Immunohistochemical classification of the localization of laminin in the thickened bronchial epithelial basement membrane of deceased bronchial asthma patients

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Abstract To ascertain histological changes in the basal lamina of the bronchial epithelial basement membrane in patients with severe bronchial asthma, an immunohistochemical study was conducted in 43 patients who died of bronchial asthma. Antibodies against laminin, a component of the lamina lucida, were utilized. The results revealed various patterns for immunoreactivity to laminin in the thickened basement membrane layer. We were able to classify these reactivities into four patterns. In Pattern A, laminin reactions branched vertically in relation to the thickened basement membrane layer. In Pattern B, laminin reactions formed lines along the lower margin of the thickened basement membrane layer. In Pattern C, laminin reactions formed lines along the upper margin of the thickened basement membrane layer. Finally, in Pattern D, no laminin reactions were observed. In addition, relationships between immunohistological characteristics of laminin and findings such as epithelial cell shedding, basal cell proliferation and basement membrane layer thickening were investigated. In many Pattern A patients, epithelial cell shedding was observed, but goblet cell hyperplasia and basal cell proliferation were barely detectable. Conversely, in numerous Pattern D patients, epithelial cell shedding was barely seen, but goblet cell hyperplasia and basal cell proliferation were marked. Hence, Patterns A and D were on opposite ends of the spectrum of morphological characteristics associated with severe bronchial asthma. In Patterns B and C, laminin reactions formed lines along the lower and upper margin of the thickened basement membrane layer, respectively. However, no marked differences existed in epithelial cell shedding and basement membrane layer thickening. The present study is thus the first to clarify that laminin reactions in the thickened basement membrane layer vary, and this feature is unique to the bronchi of patients with severe bronchial asthma. © 2003 Elsevier Science Ltd. All rights reserved.

Keywords immunohistochemistry; laminin; severe bronchial asthma; epithelial basement membrane.

INTRODUCTION

The principal components of the basement membrane are glycoproteins such as collagen type IV, glycosaminoglycan, laminin, fibronectin and entactin (1–5). Under electron microscopy, the basement membrane has three layers: lamina lucida, lamina densa and lamina reticularis, in order from the cell side (5–7). Laminin, the principal component of the lamina lucida, is secreted from endothelial and epithelial cells, and binds strongly with collagen type IV (8–10) to play an important role in the adhesion, differentiation and migration of these cells (4,9,10). When injury causes epithelial cells to detach, cells are regenerated along the existing basement membrane. Since a lack of the basement membrane hinders cell regeneration, this membrane is considered to act as an anchor for cell proliferation (8).

For a long time, basement membrane layer thickening has been considered one of the typical morphological changes in the bronchi of patients with bronchial asthma (II). This thickening is seen as a uniform amorphic thick layer under light microscopy. Nonetheless, electron microscopy reveals thickening of the lamina reticularis, while the basal lamina, consisting of the lamina lucida and lamina densa, does not thicken (12,13).

Many studies have been conducted to investigate histological changes in the lamina reticularis (12–22), but
few studies have investigated the basal lamina (12,13), and virtually no studies have examined the basal lamina of patients with severe bronchial asthma. The present study immunohistochemically investigated laminin, the principal component of the lamina lucida, to ascertain histological changes in the thickened basement membrane layer in the lungs of individuals who died of severe bronchial asthma. The results demonstrated that immunoreactivity to laminin in the basement membrane layer was not uniform in all patients, showing a great degree of variation.

MATERIALS AND METHODS

Subjects were 43 patients who died of bronchial asthma and were autopsied at the Tokyo Medical Examiner’s Office during about 10 years since 1989. The subject group comprised 30 men and 13 women, ranging in age from 22 to 74 years (mean: 50 years) at time of death. In every patient, autopsy revealed lung hyperinflation and mucus plug in the bronchial lumen, and pathological findings revealed severe bronchial lesions. No other lethal abnormalities were observed in other organs. Although the duration of bronchial asthma could not be ascertained, inspection reports yielded information about the health status of most patients at the time of death. The general health of two patients was fine; 2 patients were sick but not receiving medical care; 1 patient had received medical care in the past, but not at the time of death; and 36 patients were receiving treatments. Health status at time of death was unknown for 2 patients. For controls, 20 patients younger than 65 years, who were autopsied within 24 h of death at our department, were subjected to the same set of tests. These control patients had not died of respiratory organ injuries such as carbon monoxide poisoning, burns, asphyxiation caused by neck compression, or drowning, and they did not display pulmonary abnormalities.

