchial asthma; DM, diabetes mellitus; LC, lung cancer; IPF, idiopathic pulmonary fibrosis; BP, bacterial pneumonia; TB, pulmonary tuberculosis; SA, sarcoidosis.

TABLE 3. Mann–Whitney's U-test

Variables	P value
Gender	0·0842
Bronchial asthma	0·0180*
Diabetes mellitus	0·0038*
Lung cancer	0·0811
Idiopathic pulmonary fibrosis	0·9831
Bacterial pneumonia	0·4399
Pulmonary tuberculosis	0·2185
Sarcoidosis	0·0587

*, Statistically significant, compared to patients without bronchial asthma and diabetes mellitus.

idiopathic pulmonary fibrosis, bacterial pneumonia, pulmonary tuberculosis or sarcoidosis. Since some patients had more than one disease, the effects of gender, bronchial asthma, diabetes mellitus, lung cancer, idiopathic pulmonary fibrosis, bacterial pneumonia, pulmonary tuberculosis and sarcoidosis on thickness were evaluated using Mann–Whitney's U-test (Table 3). As reported previously, the basement membrane in patients with bronchial asthma was significantly thicker than in patients without bronchial asthma (8·193 ± 1·362 μ *vs* 5·145 ± 0·233 μ, P=0·0180) (mean ± SEM). Also, the basement membrane in patients with diabetes mellitus was significantly thicker than in patients without diabetes mellitus (7·217 ± 0·753 μ *vs* 4·968 ± 0·235 μ, P= 0·0038). Although the basement membrane in men (n=122) appeared thicker than in women (n=49), a

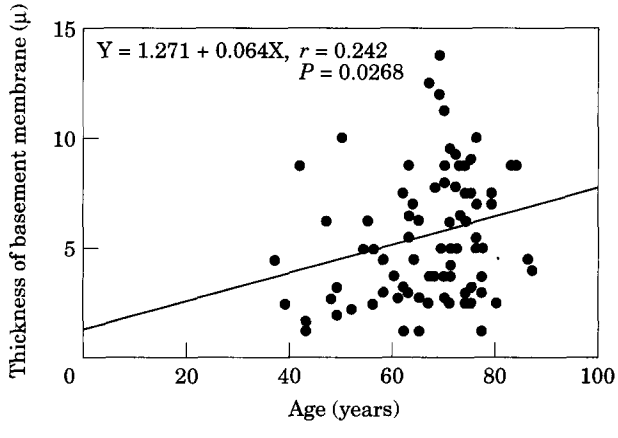

FIG. 2 Relationship between the thickness of basement membranes of bronchial epithelial cells and age in patients with lung cancer. Thickness was weakly correlated with age.

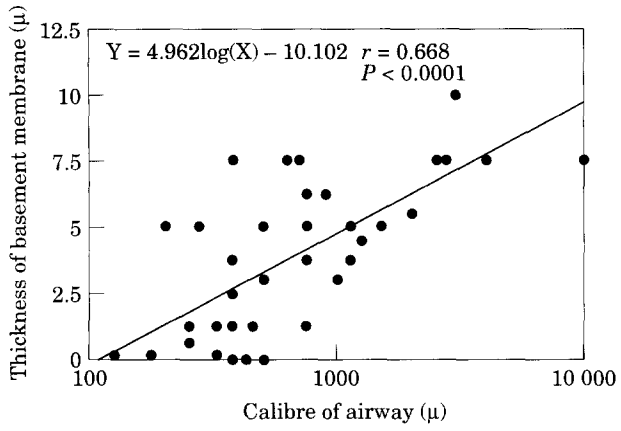

FIG. 3 Relationship between the thickness of basement membranes of bronchial epithelial cells and the calibre of the airway lumen in nine autopsy cases. Thickness was correlated with calibre of airway lumen.

significant difference was not found ($5{\cdot}524 \pm 0{\cdot}284\,\mu$ and $4{\cdot}638 \pm 0{\cdot}397\,\mu$, respectively, $P=0{\cdot}0842$). Figure 2 shows the relationship between thickness of the basement membrane and age in patients with lung cancer. The thickness of the basement membrane weakly correlated with age ($r=0{\cdot}242$, slope$=0{\cdot}064$, $P=0{\cdot}0268$).

