Alteration of Fibroblast Architecture and Loss of Basal Lamina Apertures in Human Emphysematous Lung

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Rationale: In normal human lung, single alveolar fibroblasts link capillary endothelium to type 2 pneumocytes through apertures in the endothelial and epithelial basal laminae. These fibroblasts are hypothesized to play a role in cellular communication between the endothelium and epithelium and are positioned to provide leukocytes a surface on which they may migrate through the interstitium.

Objectives: To determine whether fibroblasts link the endothelium to the epithelium in emphysematous lung and to compare basal lamina aperture frequency with previously published results.

Methods: We performed transmission electron microscopy serial section three-dimensional reconstructions of emphysematous regions of human alveolar wall and a quantitative analysis of basal lamina apertures beneath 403 type 2 pneumocytes.

Measurements and Main Results: Our three-dimensional reconstruction demonstrated that the fibroblasts subtending type 2 pneumocytes in emphysematous lung no longer link these epithelial cells to the capillary endothelium through basal lamina apertures. Basal lamina apertures may be absent below some type 2 pneumocytes. Our morphometric analysis showed that their frequency and area beneath type 2 pneumocytes is significantly reduced in emphysematous regions when compared with nonemphysematous regions of matched control lung.

Conclusions: We conclude that the endothelial/fibroblast/epithelial linkage is disrupted in emphysematous human lungs and postulate this disruption may disturb leukocyte migration and account for their accumulation in the alveolar interstitium of emphysematous lung tissue.

Keywords: basal lamina apertures; emphysema; fibroblasts; leukocyte migration; transmission electron microscopy

Emphysema is a term used to describe the destruction of alveolar walls and is one of the major pathologic features of chronic obstructive pulmonary disease (1). The traditional hypothesis for the pathogenesis of cigarette smoke-induced emphysema states that there is an imbalance between proteolytic and antiproteolytic mechanisms. However, there is a growing body of evidence that suggests an imbalance between oxidative damage and antioxidative repair mechanisms and an imbalance between proliferation and apoptosis of alveolar cells may have important roles in its pathogenesis (2–8). Histologically, emphysema is described by the permanent destruction of alveolar walls with abnormal enlargement of the airspaces (9). Although the chronic inflammatory response plays a central role, the mechanisms of alveolar wall remodeling are unknown.

Although the current definition of emphysema does not include the presence of alveolar fibrosis, it is now known that fibrosis is present even with alveolar wall loss. Among the ultrastructural changes in emphysema, there is a net deposition of collagen and elastin in alveolar walls (10, 11). These data suggest that the alveolar fibroblast is central to the remodeling process of cigarette smoke-induced emphysema.

In normal human alveoli, there exist two populations of fibroblasts: structural and myofibroblasts (12, 13). We have shown in rabbit and human lung that myofibroblasts simultaneously bridge the interstitium to link epithelium and the endothelium through apertures in the respective basal laminae (14, 15).

For leukocytes to migrate, they must cross a number of barriers in the alveolar wall. Our previous work indicates that after diapedesis, leukocytes follow a pathway whereby they enter the interstitium through endothelial basal lamina apertures, travel along the myofibroblast, leave the interstitium via type 2 cell basal lamina apertures, and emerge adjacent to type 2 pneumocytes (14, 16). In this manner, leukocytes are able to migrate through lung parenchyma without causing permanent tissue destruction or scarring.

We hypothesized that there may be significant alterations of this myofibroblast architecture in emphysematous regions of human lung that may significantly affect or disrupt leukocyte migration, leading to their accumulation in emphysematous tissues (17). Here, they may contribute to the remodeling characteristic of emphysema.

