Cellular and Connective Tissue Changes in Alveolar Septal Walls in Emphysema

GORDANA VLAHOVIC, MICHAEL L. RUSSELL, ROBERT R. MERCER, and JAMES D. CRAPO

Duke University Medical Center, Durham, North Carolina; National Institute of Occupational Safety and Health, Morgantown, West Virginia; and National Jewish Medical and Research Center, Denver, Colorado

Emphysema is commonly defined as enlargement of airspaces distal to terminal bronchioles accompanied by destruction of alveolar walls, but without obvious fibrosis. Morphometric techniques were used to correlate changes in components of the alveolar septa surrounding enlarged airspaces in human emphysema with the mean linear intercept (Lm) of those airspaces. Alveolar and capillary surface density decreased with increased Lm, but the ratio of these surface densities to each other remained close to normal for mild to moderate increases in Lm. This suggests that the decreased gas exchange observed in emphysema is initiated by a total loss of septa and not by selective pathological changes of the microvasculature. Increases in septal wall thickness directly correlated with increases in Lm. For the mild to moderate emphysema lesions included in this study, an increase of 100% in Lm correlated with a 130% increase in the relative volume of the alveolar septal interstitium. Significant increases occurred in both elastin (0.14 to 0.56 $\mu m^3/\mu m^2$ basement membrane [BM]) and collagen (0.49 to 1.63 $\mu m^3/\mu m^2$ BM). The increase in elastin and collagen raises the possibility of a remodeling process in the connective matrix in alveolar walls. Whether or not the new connective tissue represents a disordered, nonfunctional regional response needs to be determined.

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Emphysema is characterized by abnormal enlargement of the respiratory regions of the lung distal to terminal bronchioles, accompanied by destruction of the walls, and with loss of tissue per unit volume (1–4). There are two major forms of emphysema: panacinar and centroacinar emphysema (1–4). Panacinar emphysema involves airspace enlargement throughout the acinus and is thought to commonly arise as a result of a deficiency in synthesis or secretion of α_1-proteinase inhibitor (α_1-PI). The most frequently observed form of emphysema, centroacinar emphysema, develops in the central portions of the acinus in close proximity to respiratory bronchioles and is predominantly associated with prolonged exposure to cigarette smoke (5–7). The pathogenesis of emphysema is still unknown: the most accepted hypothesis is based on an imbalance in proteases and antiproteases. That hypothesis is that tissue injury results from the actions of excess proteolytic enzymes liberated from inflammatory cells such as neutrophils and monocytes (8–10).

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Correspondence and requests for reprints should be addressed to James D. Crapo, M.D., National Jewish Medical and Research Center, 1400 Jackson Street, Denver, CO 80206. E-mail: CRAPOJ@njc.org

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The role of cigarette smoking in the formation of emphysema is partly explained by recruitment of polymorphonuclear leukocytes and monocytes in the lower respiratory tract either as a consequence of epithelial injury caused by smoke or as a response to chemicals in smoke (5–7). The targets of proteolytic enzymes and free radicals liberated from polymorphonuclear leukocytes and monocytes are collagen, elastin, proteoglycans, and α_1-PI, respectively. Damage to the proteolytic enzyme inhibitor accelerates and augments development of emphysema.

Lung changes characterizing emphysema have been studied in both humans and animals via different methodologies (9, 11). Most of the findings in these studies suggest that connective tissue, especially elastin, is a major target of destruction in emphysema. The architectural rearrangement and loss of gas exchange surface caused by elastin degradation in emphysema is generally thought to be irreversible. Animal models of emphysema have been created by intratracheal administration of pancreatic elastase, and these models demonstrate synthesis of new elastin (12). The enhanced deposition of elastin in these models has been used as a basis to challenge the relevance of these animal models to human emphysema. It is not known how the development of emphysema in humans correlates with specific structural changes of the lung parenchyma, such as whether or not destruction of the vascular bed is an early event and whether or not early interstitial changes involve production or loss of either of the primary connective tissue elements, collagen and elastin.

The goal of this study was to investigate structural changes of the walls of enlarged airspaces occurring in areas of mild and moderate human emphysema to determine if enhanced deposition or degradation of connective tissue occurs. Morphometric techniques combining light and electron microscopy were

used to quantify changes in interstitial elastin and collagen, interstitial inflammatory cells, endothelial cells, and alveolar epithelial cells in areas of mild to moderate emphysema. This comparison of structural changes in lung tissue from areas of airspace enlargement to the structure of normal lung demonstrates that specific connective tissue changes are part of the early pathological events in emphysema.