As a general rule, a 10% formaldehyde solution was injected via the bronchi into the lungs at a low pressure for fixation. However, this could not be performed in some patients. After fixation for 2–4 days, a total of 15 specimens were collected from three locations in each left and right lung, and created an incision perpendicular to the longitudinal axis of the airway. Each formalin-fixed specimens were embedded in paraffin and stored at room temperature. Then, 3–4 μm sections were prepared from each specimens, deparaffinized, and subjected to immunohistochemical staining using anti-laminin antibodies. Since changes associated with bronchial asthma have been reported in the segmental and subsegmental bronchi (23–25), the cross-sections of the subsegmental bronchus of each patient were used. Immunostaining was performed using a Vectastain ABC-PO mouse IgG kit (Vector Laboratories Inc., Burlingame, USA) according to the avidin–biotin–peroxidase complex (ABC) method. After deparaffinization, endogenous peroxidase deactivation was performed using 3% hydrogen peroxide solution for 3 min. Next, these sections were treated with 0.5% trypsin for 20 min to activate antigens. In order to block nonspecific reactions, normal horse serum was allowed to react with sections for 20 min. As primary antibody, mouse anti-human laminin monoclonal antibody (Daiichi Fine Chemical Co., Ltd., Toyama, Japan) was diluted to 6.25 μg/mL and allowed to react with the sections at 30°C overnight. After washing the sections using phosphate buffered saline (PBS), biotin-labeled secondary antibody (horse anti-mouse IgG antibody) was allowed to react with the sections at 37°C for 30 min. Then, the avidin–biotin–peroxidase complex was allowed to react with the sections at 37°C for 30 min., and the resulting sections were immersed in a DAB solution (0.02% 3, 3’-diaminobenzidine tetrahydrochloride, 5% H2O2 in 0.05 M Tris-HCl, pH 7.6) (Dojindo Laboratories Inc., Kumamoto, Japan). After staining nuclei using hematoxylin, ENTENELLAN (Merck KgaA, Darmstadt, Germany) was used for mounting, and photographs were taken under a light microscope (PROVIS AX80, OLYMPUS, Tokyo, Japan).

Furthermore, with some specimens, the relationship between immunoreactivity to laminin and such findings as, goblet cell hyperplasia, epithelial cell shedding, basal cell proliferation and basement membrane layer thickening, was investigated.

RESULTS

Figure 1 shows laminin reactions in the subsegmental bronchus of a normal lung. The surface of the bronchus was covered with pseudostratified ciliated epithelia, and laminin reactions formed lines under the epithelial cells.

**Fig. 1.** Immunohistochemical staining with antibody anti-human laminin in normal bronchus. Antibody anti-laminin shows strong positivity along the epithelial cells. Thick laminin reaction lines under rows of pseudostratified ciliated epithelia (ABC method, original magnification × 700).
Some laminin reactions were also seen in the lamina propria.

Laminin reactions detected in the thickened basement membrane layer of the subsegmental bronchus of bronchial asthma patients were divided into four patterns: In Pattern A, laminin reactions branched out vertically in relation to the basement membrane layer; in Pattern B, laminin reactions formed lines along the lower margin of the basement membrane layer; in Pattern C, laminin reactions formed lines along the upper margin of the basement membrane layer; and in Pattern D, no laminin reactions were seen. When different patterns were seen, a predominating pattern was selected for classification purposes.

In the lamina propria, findings such as, inflammatory cell infiltration, edema, vasodilatation, smooth muscle hypertrophy and submucosal gland hypertrophy were seen, but there were no marked differences among the four patterns. Nonetheless, as will be discussed later, there were slight differences in the degree of capillary proliferation among the four patterns.