The purpose of this study was twofold: (1) to determine whether the relationship between these myofibroblasts, type 2 pneumocytes, and adjacent capillary endothelium exists as in nonemphysematous lung or has been altered in frankly emphysematous areas of lung and (2) to define qualitatively and quantitatively the population of apertures in the epithelial basal lamina beneath type 2 pneumocytes for comparison with our previously published results from nonemphysematous human lung (15). To do this, we performed two three-dimensional (3D) serial section reconstructions of alveolar walls using transmission electron microscopy and conducted a morphometric analysis of the relative surface area of apertures beneath type 2 pneumocytes. Some of the results of these studies have been previously reported in the form of an abstract (18).

METHODS

Tissue Acquisition and Processing

Emphysematous lung samples were acquired from the James Hogg iCAPTURE Centre Lung Registry as described elsewhere (19, 20). Briefly, lung samples came from patients undergoing lung resections or lobectomies at St. Paul's Hospital due to small peripheral lung tumors. The tissue used for the present study came from emphysematous regions of lung from the same patients used in our previous study of nonemphysematous lung (15). Emphysematous regions are identified from the patients' computed tomography scan, and a 1-cm³ piece of emphysematous lung is taken well away from any tumor. The piece is cut into 1-mm³ pieces and processed and stained for transmission electron microscopy as described elsewhere (15).

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3D Reconstructions

Two blocks from emphysematous areas of two patients were chosen for serial sectioning on an RMC MT-6000-XL ultramicrotome (Sorvall Microtomes, Wilmington, DE), and 3D reconstructions were performed as previously described (15).

Morphometric Analysis of Aperture Size, Frequency, and Area

To estimate the size and frequency of basal lamina apertures beneath type 2 pneumocytes, a total of 21 blocks from five patients (seven, five, three, one, and five blocks per patient, respectively) were thin sectioned. At least 50 cells from each patient were assessed. In each section, digital images of all epithelial cell profiles that had lamellar bodies, and by definition were type 2 pneumocytes, were taken with a Bioscan digital camera (Model 792; Gatan, Inc., Pleasanton, CA) at 5,800 $\times$ magnification.

We use the term "aperture" to describe the basal lamina fenestrae because they are holes in a thin layer. In thin section, the basal lamina appears as a linear structure; therefore, a discontinuity in this line is best described as a "gap." The number of gaps beneath all 403 type 2 pneumocytes was counted, and the frequency of apertures was calculated by dividing the total number of gaps by the total number of type 2 pneumocytes as described previously (15). The linear length of the basal lamina beneath the type 2 pneumocytes and the length of the gaps in thin section were measured using Image Pro Plus software (Media Cybernetics, Silver Spring, MD).

To estimate the relative surface area of apertures beneath the type 2 pneumocytes, the cycloid line intercept morphometric method was used as described previously (15). The relative surface area of apertures was calculated by dividing the number of intercepts with gaps by the total number of intercepts with type 2 pneumocyte basal lamina and multiplying by 100. For each of the two reconstructions, color segmentation by the Image Pro Plus software allowed direct measurement of the relative surface area of apertures beneath the reconstructed type 2 pneumocytes.

Statistics

Data were analyzed using one-way analysis of variance followed by and paired sample *t* test to compare this new data with that of our previously published data in nonemphysematous human lung (15). All data are presented as mean $\pm$ SEM.

RESULTS

Our observations of frankly emphysematous tissue revealed that most parenchymal walls are dramatically thickened, although occasionally walls of more normal thickness were observed. The thickening of alveolar walls consisted primarily of increased connective tissue deposition that included a population of migratory leukocytes. The reconstructed alveolar walls were from areas of characteristically thickened alveolar walls, whereas the morphometric analysis involved random sampling of all of the tissue from frankly emphysematous regions.

3D Reconstructions

The two reconstructions (Figures 1 and 2) illustrate aspects of the altered fibroblast organization relative to the type 2 pneumocytes and adjacent capillaries. In Figures 3 and 4, connections between the fibroblasts, epithelium, endothelium and extracellular matrix (ECM) are illustrated in individual serial sections from reconstruction number one. In Figure 5, a single section from the first reconstruction illustrates the layering of fibroblast extensions with collagen and elastic elements.