METHODS

Human lung tissue was obtained from surgically resected lobes. Five specimens were selected from areas not involved with tumor from lung lobes removed for cancer from each of seven human patients. The characteristics of the patients, including their primary diagnoses, are given in Table 1. Six of seven patients were smokers prior to the surgery. Pulmonary function test changes suggested mild to moderate chronic obstructive pulmonary disease (COPD) in six of the patients, and these changes correlate with their history of cigarette smoking. Total lung capacity (TLC) was measured by planimetry using chest films (13) and was found to be increased in four of the seven patients.

TLC Calculation

Posteroanterior and lateral chest films were used for calculation of TLC using the planimetry method (13). Predicted values of the TLC based on sex, age, and height were from the Intermountain Thoracic Society (14). The percent change of TLC relative to its predicted value for each patient is shown in Table 1.

Tissue Preparation

Lung fixation was performed by instilling 2% glutaraldehyde in 0.085 M sodium cacodylate buffer (pH 7.4) through syringes which were inserted into the airways of collapsed lobes or lung segments. The fixative was instilled quickly enough to achieve rapid filling of lung tissue while the lung was closely observed to avoid overdistension. This fixative is sufficiently rapid that the alveolar tissue becomes rigid within seconds and maintains full inflation even though there is a cut surface on the surgically obtained lung lobes. We have found that this technique leads to high-quality fixation at a uniform degree of inflation. This technique can be estimated to result in a lung segment inflation equal to 60 to 70% of TLC (15, 16). Glutaraldehyde is such a rapid fixative that the alveolar region fixes during the instillation process and while the fluid pressure at the front of the fixative flow is lower than the airway pressure. Thus, the factors that determine the degree of lung inflation when fixing by the instillation of glutaraldehyde are the speed with which the fixative is instilled and the compliance of the alveolar walls, which determines the pressure at which an alveolus stops filling and preferentially forces the liquid fixative to move distally through the lung (15, 16). The exact degree of inflation of the fixed tissue cannot be determined when the study is done on surgically obtained cut lung segments or lobes. The critical factor is that all lung specimens are inflated to a reasonably similar degree so that the comparisons of alveolar septal density and composition are valid. To account for possible small variations in the degree of inflation of the fixed lung segments, all data on alveolar septal composition were normalized by dividing the tissue volume densities by the surface density of the alveolar epithelial basement membrane.

After instillation of fixative, the tissue remained in the fixative for 24 h before being cut into 1-cm-thick slices in the sagittal plane. From the slices for each lobe or lung segment, 10 blocks (1.5 cm × 1.5 cm × 1.5 cm) of tissue were randomly chosen and cut out. Thus, a total of 70 tissue blocks were selected from the seven lobes or lung segments resected from seven subjects. Half of each block was used for paraffin sectioning. Fixation quality of the selected tissue blocks was estimated by examining their corresponding paraffin sections by light microscopy. The purpose of that examination was to exclude from the study any tissue block that was not well fixed. Based on quality of fixation, six of the 70 samples were rejected from the study. Five tissue blocks were then randomly selected from the remaining well-fixed tissue blocks for each lobe or lung segment. By this process, 35 total blocks were picked from the seven lobes or lung segments. These tissue blocks were sequentially washed in 0.085 M sodium cacodylate buffer, postfixed in 2% OsO_4, and rinsed in buffer again. They were dehydrated through 50%, 70%, and 100% ethanol, transferred to propylene oxide, and gradually infiltrated with 100% Epox-812 (Ernest F. Fulham, Latham, NY).

Isolation of the Regions Studied

The Epox tissue blocks were softened with mild heat (40° C) and then cut into slices approximately 0.3 mm thick. Each slice was examined with a dissecting microscope. Three regions per tissue block (35 tissue blocks total) were isolated to be studied. These regions were chosen based on their manifesting the average degree of airspace enlargement as qualitatively seen over the entire slice. A series of 1 × 1 mm cubes were dissected from each of those regions, and then one was randomly selected for further study. Thick sections (0.5 μm) were cut for light microscopy using a diamond knife, placed on a glass slide, and stained with toluidine blue. From the same regions, thin sections (87 nm) were then cut, placed on 200-mesh formvar-coated grids, and stained with lead citrate and uranyl acetate for electron microscopy. The morphometric data obtained from each group of three studied regions was averaged to create the data set for the corresponding tissue block.