In Pattern A, laminin reactions formed lines that ran vertically in relation to the basement membrane layer. Individual lines were narrow and thin. Also, some laminin reactions surrounded capillaries in the lamina propria and extended to the basement membrane layer. Although laminin reactions formed a single narrow thin line in the lamina propria, they branched out into several lines in the basement membrane layer. The tip of these branches extended mostly vertically or at an angle, but horizontally in some cases (Fig. 2). This pattern was seen around the entire circumference of the basement membrane layer in four patients and in one to two-thirds of the entire circumference in the remaining patients. In these patients with Pattern A reactions that did not surround the entire circumference, Pattern B (laminin reactions along the lower margin of the basement membrane) or Pattern D (no laminin reactions) was also seen. Capillary proliferation in the lamina propria, mostly ranging from moderate to severe in severity, was seen in almost all patients. Pattern B laminin reactions were seen along the lower margin of the basement membrane layer. Although most laminin reactions appeared to form lines, there were some notable spots, and where lines were terminated, some lines extended vertically in relation to the basement membrane layer. In all five patients, reactions were present partially, not all around the circumference of the basement membrane layer. Pattern A, C or D was observed where Pattern B laminin reactions were not detected. However, there was no connection between laminin reactions in the lamina propria and those in the basement membrane layer (Fig. 3). Also, when compared to the other patterns, thickening of the basement membrane layer was relatively milder for Pattern B. Furthermore, as shown in Fig. 4, Pattern C was characterized by strong thick laminin lines along the upper margin of the basement membrane layer. Pattern C reactions were seen around the entire circumference of the basement membrane layer in one patient, about 1/3 the circumference in two patients, and 2/3 the circumference in three patients. When Pattern C reactions were partially seen around the circumference of the basement membrane layer, Pattern A-like reactions, where laminin reactions branched out in the basement membrane layer and extended narrowly and thinly, were seen in three of the six patients. These laminin reactions in the lamina propria were narrow but strong, extending toward the lower margin of the basement membrane layer in a reticular feature.

Also, in Pattern D, although basement membrane layer thickening was most pronounced, no laminin reactions along the lower margin of the basement membrane layer for deceased bronchial asthma subject. Laminin reactions run perpendicular to the thickened basement membrane layer. Laminin reactions surrounding capillaries in the lamina propria extend into basement membrane layer in some areas (ABC method, (original magnification × 700).

FIG. 2. Pattern A immunoreactivity for laminin of the basement membrane layer for deceased bronchial asthma subject. Laminin reactions run perpendicular to the thickened basement membrane layer. Laminin reactions surrounding capillaries in the lamina propria extend into basement membrane layer in some areas (ABC method, (original magnification × 700).

FIG. 3. Pattern B immunoreactivity for laminin of the basement membrane layer for the deceased bronchial asthma subject. Laminin reaction lines along lower margin of basement membrane layer. Although reactions appear to form lines, some pronounced dots are apparent. Among truncated lines, some reactions extend vertically (ABC method, (original magnification × 700).
Capillary proliferation was mild in the lamina propria, and only narrow reticular laminin reactions were seen around capillaries (Fig. 5). In most Pattern D patients, no laminin reactions were seen around the entire circumference of the basement membrane layer, but some Pattern A-like reactions were seen in six patients.

Table I shows the number, sex, age and health status for the patients with each pattern. There were no marked differences in these parameters among the four patterns.

Table 2 shows the laminin reactions and epithelial findings for the 43 patients classified into the four laminin reaction patterns. In the Pattern A patients, relatively severe epithelial cell shedding was seen, and thickening of the basement membrane layer was mostly moderate or severe. In the pattern B and C patients, there were no clear differences in epithelial cell shedding and basement membrane layer thickening. In the Pattern D patients, epithelial cell shedding was hardly seen, but marked goblet cell hyperplasia was seen in many patients. Furthermore, basal cell proliferation and markedly thickened basement membrane layer were seen among the Pattern D patients.

**DISCUSSION**

Bronchial asthma is characterized by reversible obstructive changes, reactive acceleration and chronic inflammation of the airway (26–28). In persistent or severe bronchial asthma, various morphological changes, such as, epithelial cell shedding, goblet cell hyperplasia and basement membrane layer thickening, are seen (11,12,29,30). These changes can serve as indicators of the severity of bronchial asthma in airway remodeling (11,18,21,29).

