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Correlation of lung surface area to apoptosis and proliferation in human emphysema

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ABSTRACT: Pulmonary emphysema is associated with alterations in matrix proteins and protease activity. These alterations may be linked to programmed cell death by apoptosis, potentially influencing lung architecture and lung function.

To evaluate apoptosis in emphysema, lung tissue was analysed from 10 emphysema patients and six individuals without emphysema (normal). Morphological analysis revealed alveolar cells in emphysematous lungs with convoluted nuclei characteristic of apoptosis. DNA fragmentation was detected using terminal deoxynucleotide transferase-mediated dUTP nick-end labelling (TUNEL) and gel electrophoresis. TUNEL revealed higher apoptosis in emphysematous than normal lungs. Markers of apoptosis, including active caspase-3, proteolytic fragment of poly (ADP-ribose) polymerase, Bax and Bad, were detected in emphysematous lungs. Linear regression showed that apoptosis was inversely correlated with surface area. Emphysematous lungs demonstrated lower surface areas and increased cell proliferation. There was no correlation between apoptosis and proliferation, suggesting that, although both events increase during emphysema, they are not in equilibrium, potentially contributing to reduced lung surface area.

In summary, cell-based mechanisms associated with emphysematous parenchymal damage include increased apoptosis and cell proliferation. Apoptosis correlated with airspace enlargement, supporting epidemiological evidence of the progressive nature of emphysema. These data extend the understanding of cell dynamics and structural changes within the lung during emphysema pathogenesis.

KEYWORDS: Caspase, cell death, cell proliferation, morphometry, terminal deoxynucleotide transferase-mediated dUTP nick-end labelling

A primary hypothesis for the pathogenesis of pulmonary emphysema is protease–antiprotease imbalance [1, 2]. This model suggests that alterations in levels of extracellular matrix (ECM)-degrading enzymes and their inhibitors lead to a loss of matrix components of the lung. The hypothesis is supported by studies demonstrating elevated expression of proteases and loss of ECM in the emphysematous lung compared with normal [3–6]. However, the hypothesis does not elucidate all aspects of emphysema pathogenesis, such as the role of latent adenoviral infection [7]. It also does not explain the accelerated, destructive nature of emphysema, even after patients stop smoking [8, 9].

To clarify whether alternative cellular mechanisms contribute to emphysema pathogenesis, investigators have focused on the role of programmed cell death by apoptosis [10–12]. YOKOHORI *et al*. [12] detected increased apoptosis of alveolar wall epithelial cells (although not endothelial cells in emphysema lung). KASAHARA and MATSUSHIMA [11] demonstrated apoptosis of alveolar septal and endothelial cells accompanied by decreased expression of vascular endothelial growth factor (VEGF) in emphysematous lung tissues. Building on earlier work, which showed that blocking VEGF receptor signalling-induced apoptosis and subsequent emphysema in rats [10], scientists identified significantly reduced levels of VEGF in the induced sputum of emphysema patients compared with that of normal individuals and patients with asthma [13]. These studies suggest that loss of endothelial cell maintenance factors contribute to the specific pathogenesis of emphysema. In addition, the role of apoptosis in emphysema has recently been found to be not merely correlative, but potentially causative [14]. Direct intratracheal delivery of active caspase-3 led to loss of alveolar epithelia and emphysema-like structural changes. Apoptosis

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occurred in the absence of inflammation, demonstrating the specific role of programmed cell death in this disease [14]. The present study was undertaken to assess the relationship between lung cell apoptosis, proliferation, and tissue surface area in patients with pulmonary emphysema.

MATERIALS AND METHODS

Human lung samples

Human lung tissues were collected from 16 patients at New York Columbia Presbyterian Medical Center, New York, NY, USA, under institutional guidelines. Ten samples were from patients with pulmonary emphysema: five obtained from lung transplantation and five from lung volume reduction. Patients had stopped smoking $\geqslant$6 months prior to surgery. Six normal samples were from accidental death victims or unused donor lungs harvested for transplant. For comparison, lung tissues were obtained from patients with nonspecific interstitial pneumonitis (n=6), eosinophilic granuloma (n=3), hypersensitivity pneumonitis (n=3) and lymphangioleiomyomatosis (n=2).