Light Microscopy

Using light microscopy and the 0.5-μm sections, mean linear intercept (Lm) was determined for each region studied. Each entire 0.5-μm section was photographed and printed on 11 × 14-inch photographic pa-

TABLE 1

CHARACTERISTICS OF THE PATIENTS INCLUDED IN THE STUDY

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
Sex	Male	Female	Female	Male	Female	Male	Female
Age	70	73	50	65	54	53	72
Height, cm	175	173	173	178	173	183	168
Weight, kg	77.8	53	100	74	73	61	68
Smoker, length, yr	Yes (35)	Yes†	No	Yes (40)	Yes (43)	Yes (20)	Yes‡
Diagnoses	Adenocarcinoma; emphysema; SLE*	Squamous cell carcinoma; asthmatic bronchitis	Bronchial carcinoid of the left upper lobe	Squamous cell carcinoma; mild COPD; MI history, 2×	Adenocarcinoma; moderate centrilobular emphysema	Bronchoalveolar carcinoma	Adenocarcinoma
FVC, % pred	90	112	119	91	85	103	86
FEV_1, % pred	74	69	108	76	70	58	81
FEV_1/FVC, %	82	46	73	66	64	56	71
TLC, % pred	90	132	68	119	110	132	97

Definition of abbreviations: MI = myocardial infarction; SLE = systemic lupus erythematosus.

* Patient in remission since 1986.

† The patient has a 25 to 50 pack-year smoking history.

‡ Heavy smoker at 1 to 2 packs per year for unknown number of years.

per (×157 final magnification). An overlay consisting of horizontal and vertical, parallel lines was placed over the printed image of each region. All intercepts with alveolar septal tissue were counted. The total length of all the lines together divided by the number of intercepts gives the mean linear intercept for the region studied (Lm_r). The overall mean of the Lm for each of the three regions studied for each tissue block was used as the Lm for the corresponding tissue block. Based on the calculated Lm, all 35 human tissue blocks studied were divided into three groups (Groups 1, 2, and 3) which correspond to increasing degrees of airspace enlargement (mild to moderate emphysema).

Electron Microscopy

Tissue from each region was randomly selected for study by photographing the upper left corner of every other grid square in a checkerboard pattern through each entire section using a Zeiss 10-C transmission electron microscope (Thornwood, NY). Only grid spaces completely covered by the section were included. Parenchymal structures such as blood vessels greater than 25 μm in diameter or airways were not photographed. The micrographs were printed at an enlargement of 8,500× on 11 × 14-inch photographic paper. A counting overlay consisting of 112 lines, each 2 cm long, was printed on each micrograph.

Morphometric Analysis

The general approaches used in this type of morphometric analysis have been reported in detail (17–21). The volume density (Vv) and surface density (Sv) of alveolar tissues were determined by point and intercept (line intersection) counting as previously described (18, 19, 22). The alveolar tissue was separated into epithelium, interstitium, and endothelium. The interstitium was divided into interstitium—cellular (fibroblasts, interstitial macrophages, polymorphonuclear leukocytes, and other interstitial cells), and interstitial matrix (elastin, collagen, and acellular space).

To compare data from the various groups in an unbiased manner, all data was normalized to the alveolar epithelial basement membrane surface area. We divided volume density (Vv) by the surface density of alveolar epithelial basement membrane (SvBM) of the same sample.

Statistical Analysis

All comparisons of statistical significance reported in Table 2 are comparisons of Groups 1, 2, and 3. Duncan's multiple comparison test (23) was used to evaluate significant changes occurring between the studied groups. All tests were 2-sided tests, and $p < 0.05$ was considered to be significant.