Figure 3 shows the relationship between the thickness of the basement membrane and the calibre of the airway lumen in lung specimens obtained from nine autopsy cases. Their mean age at death was $61{\cdot}8 \pm 2{\cdot}6$ years (mean ± SEM). The thickness of the basement membrane correlated well with the calibre of the airway lumen, and could be expressed as $Y = 4{\cdot}962 \times \log X - 10{\cdot}102$ ($r=0{\cdot}668$, $P<0{\cdot}0001$), where Y shows the thickness of the basement membrane (μ), and X shows the calibre of the airway lumen (μ).

Discussion

The lung contains a remarkable assortment of basement membranes, including those of the alveolar wall, pulmonary arteries, veins and microvasculature, as well as those surrounding smooth muscle, glands and nerves (7). Although many electron microscopists have referred to the basement lamina (basal lamina), alone, as the basement membrane (2), this study considered the basement lamina and the reticular lamina together as the basement membrane (2,3). The basement lamina is a glycoprotein layer approximately $0{\cdot}1\,\mu$m thick, composed of fine filaments containing type IV collagen, and it cannot be resolved by light microscopy. The underlying tissue, the reticular lamina, is composed of a condensed ground substance and small irregular bundles of collagen fibrils (2). Usually, it can be visualized with light microscopy.

Alterations in the lung basement membranes occur in various conditions. It is the reticular lamina and not the basement lamina which becomes thickened in various pulmonary diseases. The sites from where samples are taken is an important factor which may affect the analysis of data. The trachea and proximal bronchus have been reported to have prominent basement membranes compared with those of the distal bronchioles (12), and the results of this study in autopsy cases confirmed this finding. To minimize the effects of differences in airway lumen calibres, samples obtained from the trachea, main and lobar bronchus and respiratory bronchioles were discarded.

Aging increases basement membrane deposits at several anatomic sites in humans (8,10), and it also may be an important factor in the thickness of the bronchial basement membrane (7,8,10). The present study found that the thickness of bronchial basement membranes weakly correlates with age when evaluated in patients with lung cancer.

Gender is another factor that appears to affect the thickness of the basement membrane. Shindo *et al.* (14) demonstrated, by electron microscopy, that the thickness of the glomerular basement membrane in patients with recurrent and persistent haematuria and nephrotic syndrome was significantly greater in males than in females. Female steroids, including oestrogen, modulate connective tissue deposition (15). Although bronchial basement membranes in males appeared to be thicker than in females in this study, no significant difference was found.

Electron microscopic studies have shown that there is a uniform thickening or reduplication of the basement membrane in glomeruli, capillaries, alveoli and around nerves in diabetes mellitus (4,7,10). Cagliero *et al.* (16) reported that cultured human umbilical vein endothelial cells supplemented with high glucose showed increased expression of basement membrane components. A study of pulmonary function in patients with insulin-dependent diabetes demonstrated decreased elastic recoil, decreased volumes and decreased pulmonary diffusing capacity (17), which may be secondary to alterations in interstitial connective tissue or pulmonary surfactant (7). However, there have been no reports in the literature describing the thickness of bronchial basement membranes in diabetics. This paper shows, by light microscopy, that the thickness of the bronchial basement membrane in diabetic patients is more prominent than that in non-diabetic patients. However, no close correlation between fasting blood sugar or HbA1c and basement membrane thickness was found (data not shown).

Light microscopy has shown thickening and hyalinization of the reticular layer underlying the epithelial basal lamina of bronchial mucosa, which quite likely represents a salient feature of asthma (8). This study also demonstrated the significant thickness of the bronchial basement membrane in asthmatic patients, compared with non-asthmatic patients. It has been reported that thickened basement membranes in diabetic patients can be detected only by electron microscopy, and not by light microscopy (7,10). However, the thickened reticular lamina in asthmatic patients has been identified by light microscopy (5,6). In this study, thickening of the bronchial basement membranes in diabetic and asthmatic patients could be seen by light microscopy, and these specimens could not be distinguished morphologically.

In malignant neoplasms, basement membranes are newly formed around tumour cell nests, or pre-existing basement membranes are degraded by the invasion and metastasis of tumour cells (4,18). Basement membranes in patients with lung cancer were not clearly shown to be thicker than in patients without lung cancer in the present study.

Basement membranes in patients with sarcoidosis tend to be thin compared with those in other groups. This is partly due to a mean age of 38·5 years and female predominance in patients with sarcoidosis.

For clarification of the pathophysiology in those patients, more attention should be directed towards assessing the thickness of bronchial basement membranes in biopsy specimens.

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