First reconstruction: 140 serial sections. In the first reconstruction of 140 serial sections, the type 2 pneumocyte does not seem to be simply cuboidal, but rather of a more variable shape (Figure 1C). The fibroblasts reconstructed here in emphysematous lung are oriented parallel to the orientation of the alveolar wall (Figures 1A and 1B). The orientation of the fibroblast is particularly clear when only half of the stack is viewed at a time (Figure 1B). The

fibroblast extensions alternate between layers of collagen and elastic fibers in a repetitive pattern throughout the alveolar wall.

We observed five apertures in basal laminae; four apertures were observed on the basal surface of this type 2 pneumocyte (Figure 1D, *arrows* numbered 1–4), and one aperture was observed in the endothelial basal lamina. These apertures permitted cell–cell and cell–ECM contacts.

One of the epithelial cell apertures permitted contact between the interstitial fibroblast and the type 2 pneumocyte (Figures 1D, *arrow* labeled 1, and 4B). On the basis of gap width and number of serial sections in which it appeared, the aperture measured 110 by 900 nm. A second aperture measured 155 by 800 nm, through which an extension of the type 2 pneumocyte protruded and seemed to contact fibrillar ECM elements (type 1 collagen and/or elastin) (Figures 1D, *arrow* labeled 2, and 3B). From the reconstruction, we determined that two apertures in the epithelial basal lamina at the margin of the type 2 pneumocyte permitted three-way contacts between the fibroblast, an adjacent type 1 pneumocyte, and the type 2 pneumocyte (Figures 1D, *arrows* labeled 3 and 4, and 4A). They measured 140 by 300 nm and 250 by 1,200 nm. All of the cell–cell contacts were between epithelial cells and a single fibroblast. Contacts with multiple fibroblasts were not observed. In the endothelial basal lamina, there was an aperture through which an extension of the endothelial cell protruded and contacted an elastic fiber (Figure 3A). This endothelial aperture measured 140 by 800 nm. Because the widths are much shorter than the lengths, the apertures are more slitlike than round or oval.

The fibroblast in this reconstruction contacted no endothelial cells, pericytes, or type 1 pneumocytes; however, this fibroblast did contact an adjacent fibroblast via a cytoplasmic extension (Figure 3B). This contact had morphologic characteristics of an adherens-type junction that included increased electron densities of the opposing plasma membranes of each cell. We do not believe that this is part of a fibroblast adherens contact with itself because we did not observe cytoplasmic confluence between these cytoplasmic extensions in any portion of the 140 serial sections. This minimizes the possibility that it occurs somewhere beyond the 140 serial sections.

Second reconstruction: 170 serial sections. Although in this second reconstruction the type 2 pneumocyte is more regular in shape than in the previous one, it is remarkable in that there were no apertures present in its basal lamina through which it could contact interstitial fibroblasts (Figures 2C and 2D). In addition, we did not observe any contacts between the reconstructed fibroblast and endothelial, pericyte, or epithelial cells. Although they are not seen as clearly as in the first construction, most of the fibroblast extensions in this reconstruction are oriented parallel to the surface epithelial cell basal laminae (Figure 2A). This is because this reconstruction is located at an alveolar wall intersection. However, the fibroblast extensions alternate between layers of collagen and elastic fibers in the same repetitive pattern throughout the alveolar wall (similar to Figure 5). The apparent notch in the type 2 pneumocyte (Figure 2C) is a type 1 pneumocyte cytoplasmic extension that reaches over the type 2 pneumocyte.

Morphometric Analysis of Aperture Size, Frequency, and Area

After measuring approximately 5,400 linear micrometers of basal lamina (fractions of total contributed by each of the five patients were 12, 17, 15, 19, and 36%) below 403 type 2 pneumocytes, we found 54 gaps in the basal lamina averaging 1.00 ± 0.28 μ m wide. This gap size is not significantly different from our previously published values of 1.00 ± 0.10 for nonemphysematous regions ($p = 0.86$).