RESULTS

Based on calculated Lm all 35 investigated lung tissue blocks were classified into three groups. The Lm and all morphometric data obtained for each block represent the mean of data derived from three regions that were randomly selected from each block (Table 2). The severity of emphysema in each local domain correlates with an increase of Lm. The results demonstrate loss of alveolar and capillary surface density with increase of Lm. The ratio of alveolar to capillary surface density showed a small change from 1.42 in normal human lung to 1.93 in the most severe areas of emphysema (Group 3). This change was progressive and consistent as the severity of emphysema increased, and raises the possibility that there was a modest excess loss of capillary surface in comparison to alveolar surface; however, these changes in surface area ratios were not large and did not reach statistical significance. Epithelial and endothelial volume densities did not change with increase of Lm but both interstitial—cellular and interstitial matrix (elastin and collagen) were significantly increased. The data from normal human lung were taken from that previously published (24) and include several smokers, which may have some effect on the "normal" data.

The surface densities of both alveolar epithelium and capillaries significantly decreased in areas of mild (Group 2) to moderate (Group 3) emphysematous lesions (Table 2). These data indicate loss of alveolar tissue per unit volume with increased Lm. However, the volume of both epithelium and endothelium in the alveolar septum, when normalized to the alveolar surface area, did not change in areas of mild or moderate emphysema. In addition, the ratio of alveolar epithelial surface area to capillary surface area showed no statistically significant change from normal to mild or moderate emphysematous regions.

A slight increase in the volume of type 2 cells per unit alveolar surface occurred in correlation with an increasing Lm;

TABLE 2

AIRSPACE WALL STRUCTURE CHANGES WITH FACILITATION OF EMPHYSEMATOUS LESION*

Sample Classification Based on Lm	Normal Human Lung†	Group 1 (*n = 13*)	Group 2 (*n = 13*)	Group 3 (*n = 9*)
Mean linear intercept		0.20 ⩽ Lm ⩽ 0.26	0.26 < Lm ⩽ 0.39	Lm > 0.39
Surface density, cm^2/cm^3				
Alveolar epithelial BM surface (SA_{BM})	214 ± 77.76	271.95 ± 48.36	159.032 ± 24.56‡	108.593 ± 34.88‡§
Capillary surface	151 ± 58.89	162.1 ± 47.35	88.536 ± 32.11‡	56.395 ± 20.94‡
Arithmetic mean	2.25 ± 0.81	1.763 ± 0.761	2.568 ± 0.947	5.234 ± 2.624‡
Thickness, interstitium μm				
Volume density normalized, $(\mu m^3)/_{SA}$ (μm^2)				
Epithelium		0.4383 ± 0.101	0.4672 ± 0.113	0.4664 ± 0.189
Type I	0.331 ± 0.126	0.2334 ± 0.042	0.2449 ± 0.056	0.2188 ± 0.077
Type II	0.218 ± 0.107	0.2036 ± 0.092	0.2202 ± 0.108	0.2458 ± 0.156
Endothelium	0.405 ± 0.2	0.3074 ± 0.047	0.2946 ± 0.056	0.3237 ± 0.093
Interstitium	1.211 ± 0.416	1.383 ± 0.552	1.929 ± 0.542	3.994 ± 1.955‡
Cells	0.375 ± 0.146	0.3946 ± 0.153	0.4509 ± 0.077	0.8482 ± 0.31‡
Fibroblasts		0.3074 ± 0.089	0.3443 ± 0.07	0.5946 ± 0.239‡
Macrophages		0.0769 ± 0.064	0.0794 ± 0.315	0.2185 ± 0.103‡
Interstitial matrix	0.836 ± 0.27	0.9880 ± 0.408	1.478 ± 0.516	3.145 ± 1.768‡
Elastin		0.1444 ± 0.052	0.288 ± 0.217	0.5642 ± 0.384‡
Collagen		0.4932 ± 0.288	0.6668 ± 0.326	1.625 ± 1.083‡

Definition of abbreviations: BM = basement membrane; Lm = mean linear intercept; n = number of blocks of tissue studied.

* Group 1 represents near-normal Lm; Group 2 is mild and Group 3 is moderate emphysema as graded by the Lm.

† The normal lung data were taken from prior published material (20). Results for densities are normalized to the alveolar epithelial BM surface area.

‡ $p \leqslant 0.005$ for comparison to data in Group 1 (normal lung).

§ $p \leqslant 0.005$ for comparison to data in Group 2.