Immunohistochemical staining for apoptosis and cell proliferation

Surgically excised tissues were fixed in formalin and paraffin embedded. Cell proliferation was assessed by immunostaining sections with an antibody to human proliferating cell nuclear antigen (PCNA); clone PC10; Sigma, St. Louis, MO, USA). Biotinylated secondary antibody was detected using an avidin-biotin complex (Vector Laboratories, Burlingame, CA, USA). The ratio of PCNA-reactive cells over total cells (proliferation index) was measured at $\times$40 magnification. Immunostaining was also performed using antibodies against human Bad and Bcl-2 (Transduction Laboratories, Lexington, KY, USA), and Bax (PharMingen, San Diego, CA, USA). Sections were counterstained with haematoxylin.

Ultrastructural analysis of lung tissue

Transmission electron microscopy was performed on explanted lung tissues fixed in 2.5% glutaraldehyde. Tissues were rinsed in PBS and post-fixed cold in 2% osmium tetroxide. Ultra-thin sections were viewed with a transmission electron microscope (1200 EX II, 80 KV; Jeol, Sundbyberg, Sweden).

Morphometric analysis of airspace enlargement

Mean linear intercept, internal surface area and fractional lung volume were calculated in haematoxylin- and eosin-stained lung sections (5 μm), according to well-established stereology methods [15–18]. Digital images were captured using a Macintosh computer and Image Pro imaging software (Media Cybernetics, San Diego, CA, USA). A semitransparent grid of horizontal lines spaced at 1-mm intervals was overlaid onto 10 different areas of each $\times$40 serial lung section. Counts of intersections of these lines with alveolar walls were used to determine the internal surface area. Counts of the number of times the endpoints of the lines touched an alveolar wall were used to calculate alveolar fractional lung volumes. Data were collected from 10 random fields per tissue section.

In situ labelling of DNA cleavage

DNA cleavage was determined using terminal deoxynucleotide transferase (TdT)-mediated dUTP nick-end labelling (TUNEL) assays. Serial lung sections were subjected to biotin-streptavidin-horseradish peroxidase or fluorescein labelling of DNA strand breaks by TUNEL, using the Dead End Colorimetric Apoptosis Detection System (Promega, Madison, WI, USA), and the *In Situ* Cell Death Detection Kit (Boehringer Mannheim, Indianapolis, IN, USA). As a positive control, lung sections were treated with RNAse-free DNAse I (Boehringer Mannheim). As a negative control, the TdT enzyme was omitted in parallel reactions. The ratio of TUNEL-positive cells to total cells (apoptotic index) was measured from >3,000 parenchymal cells of each lung sample at $\times$40 magnification.

Assessment of DNA fragmentation

To assay DNA laddering, four emphysematous and three normal lung samples (100 mg wet weight) were digested in 1.2 mL digestion buffer (0.1 mg·mL^{-1} proteinase K, 10 mM Tris-HCl, 0.1 M NaCl, 25 mM ethylene diamine tetra-acetic acid (EDTA), 0.5% sodium dodecyl sulphate, pH 8.0). After protein extraction with phenol-chloroform/isoamyl alcohol and dialysis against 10 mM Tris-HCl, 1 mM EDTA, pH 8.0, samples were incubated with 1 μg·mL^{-1} of RNAse A for 1 h at 37℃, and dialysis was repeated. DNA (30 μg) was electrophoresed on 1.4% agarose gels containing 0.1 μg·mL^{-1} of ethidium bromide. Only seven samples were assessed due to tissue loss during processing and DNA purification and/or inadequate starting material from the remaining samples.

Immunoblotting of lung homogenates

Intact and cleaved caspase-3 expression was determined in six emphysematous and five normal lung samples homogenised in protein extraction buffer (20 mM Tris-HCl, pH 7.4, 0.15 M NaCl, 0.02% NaN_3, 1% NP-40, 1 mM phenylmethanesulfonyl fluoride, 2 mM *N*-ethylmaleimide, 10 μg·mL^{-1} leupeptin, 1 μg·mL^{-1} aprotinin, 10 μg·mL^{-1} pepstatin A, 1 mM EDTA). Western blot was performed on protein (120 μg) using antibodies to human caspase-3 (clone C31720; Transduction Laboratories), caspase-3 (clone 67341A; PharMingen), and proteolytic fragment of poly (ADP-ribose) polymerase (PARP, clone G734; Promega), a substrate of caspase-3.

Statistical analysis

Results of morphometry were analysed using an unpaired t-test or one-way ANOVA. Linear regression and Pearson correlation were used to assess the relationship between surface area and apoptotic and proliferation indices. Values for the Pearson correlation (r) approach a value of +1.0 or -1.0, depending on whether the factors are directly or inversely related, respectively. Linear regression values (R^2) approach an optimal value of +1.0 and indicate how well the data fit the regression curve. Data were presented as mean$\pm$SD. A p-value $<$0.05 was considered significant.