Figure 1. Three-dimensional reconstruction of 140 serial thin sections of human emphysematous alveolar wall. (A) Cross-sectional view perpendicular to the alveolar surface of the reconstruction showing length and thickness of the alveolar wall. The type 2 pneumocyte (T2, blue) sits on thin epithelial basal lamina (purple, arrowheads), which is covered by type 1 epithelium (T1, aqua). Endothelium (E, red) lines the capillary lumen, which extends into the plane of the image. In the alveolar wall, the subtending fibroblast (F, green) has numerous cell extensions that lie parallel to the axis of the alveolar wall. (B) The same perspective as that in (A) except that only half the image stack is present. It reveals the cell body of the fibroblast (F) and cytoplasmic extensions (green). (C) View of the epithelium tilted about 45° toward the viewer

to reveal the irregularly shaped type 2 pneumocyte (T2, blue). The type 2 pneumocyte is surrounded by type 1 epithelium (T1, aqua), which obscures the borders of the type 2 pneumocyte. (D) Reconstruction of the epithelial basal lamina (BL, purple) viewed from directly above the luminal surface of the alveolus. This view shows epithelial basal lamina (BL, purple) and four apertures in the basal lamina (white arrows numbered 1–4) through which cell–cell and cell–extracellular matrix (ECM) interactions occur.

Figure 2. Three-dimensional reconstruction of 170 serial thin sections of human emphysematous alveolar wall. (A) Cross-sectional view perpendicular to the alveolar surface of the reconstruction showing length and thickness of the alveolar wall. The low cuboidal type 2 pneumocyte (T2, blue) sits on thin epithelial basal lamina (purple, arrowheads). The capillary endothelium (E, red) lines the capillary lumen, which extends into the plane of the image. The subtending fibroblast (F, green) lies in the interstitium of an alveolar wall intersection. The lateral fibroblast extensions (a and b) parallel the reconstructed alveolar wall, and the vertical extensions (c) parallel the intersecting alveolar wall. (B) View of the epithelium tilted about 45° toward the viewer to reveal the low cuboidal type 2 pneumocyte (T2, blue) surrounded by type 1 epithelium (T1, aqua), which

obscures the borders of the type 2 pneumocyte. (C) Epithelial surfaces viewed from the perspective of the alveolar lumen. The type 2 pneumocyte (T2, blue) is surrounded by type 1 epithelium (T1, aqua). (D) The same view as in C with the epithelium digitally removed to reveal the intact basal lamina with no apertures (BL).

Figure 3. Ultrastructural details from the first reconstruction (see Figure 1). (A) An extension of the capillary endothelial cell (En) protrudes through an aperture in its basal lamina (*between arrowheads*) to contact an elastic fiber (EF) in the interstitium. The capillary lumen (CL) can be seen at the right. (B) Cytoplasmic extensions of the reconstructed type 2 pneumocyte (T2) that reaches into the interstitium to contact fibrous ECM elements through a gap in the epithelial basal lamina (*between arrowheads*). (C) An adherens-like contact between an extension of the reconstructed fibroblast and a cellular extension of a neighboring fibroblast (*between arrowheads*). The extensions are surrounded by layers of collagen (C) and elastic fibers (EF) on opposite sides, a pattern that repeats throughout the alveolar wall. Scale, 500 nm.

After calculating the frequency of apertures seen in the type 2 pneumocyte basal lamina using the number of gaps and the number of type 2 pneumocytes found, we estimated the frequency of apertures to be 0.13 ± 0.04 per type 2 pneumocyte. We interpret this to mean that about 1 out of 10 type 2 pneumocytes observed should have at least one aperture in its basal lamina. We have counted these parameters from five patients and a total of 21 blocks. The measurements upon which gap frequency and size were determined are shown in Table 1.