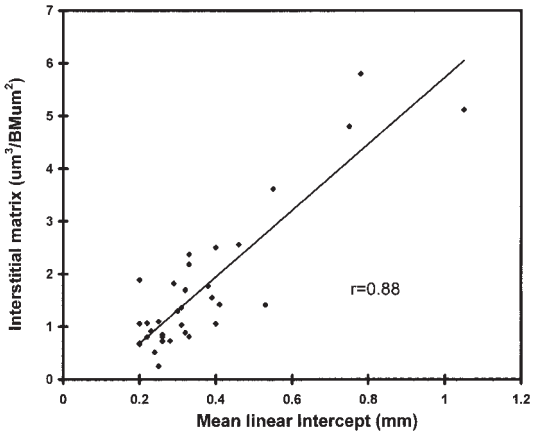

Figure 1. Correlation of Lm with the average thickness of the alveolar interstitial matrix. Matrix is expressed as volume per unit surface area of alveolar epithelial basement membrane.

however, this was not statistically significant given the sample sizes used in these studies. If this change is biologically significant, it would suggest stimulation of and/or turnover of type 2 cells in response to the development of emphysema.

The data reported in Table 2 demonstrate that the whole septal wall, but more specifically the interstitium becomes thicker in mild to moderate emphysematous lesions. Figure 1 shows the correlation of alveolar interstitial thickness with Lm. An increase of alveolar wall thickness is a known factor in suppressing the efficiency of gas exchange in emphysema. Figures 2 and 3 show low magnification views of the alveolar regions of a normal lung region and an emphysematous lung region (Group 2) from this study. Figure 3 illustrates the characteristic septal wall thickening in a diseased area. The interstitium in such areas coincides with a substantially increased Lm in the range of 130% to 330% greater than the interstitium in normal areas.

The most impressive finding in the study of the septal walls of the emphysematous lesions was high levels of both elastin (from 0.14 to 0.56 $\mu m^3/\mu m^2$ basement membrane [BM]) and collagen (from 0.49 to 1.63 $\mu m^3/\mu m^2$ BM) (Table 2). This is a three- or fourfold increase over normal for both elastin and collagen. The design of this study does not allow one to clearly

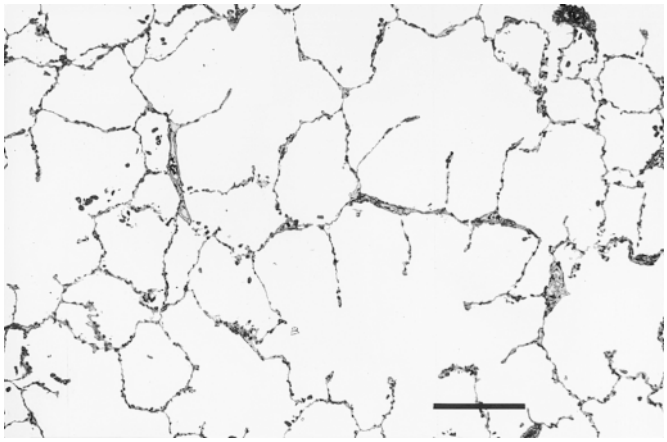

Figure 2. Light microscopic photomicrograph of normal human lung. The section was stained with toluidine blue. *Bar* = 200 μm.

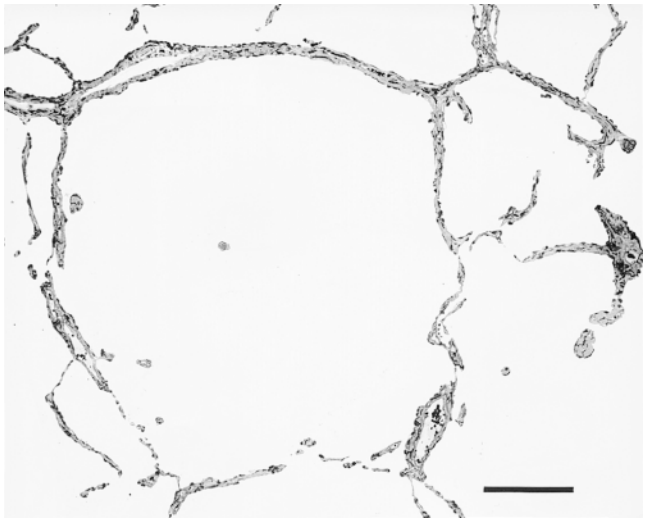

Figure 3. Light microscopic section showing the enlargement of gas exchange spaces that is characteristic of emphysema. This example illustrates the pattern of thickened interstitium observed in emphysematous lesions in human lungs. The section was stained with toluidine blue. *Bar* = 200 μm.