Thickening of the bronchial epithelial basement membrane layer is considered one of the histological changes associated with airway remodeling (11,18,21,29). The results of several electron microscopic studies have shown that this thickening is caused by abnormal deposition of interstitial collagen types I, III and V and extracellular matrix such as fibronectin and tenascin, in the lamina reticularis under the bronchial epithelia (12–21). This is referred to as subepithelial fibrosis because it is caused by the proliferation of collagen fibers (12–22,26). Also, increases in collagen and collagen deposition are believed to be caused by the interaction between airway epithelial cells and fibroblasts/myofibroblasts (17), but the
details of this interaction are not known (20,22). In bronchial asthma patients, it has been known that laminin reactions localize notably along the upper margin of the basement membrane layer (31,32), and that collagen type IV also localizes in the same area (33). In this study, we focused our attention on the thickened basement
membrane layer, and investigated histomorphological changes in the lamina lucida immunohistochemically using anti-laminin antibodies. The results showed that the localization of immunoreactivity in severe bronchial asthma is not as simple as previously thought and that laminin reactions can roughly be classified into four patterns.

As shown in Table 2, there was a relationship with epithelial cell shedding. In Pattern A, epithelial cell shedding was seen in 7 of the 15 patients, while goblet cell hyperplasia was only seen in 3 of the 15 patients. In Pattern A, lines either extended vertically in relation to the basement membrane layer or they originated from the lamina propria and branched out in the basement membrane layer. Also, capillary proliferation in the lamina propria was observed in almost every patient. Pattern A laminin reactions could form as follows: the basement membrane under epithelial cells is made of epithelial cells and fibroblasts (5, 7), and since epithelial cells are generally detached and eliminated in Pattern A patients, it is difficult to form a basement membrane. Nonetheless, as shown in Fig. 2, in some patients, laminin reactions in the lamina propria, in particular around capillaries, were marked and extended into the thickened basement membrane layer. We believe this is one of the reasons for seeing characteristic immunoreactivity to laminin in deceased bronchial asthma patients. In patterns B and C, laminin reactions formed lines extending under epithelial cells: in Pattern B, lines formed along the lower margin of the basement membrane layer, whereas in Pattern C, lines formed along the upper margin of the basement membrane layer. Although laminin reactions were totally different between these two patterns, there were no clear differences in epithelial cell shedding and basement membrane layer thickening. We could not, however, gather enough data to identify the cause of the difference between Patterns B and C. Furthermore, in Pattern D, although immunoreactivity to laminin in the basement membrane layer was not seen at all, the proliferation of basal and goblet cells was clearly elevated in many patients when compared to the other three patterns. One of the causes for the absence of immunoreactivity to laminin in the thickened basement membrane layer could be that since capillary proliferation was hardly seen in the lamina propria in Pattern D patients, the level of laminin did not increase in the lamina propria. Also, this has not been documented but it is possible that goblet cells, which form epithelia, are not capable of producing laminin. The degree of basement membrane layer thickening for Pattern D was severer than that for the other patterns, but the reason for this was not clear.

Between Patterns A and D, there were clear differences in the four items shown in Table 2. In other words, in Pattern A, epithelial cell shedding was seen, but clear signs of goblet cell hyperplasia and basal cell proliferation were not seen. On the other hand, in Pattern D, epithelial cell shedding was hardly seen, but goblet cell hyperplasia and basal cell proliferation were marked. As a result, Patterns A and D may be on the opposite ends of the morphological changes associated with severe bronchial asthma.

The above findings clarify that immunoreactivity to laminin in the basement membrane layer of patients with severe bronchial asthma shows a great degree of variation. The prerequisite for having basement membrane layer thickening, one component of airway remodeling, is the presence of the basal lamina, but some patients showed no immunoreactivity to laminin (Pattern D) or did not have a normal laminin structure (Pattern A). As a result, we concluded that conditions were too severe for remodeling to occur in patients with severe bronchial asthma. In other words, the histological characteristics of laminin are very unique in patients with severe bronchial asthma.

REFERENCES