Our previous morphometric analysis of nonemphysematous lung from the same patients showed an aperture frequency of 0.54 ± 0.14 per type 2 cell, which decline significantly in emphysematous regions to 0.13 ± 0.04 per type 2 pneumocyte ($p = 0.03$). Relative areas beneath type 2 pneumocytes occupied by apertures declined significantly from $5.58 \pm 1.51\%$ in nonemphysematous regions to $2.71 \pm 0.42\%$ in emphysematous regions ($p = 0.04$). This demonstrates a regional difference in aperture frequency and area within the lungs of these patients.

Figure 4. Ultrastructural details from the first reconstruction (see Figure 1). (A) A conjunction of cytoplasmic extensions of a type 1 pneumocyte (T1), type 2 pneumocyte (T2), and subtyping fibroblast (F) through a single basal lamina aperture (*between arrowheads*). *Inset*: A line diagram portraying the three cells and discontinuous basal lamina (*stippling*). (B) A fibroblast/type 2 pneumocyte contact adjacent to a mechanical fibroblast/basal lamina adherens-like contact, which anchors it to the basal lamina. The extension of the type 2 pneumocyte (T2) protrudes through an aperture in the epithelial basal lamina (*between arrowheads*) to make contact with the subtyping fibroblast (F). *Inset*: A line diagram portraying the type 2 cell extension through the basal lamina (*stippling*) gap contacting the fibroblast and the fibroblast/basal lamina adherens junction (*white lines*). Scale, 500 nm.

By direct measurements using color the segmentation of Figures 1D and 2D, we have determined that the apertures account for 2.78% of the area of the basal lamina beneath the type 2 pneumocyte in the first reconstruction and 0% in the second reconstruction. Alternatively, using the line intercept technique, we estimate that the apertures account for $2.71 \pm 0.42\%$ of the area beneath type 2 pneumocytes.

DISCUSSION

This study demonstrates disruption of the myofibroblast architecture in frankly emphysematous regions of human lung compared with nonemphysematous regions from the same patients. This study establishes that there are significantly fewer connections between alveolar fibroblasts and type 2 pneumocytes and that no contacts are observed between fibroblasts and endothelial cells. This is because the number of basal lamina apertures

Figure 5. Image from a single thin section of alveolar wall used in the first 3D reconstruction. Fibroblast cell extensions (F) alternate between layers of collagen (C) and elastic fibers (EF) throughout the alveolar wall. The nucleus and cell body of the reconstructed fibroblast (*) and type 2 pneumocyte (T2) on the alveolar surface can be seen. The endothelial cell (En) to the left of the type 2 pneumocyte is part of the reconstructed capillary. An interstitial monocyte (M) is in close apposition to the fibroblast cellular extension directly beneath it (arrows).

was significantly decreased or absent, causing the type 2 pneumocytes and endothelial cells to reduce or lose contact with the interstitium. Our 3D reconstructions demonstrate that the orientation of the myofibroblast cytoplasmic extensions is parallel to the alveolar wall. These extensions are intimately associated with alternating layers of collagen and elastic fibers in a repetitive, layered pattern throughout the alveolar wall, which is more characteristic of irregular dense connective tissue rather than loose connective tissue of alveolar wall. A further observation from our 3D reconstructions from emphysematous lung is that endothelial cells and type 2 pneumocytes sometimes contact ECM elements through the few remaining apertures. Thus, we have demonstrated that fibroblast–epithelial cell contacts decline in frankly emphysematous regions and that there seems to be a shift in fibroblast phenotype to one of ECM synthesis and support.