determine whether the increase in elastin and fibrous collagen in alveolar walls of emphysematous lesions represents new synthesis of these connective tissue elements or a preferential loss of the thin delicate interalveolar septal walls with retention of interacinar or intersegmental walls that have high connective tissue content. Qualitatively, the walls of emphysematous lesions in Group 3 samples showed structural changes that were not readily found in the alveolar regions of normal

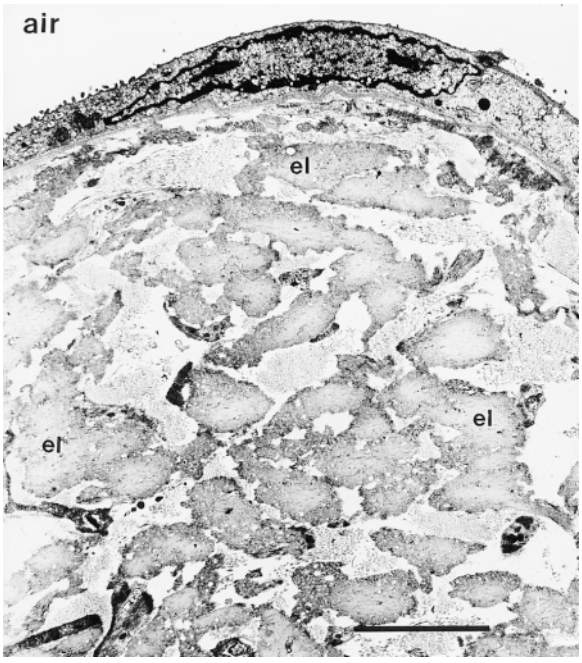

Figure 4. Electron micrograph of an alveolar septal wall from an emphysematous region of the lung. This micrograph demonstrates an area having a high concentration of elastin (el) distributed throughout the interstitium. Thin sections for electron microscopy were stained with uranyl acetate and lead citrate. *Bar* = 5 μm.

lungs, suggesting that new connective tissue synthesis is at least a component of the remodeling process. The net result of loss of fine alveolar walls and restructuring of the remaining tissue is a wall along emphysematous lesions that has greater amounts of connective tissue per unit surface area. The increase in connective tissue directly correlates with the severity of the emphysema. Figure 4 demonstrates the enhanced elastin that can be seen by electron microscopy in the alveolar septal walls from a diseased region of human lung tissue. Figures 5 and 6 further illustrate the patterns of elastin and collagen where they are found in high concentrations in emphysematous regions. A significant level of connective tissue synthesis in diseased areas suggests a remodeling process of the alveolar walls. Interstitial fibroblasts are often found in close proximity to the elastin (Figure 6), and they are likely important in tissue remodeling associated with emphysematous lesions. As shown in Table 2 there is a statistically significant increase in the volume of interstitial cells in lung regions having a high Lm and enhanced collagen and elastin. A close association with an increasing Lm was also observed for enhanced numbers of interstitial macrophages. In the specimens representing moderate emphysematous lesions (Group 3), the interstitial macrophage volume was increased almost threefold (data not shown).

DISCUSSION

There have been no previous studies that have separately quantified the components of alveolar septal walls in human emphysematous lung lesions. By combining electron microscopy and morphometric measurements of the lung parenchyma from human lung tissue expressing varying degrees of emphysema, the alveolar septal structural modifications that occur in mild to moderate emphysema are defined. A loss of total tissue, increased interstitial thickening of the remaining tissue (including increases in both elastin and collagen), and increased volumes of interstitial fibroblasts and interstitial macrophages were main findings of this study.

Destruction of alveolar septal tissue is one of the classic characteristics of emphysema. We now show that this destruction of the alveolar septum involves a simultaneous loss of alveolar surface area and capillary surface area as the magnitude of the emphysema progresses. There is not a substantial excessive destruction of the microvascular bed during the phases of emphysema that were included in the current study. The findings that the epithelial and capillary surface ratios showed minimal change with increasing Lm, suggests that the gas exchange decrease occurring in emphysema is initiated by complete loss of alveolar tissue rather than by selective pathological changes of the capillary endothelium and vascular bed.