RESULTS

Clinical features of patients

Lung tissue was obtained from 16 unrelated adults. Relevant and available clinical data and smoking histories of these individuals are provided in table 1. No difference in age was detected between the two groups.

TABLE 1 Patient characteristics

	Normal	Emphysema
Subjects n	6	10
Sex		
Male	2	5
Female	4	5
Age yrs	46±22.7	52.2±11.7
Smoking pack-yrs	NA	40.9±24.9
FEV1 % pred	NA	26.8±6.8
FEV1/FVC %	NA	33.3±4.9
$D_{L,CO}$ %	NA	43.2±20.9

Data presented as n or mean±SD. FEV1: forced expiratory volume in one second; % pred: % predicted; FVC: forced vital capacity; $D_{L,CO}$: diffusing capacity of the lung for carbon monoxide; NA: information was not available for all normal samples.

Lung surface area, mean linear intercept and fractional volume findings

Surface area measurements were made in all lung specimens using tissue morphometry, and the quantitative results are shown in table 2. As expected, lung tissue from patients with emphysema demonstrated significantly lower surface areas compared with normal lungs. Lung sections from emphysema patients demonstrated increased mean linear intercept, reduced surface area and fractional lung volumes compared with normal lung sections.

Morphological detection of apoptosis

Haematoxylin- and eosin-stained lung tissue sections from normal and emphysematous lungs are demonstrated in figure 1. Scattered throughout the emphysematous lung tissues were cells exhibiting convolution of nuclear outlines morphologically characteristic of cells undergoing apoptosis (fig. 1b). These nuclear changes were not clustered in focal regions, as is typically seen in tissue necrosis. Apoptotic cells included endothelial cells, epithelial cells and fibroblasts. Neutrophil infiltration into the alveolar space or alveolar septa was negligible.

Biochemical characterisation of apoptosis

In situ detection of DNA cleavage

TUNEL reactions from normal and emphysema lung sections are demonstrated in figure 1d–h. Both fluorescein isothiocyanate (FITC) TUNEL for sensitivity and specificity, and biotinylated TUNEL reactions for analysis of apoptotic cells in the context of the lung tissue were performed. TUNEL reactions using FITC-conjugated nucleotide (fig. 1d and e) exhibited little or no labelling in the normal lung (0.17±0.18%), while more cells with intense labelling were present throughout emphysematous tissue (2.6±1.1%; $p=0.0002$). TUNEL assays using biotinylated nucleotide (fig. 1f, g and h) showed no labelling in the normal lung, while emphysema sections were TUNEL positive (6.1±3.5%; $p<0.01$). The higher apoptotic values obtained with the biotin protocol are likely to be due to an increased background, resulting from inflammatory cell peroxidase activity. Throughout the emphysema specimens, both alveolar and mesenchymal cells exhibited positive TUNEL staining. In addition, several macrophage-like cells with TUNEL-positive cytoplasmic bodies can be seen, characteristic of phagocytosis of apoptotic cells (fig. 1h). TUNEL assays performed on lung sections from patients with diffuse interstitial lung diseases were similar to normal samples. Assays performed without TdT enzyme showed no reactivity (data not shown).

Ultrastructural analysis of emphysema lungs confirmed morphological changes of apoptosis in several cell types. The most prominent features were cytoplasmic condensation and vacuolisation, chromatin condensation and connective tissue degradation. Electron microscopy (fig. 2a) of emphysema lung tissue shows two apoptotic cells in close apposition to a healthy cell. These cells demonstrate cytoplasmic and nuclear condensation with irregularities in cell shape, characteristic of apoptosis.

The presence of apoptosis in the emphysema lung samples was next examined using biochemical analysis of DNA laddering (fig. 2b). Agarose gel electrophoresis of DNA from normal samples revealed intact high molecular weight species. In contrast, sample E2, from a patient with the most clinically and morphometrically severe emphysema, demonstrated the most extensive laddering pattern with fragments of ~180 basepairs. Samples E1, E3, and E4 came from patients with less severe emphysema than E2. These samples did not show laddering, but nonetheless did reveal a smear on the gel.