In the walls of normal human alveoli, there exist two populations of fibroblasts (12, 13). One population is believed to consist of structural fibroblasts that are oriented parallel to the alveolar wall and are involved with ECM turnover and alveolar wall structural homeostasis. The other population is believed to consist of contractile fibroblasts or myofibroblasts. These cells are oriented perpendicular to the alveolar wall, express sarcomeric proteins (especially α -actin), and have contractile functions. From our reconstructions in emphysematous lung reported here,

it seems that these myofibroblasts are absent or are reoriented so as to no longer bridge the endothelium to the epithelial type 2 pneumocyte. Both reconstructed fibroblasts were intimately associated in a pattern of repeating layers of collagen and elastin (Figure 5), and the fibroblasts were oriented parallel to the alveolar wall, like structural fibroblasts. This can be explained in several ways: (1) the myofibroblasts may have reoriented during the emphysematous remodeling process, (2) the myofibroblasts may have been lost (possibly by necrosis or apoptosis), or (3) the myofibroblasts may have altered their phenotype to that of structural fibroblasts. Distinguishing between these possibilities is beyond the scope of this article and should be examined in subsequent studies focused on lung tissues at earlier stages of remodeling, such as regions bordering frankly emphysematous areas.

Our previous work in rabbit and human lung parenchyma proposes a role for this type 2 pneumocyte–fibroblast–endothelial organization in leukocyte migration (14, 16). Our previous work suggests that leukocytes may follow a particular pathway along fibroblasts from capillary to alveolus so that they are able to migrate through lung parenchyma without causing permanent tissue destruction or scarring. Thus, after diapedesis, leukocytes enter the interstitium through endothelial basal lamina apertures, travel along the myofibroblast to leave via type 2 cell basal lamina apertures, and emerge adjacent to type 2 pneumocytes.

Our work in guinea pig, rabbit, and human small conducting airways suggests that a multicellular reticulum of fibroblasts may link these pores to endothelium of subtending microvasculature (21), much like that in the lung parenchyma. Apertures in epithelial basal lamina are also found in human bronchi (which the investigators termed “pores”) (22, 23). Leukocytes use these pores as a conduit between the interstitium and epithelium (22, 23).

The apparent shift in fibroblast phenotype in emphysematous lung may have significant consequences for leukocyte migration. On the basis of our observations, one could reasonably predict that although leukocytes may enter the interstitium through endothelial basal lamina apertures in response to inflammatory stimuli, without myofibroblasts to guide them toward the epithelium and with fewer epithelial basal lamina apertures, they may accumulate in the interstitium. In fact, leukocytes accumulate within the interstitium of emphysematous lung (17). This interstitial accumulation or trapping of leukocytes could contribute to ECM destruction and remodeling characteristic of emphysematous lung.

In addition to the role of epithelial–interstitial connections in leukocyte migration, transbasal lamina epithelial/interstitial connections seem to play some role in the development, maintenance, and repair in lung and other tissues (24–29). These contacts have been implicated in the epithelial/mesenchymal communication necessary for the embryonic development of tissues (30–35). As tissue organization is established during lung development, the numbers of such contacts and their apertures decline (35).

TABLE 1. SUMMARY OF MEASUREMENTS AND ESTIMATES OF GAP FREQUENCY AND SIZE FOR EACH PATIENT

Patient	T2 Cell Basal Lamina Length, per Patient (μm)	T2 Cells (n)	Gaps (n)	Gap Frequency (per T2 Cell)	Gap Size (μm)
1	1,052	82	25	0.30 ± 0.10	1.07 ± 0.14
2	933	68	8	0.12 ± 0.08	0.97 ± 0.28
3	792	50	4	0.08 ± 0.03	0.87 ± 0.28
4	670	57	3	0.52 ± 0.02	1.08 ± 0.52
5	1,957	146	14	0.10 ± 0.01	0.86 ± 0.12

Gap frequency and size are expressed as mean $\pm$ SEM.

Although epithelial basal lamina apertures and the cellular contacts they permit are present throughout lung development, studies in a rat model of lung injury with normal repair after hyperoxia have shown that the number of apertures and contacts decreased at the time of maximal type 2 cell division and increased to normal numbers after a peak in type 2 cell division (36). These same investigators showed that although this pattern was repeated in a rat model of lung injury with abnormal repair and fibrosis after bleomycin injury, the number of apertures was greatly decreased for a longer period (36). These findings suggest a role for epithelial–fibroblast connections in lung repair after injury and the transient nature of basal lamina apertures.