Many studies have demonstrated that degradation of elastin plays a key role in the initiation of emphysema (9, 25), although the loss of complete alveolar septal walls as a coordinated event raises questions as to whether or not elastin is the major target of destruction. The pathogenesis of emphysema involves a variety of events, including free radicals, activation of inflammation (polymorphonuclear and monocyte recruitment), and a variety of cellular derived mediators or cytokines all of which cumulatively lead to proteinase inhibitor inactivation, membrane lipid oxidation, and proteinase liberation (26). Focal lung injury, also a characteristic of emphysema, can be explained by the inhomogeneous distribution of cigarette smoke components (the major cause of acquired emphysema), antiproteinase inhibitors, antioxidants, and cellular derived oxidants in the lung (26).

The progressive loss of complete portions of the alveolar septa during the formation of emphysema suggests that gas exchange in the early stages of emphysema primarily decreases due to the loss of entire segments of alveolar septa, and not to a selective loss of the alveolar microvasculature. The ratio of capillary to alveolar surface in the septal tissue of mild and moderate emphysematous lesions remains normal. The volume of endothelial cells per unit alveolar surface in these areas compared with nearby healthy areas of human lung also remained unchanged. Thickening of the interstitium could lead

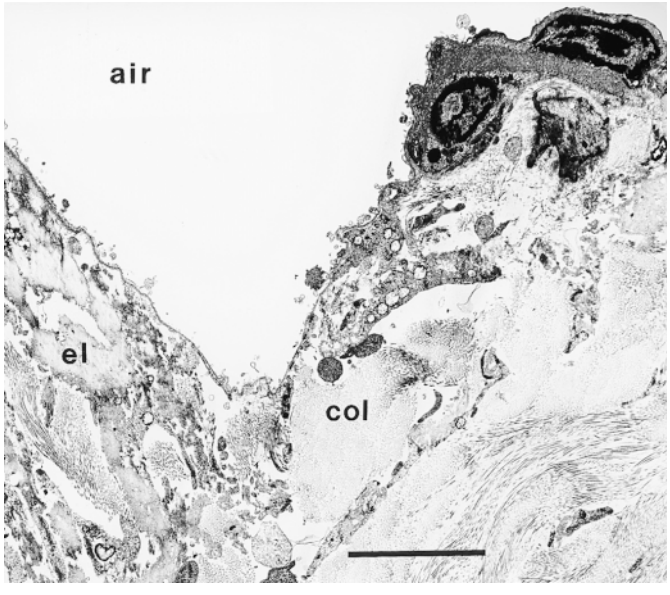

Figure 5. Electron micrograph showing collagen (col) and big patches of elastin (el) in the alveolar region of emphysematous human lung. Thin sections for electron microscopy were stained with uranyl acetate and lead citrate. *Bar* = 5 μm.

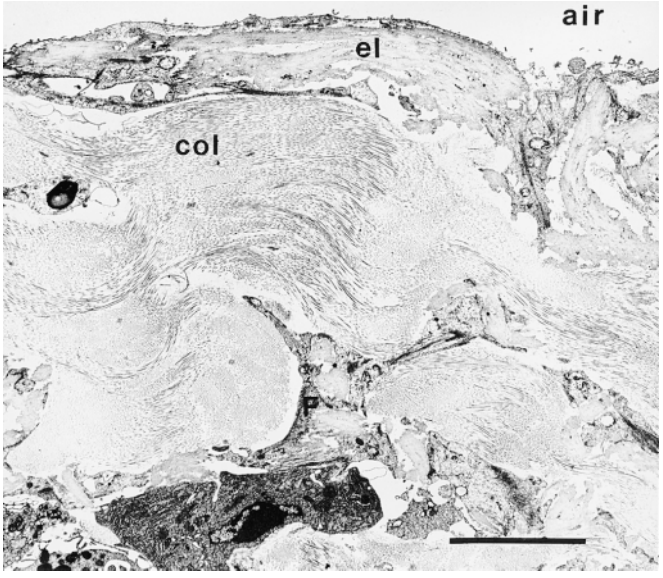

Figure 6. Electron micrographs of collagen (col) and elastin (el) in the alveolar region of emphysematous human lung. These connective tissue elements are present in high concentrations in septal walls of diseased areas. The large number of interstitial fibroblasts (F) found frequently in regions of enhanced collagen and elastin is likely important in the tissue remodeling. Thin sections for electron microscopy were stained with uranyl acetate and lead citrate. *Bar* = 5 μm.

to a partial diffusion block for gas exchange; however, the magnitude of the interstitial changes is not sufficient for the diffusion block to be functionally significant in comparison to the effects of the loss of entire alveolar septal segments.