TABLE 2 Surface area, mean linear intercept of all alveolar spaces (MLI) and volume of human lung samples

Characteristic	Normal	Emphysema	p-value
Surface area μm^2	182.0±29.8	88.5±44.8	0.0003
Volume fraction	0.275±0.095	0.17±0.08	0.051
MLI μm	0.011±0.002	0.03±0.01	0.012

Data presented as means±SD. Groups were compared using the unpaired t-test.

Expression of activated caspase-3 and cleaved PARP in lung homogenates

Caspases are aspartate-directed cysteine proteases with a pivotal role in the execution of apoptosis, not necrosis. Thus, caspase-3 Western blot analyses of normal and emphysematous lung tissues were performed (fig. 3). Pro-caspase-3 (32 kDa) was detected with similar frequency in normal and emphysematous lung homogenates (fig. 3a). These findings were confirmed with a rabbit polyclonal antibody against caspase-3 (data not shown). However, the activated subunits of caspase-3 (p17 and p12) were detected only in emphysematous lung homogenates (fig. 3b). In addition, an antibody that specifically reacts to the proteolytic fragment of PARP, a substrate of active caspase-3, demonstrated reactivity in the emphysema lung homogenates but not in normal lung (fig. 3c). The additional band running at 80 kDa may represent further degradation of PARP.