The significantly decreased or absent contacts between fibroblasts and other cells in the alveolar wall in emphysematous regions of lung may have an effect on epithelial cell–fibroblast communication. Because the fibroblast is in the position to coordinate responses to stimuli originating in the epithelium or endothelium, disruption of the contacts between fibroblasts and epithelial cell or fibroblasts and endothelial cells, communication between cells and the coordination of cellular responses may be disrupted. In fact, Knight proposed that in human bronchial airway, disturbing the epithelial cell–fibroblast communication may contribute to altered airway structure (37).

In previous qualitative studies, it was suggested that in mouse and human lung, basal lamina gaps and epithelial–fibroblast contacts are rare or not observed and that their numbers increase in cases of idiopathic pulmonary fibrosis (IPF) (38, 39). Using quantitative morphologic analysis with random sampling, we have determined the aperture frequency in resections of grossly normal lung parenchyma from patients who smoked (15). In contrast to the aforementioned authors' findings in IPF (38, 39), we observed here a significant decline in the frequency of apertures and connections between type 2 pneumocyte and fibroblasts in frankly emphysematous lung, suggesting that the remodeling of fibroblast architecture and synthetic activity of fibroblasts in emphysema differs from that in IPF.

One limitation of the present study is that we were able to perform only two 3D reconstructions of alveolar walls because of the labor-intensive nature of this method. To compensate for the low number of reconstructions, we quantified the basal lamina apertures by doing a morphometric analysis using numerous sections of hundreds of cells from five patients. The 3D reconstructions provide actual counts of contacts and allow us to identify the cells involved in these contacts with certainty. Although the sample size in morphometric analyses is significantly larger, the technique constitutes an estimate rather than a direct count. However, these complementary techniques make an outstanding tool for morphologic studies. It was reassuring that the 3D reconstructions and the morphometric analysis demonstrated that emphysematous lung has a significantly reduced number of apertures and therefore comprises less of the area beneath type 2 pneumocytes than matched control lung.

Another limitation of this study is the possibility that apertures may mechanically close in the absence of cellular extensions (epithelial, fibroblast, or migrating leukocytes), thus apparently decreasing the frequency of apertures. However, in rabbit lung, during a pneumococcal pneumonia, we have observed patent apertures from which type 2 cytoplasmic extensions have been withdrawn during the process of leukocyte migration (14). In addition, we have observed patent apertures in the basal lamina of small conducting airway epithelium of patients with asthma who lacking any cytoplasmic extensions (40). Therefore, it is unlikely that apertures are able to mechanically close and thereby evade being counted in a study such as this. Rather, we suspect that apertures no longer in use have been filled in by

the deposition of new basal lamina or were not present in this remodeled emphysematous tissue to begin with.

In this study, we have combined the advantages of serial section reconstruction on a few cells with a morphometric analysis of over 400 cells to demonstrate for the first time significant alterations of the fibroblast architecture in emphysematous lung. This altered structure may impede inflammation and compromise maintenance and repair of lung structure and function. From our 3D reconstructions, we have determined that the fibroblasts subtending type 2 pneumocytes in emphysematous lung no longer link these epithelial cells to the capillary endothelium through basal lamina apertures. Our 3D reconstructions demonstrated that basal lamina apertures may be completely absent below some type 2 pneumocytes. Our morphometric analysis showed that their frequency and area beneath type 2 pneumocytes is significantly reduced in emphysematous regions of human lung when compared with nonemphysematous regions of matched control lung. Given the proposed roles of myofibroblasts in cell–cell communication and leukocyte migration, one might predict that leukocytes may have difficulty finding their way to the airspaces, which would explain their accumulation in the interstitium of emphysematous regions of human lung. Here, they may contribute to the destructive remodeling characteristic of emphysema.

Conflict of Interest Statement: None of the authors have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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