In animal models of emphysema, repair has been shown to occur after acute injury to the lung (9, 11, 12). For instance, Mercer described gaps in elastin fibers of two hamster alveolar septal walls that occurred as a result of elastin destruction after exposure of the animals to a single dose of pancreatic elastase (12). He also illustrated enhanced deposition of elastin fibers during repair of the injured areas. This study supports the hypothesis that tissue repair and remodeling are a critical component of the process leading to emphysematous lesions. It is likely that following periods of intense elastolysis there are periods of repair in which elastin is remodeled perhaps in a disordered state and thus contributing to the loss of elastic recoil in the function of emphysematous lungs. Alternatively, phases of remission and repair, with fewer neutrophils present and macrophages predominating, could be the most common pathological expression of early phases of emphysema.

The results of the current study show a significant correlation between degree of emphysema and the thickness of interstitium in the remaining alveolar septal walls. Substantially enhanced numbers of neutrophils were not found in the tissues studied. The lack of neutrophils could be due to the fact that the tissue was obtained from patients who underwent surgery, and presurgical therapy or abstention from smoking could have reduced the frequency of acute inflammation. Two primary connective tissue components, elastin and collagen, were found to be increased in relative volume in areas of emphysema. An increase in elastin- and collagen-producing cells is also an important indicator of tissue modeling or repair (12, 27). In the current study, interstitial fibroblasts were consistently found in close proximity to areas of elastin (Figure 6) and collagen in the alveolar interstitium of diseased areas. Quite often the long processes of interstitial fibroblasts enveloped adjacent connective tissue elements. *In vitro* studies have demonstrated potential mechanisms for a direct repair process. Cultured pulmonary fibroblasts derived from neonatal rats demonstrate an increase in tropoelastin messenger RNA (mRNA) and elastin synthesis after the cells are exposed to elastase and elastase-solubilized extracellular matrix peptides. In cultured fibroblasts incubated with matrix peptides but not treated with elastase, a significant reduction of tropoelastin mRNA and elastin synthesis occurred (28). This suggests that elastin synthesis occurs at the sites where both elastase and injured extracellular matrix elements are present. On the basis of these studies, elastin synthesis would be expected to occur in the very areas where elastolysis initiates an emphysematous-like lesion. The current report of a significant correlation between enhanced elastin and collagen deposition and the local degree of emphysema illustrates this concept of remodeling, although possibly disordered, as a possible step in the creation of the emphysematous lesion.

The early stages of emphysema included in the present study were primarily characterized by the presence of macrophages rather than neutrophils. Mononuclear phagocytes usually accumulate in large numbers in the lung in response to cigarette smoking (1, 6). In this study they were found to be significantly increased in emphysematous lesions. It is believed that macrophages play a role in the pathogenesis of the alveolar septal injury that characterizes pulmonary emphysema, and that they may be important especially in the pathogenesis of chronic tissue destruction (10, 29). Their significance as a source of proteolytic enzymes and in the release of proteinase inhibitors is still questionable. It has been suggested that macrophages have elastolytic ability owing to liberation of the elastolytic enzymes metalloelastase and cathepsin L (28). Cathepsin L is significantly elevated and its mRNA highly expressed in alveolar marcrophages obtained from bronchalveolar lavage fluid from smokers compared with nonsmokers. This supports the concept that alveolar macrophages contribute to the proteolysis of elastin as part of the process of lung destruction associated with cigarette smoking.

This study shows that the walls of emphysematous lesions contain increased amounts of elastin and collagen which is consistent with either loss of interalveolar septal walls normally low in connective tissue or enhanced synthesis of elastin and collagen in emphysematous areas—or that both processes are occurring. An increase in synthesis of elastin and collagen would suggest a repair process but does not necessarily indicate functionality. Whether newly synthesized connective tissue in emphysema undergoes the full process of maturation to normal connective tissue that is able to perform its functional role or represents a disordered nonfunctional regional response needs to be determined.

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