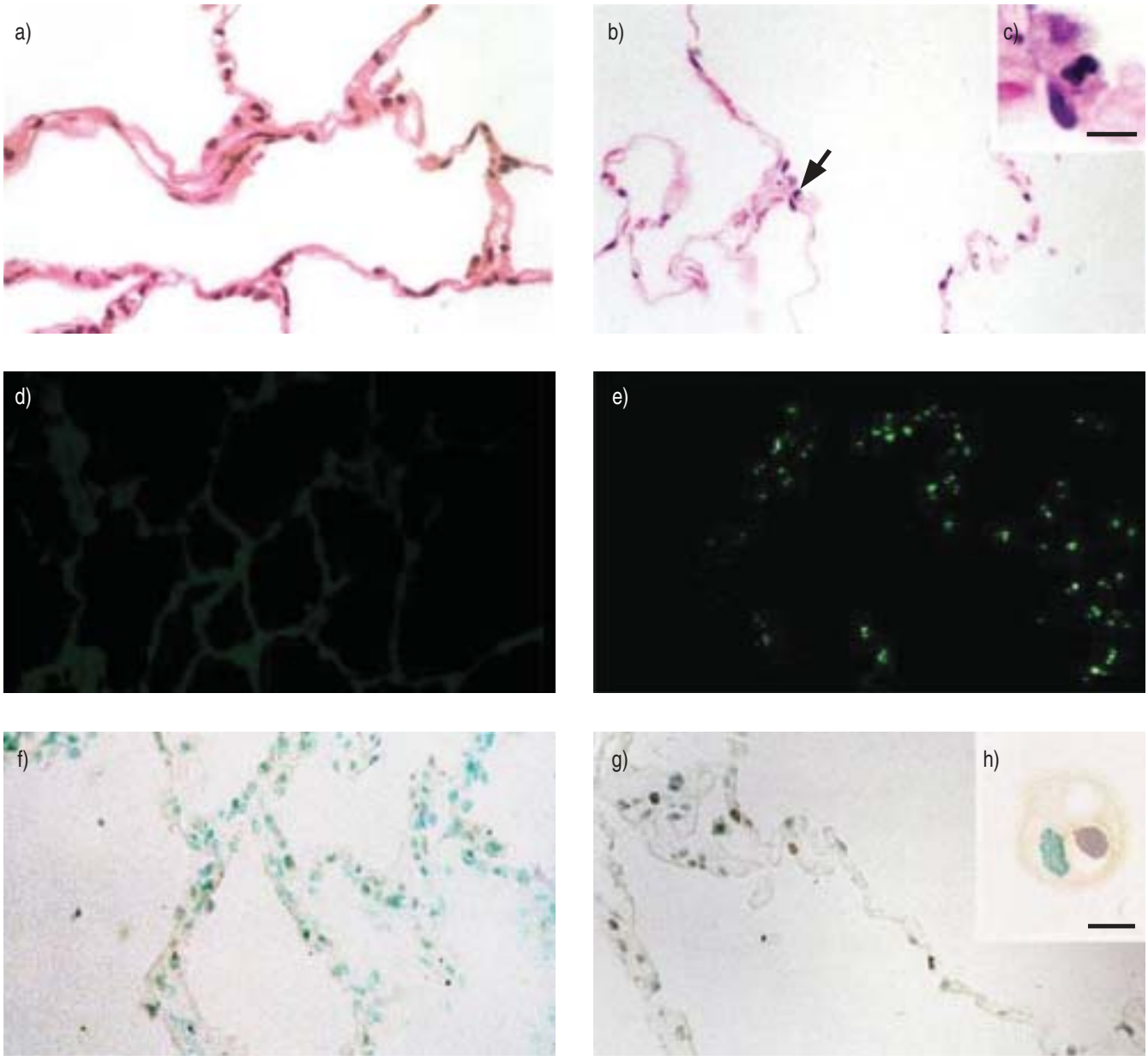

FIGURE 1. Morphological examination and terminal deoxynucleotide transferase-mediated dUTP nick-end labelling (TUNEL) staining of human lung tissue. Haematoxylin and eosin staining of normal (a) and emphysema (b) lung sections. The arrow in b) and c) identifies nuclear pyknosis and convolution. d, e) Fluorescein isothiocyanate-labelled TUNEL reactions from normal and emphysematous lung tissues. f, g) Biotinylated TUNEL reactions from normal and emphysematous lung sections. h) Identification of macrophage-like cells containing TUNEL-positive material in their cytoplasm. Scale bar=10 μm.

Expression of apoptosis-associated proteins Bcl-2, Bad, and Bax in the human lung

The initiation and progression of apoptosis occurs through several proteins of the Bcl-2 family, including Bcl-2, Bad, and Bax [19]. Immunohistochemical staining of these proteins in normal and emphysema lung tissue is demonstrated in figure 4. Bcl-2 was not detected in either normal or emphysematous lung tissue (fig. 4a and b). In addition, Bax and Bad expression were not detected in normal lung tissues (fig. 4c and f). However, expression of both proteins was detected in emphysematous lungs (fig. 4d and 4g). In emphysema samples, Bax staining was localised to alveolar epithelial cells (fig. 4d, arrows), whereas Bad staining was distributed among what appeared to be both epithelial and mesenchymal cells (fig. 4g, arrows). Interestingly, several macrophage-like cells demonstrated inclusion of Bax-positive material in their cytoplasm (fig. 4g).

Individual values of surface area, apoptosis, and cell proliferation

Values of surface area, apoptosis, and proliferation in the individual samples are presented using scatter plots (fig. 5). The distribution of surface area and apoptosis was broad among emphysema samples, but remained significantly

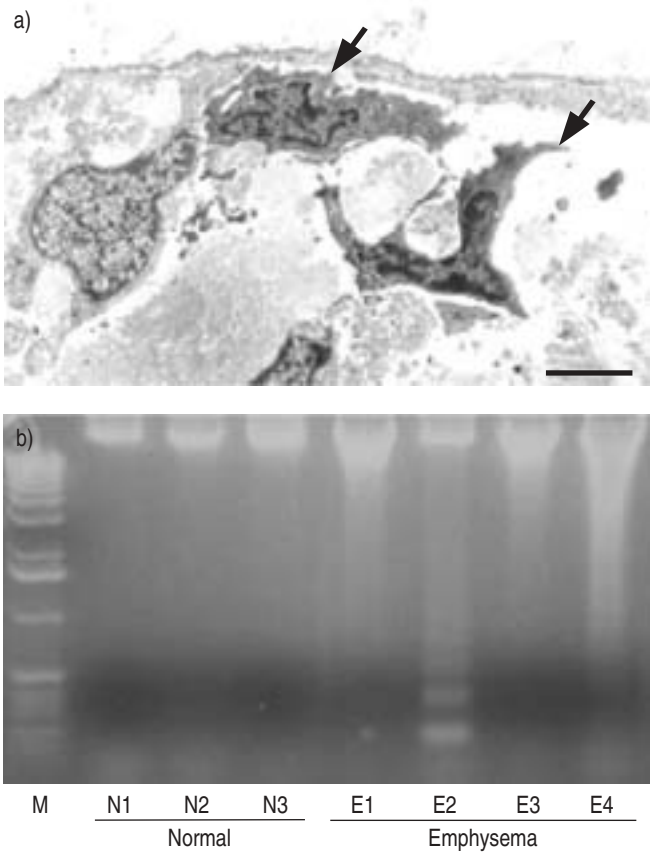

FIGURE 2. Biochemical analyses of apoptosis in human lung. a) Electron micrograph showing the ultrastructure of alveolar septa of an emphysema lung section. Apoptotic cells (arrows) adjacent to a normal cell illustrate cytoplasmic condensation and shrinkage, with condensation of nuclear chromatin. Loss of contact with the extracellular matrix contact is also observed. Scale bar=2 μm. b) DNA was isolated from three normal and four emphysematous lungs and analysed by agarose gel electrophoresis (30 μg of DNA per lane). DNA from all emphysema samples showed a smear, with sample E2 from a patient with severe emphysema revealing extensive laddering. Lane 1 shows a 1-kb DNA ladder.

different from normal lung. The emphysema samples demonstrated lower average lung surface areas than normal lungs (fig. 5a). All emphysema samples demonstrated higher apoptosis levels than the normal samples (fig. 5b). Data in figure 5b are from FITC TUNEL counts of apoptotic cells. Levels of cellular proliferation were also examined using immunohistochemistry for PCNA (fig. 5c). Interestingly, emphysematous lungs demonstrated higher levels of cell proliferation than normal lungs ($p<0.05$).

Correlation and linear regression analyses of tissue morphometry with apoptotic and proliferation indices

The results of cell morphology and tissue morphometry studies suggested a potential association between cell loss and lung architecture. Thus, the correlation between lung surface area and apoptosis (FITC TUNEL), as determined by TUNEL staining in the individual samples (fig. 6a), was plotted. Higher levels of apoptosis were detected as lung surface area decreased ($r=-0.75$; $p=0.001$). Next, the level of cell proliferation was compared relative to surface area.

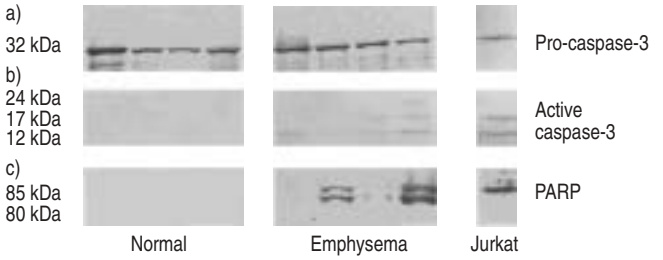

FIGURE 3. Caspase-3 activity and poly (ADP-ribose) polymerase (PARP) cleavage in human lung. Western blot analysis of homogenates from normal and emphysematous human lungs (120 μg of protein per lane). a) Pro-caspase-3 expression (32 kDa) is seen in all samples. The last lane shows reactivity in lysates from Jurkat cells stimulated by anti-Fas antibody as a positive control. b) Expression of the pro-form of caspase-3. Expression of the 12- and 17-kDa fragments with a 24-kDa intermediate form detected by a rabbit polyclonal antibody in the emphysema samples. c) Expression of the proteolytic fragment of PARP, a substrate of caspase-3, in emphysematous lung tissue homogenates but not in the normal lung. The presence of the additional band running at 80 kDa may represent further degradation of PARP.

Although cell proliferation (PCNA staining) was significantly higher in emphysematous lungs, no significant correlation between cell proliferation and surface area was detected (fig. 6b). Further, no statistical correlation between cellular apoptosis and proliferation was detected, although the positive slope of the regression line indicates that higher proliferative rates may be occurring in samples with higher rates of apoptosis (fig. 6c). These data suggest that the kinetics of cell loss (apoptosis) and cell replacement (proliferation) may be a critical feature of emphysema pathogenesis.

DISCUSSION

The present study demonstrates extensive cell death by apoptosis, in combination with connective tissue degradation, in the lung tissue of patients with pulmonary emphysema. Apoptosis was demonstrated and confirmed by several different techniques, including cell morphology, DNA fragmentation and apoptosis-related protein expression. These results confirm and extend observations made by others [10–12]. In addition, the data provide a correlation of apoptosis with a morphometric measure of the emphysematous process.

Each method of demonstrating apoptosis in the present study provides insight into the potential role of apoptosis in the pathogenesis of pulmonary emphysema. By morphological analysis, scattered apoptotic cells were visible in the absence of tissue inflammation. Ultrastructural analysis confirmed cytoplasmic and nuclear condensation in apoptotic cells. These features distinguish apoptosis from tissue necrosis [20]. In the latter, necrotic cells exhibit cellular swelling and rupture of the plasma membrane, while the nucleus remains relatively intact. In addition, in tissue necrosis, there is an associated inflammatory response. The morphological features of the cells in the emphysema tissue in this study were consistent with apoptosis.

Cellular apoptosis was confirmed by DNA fragmentation by *in situ* end labelling and gel electrophoresis. However, no specific cell type was predominately affected. These results are similar

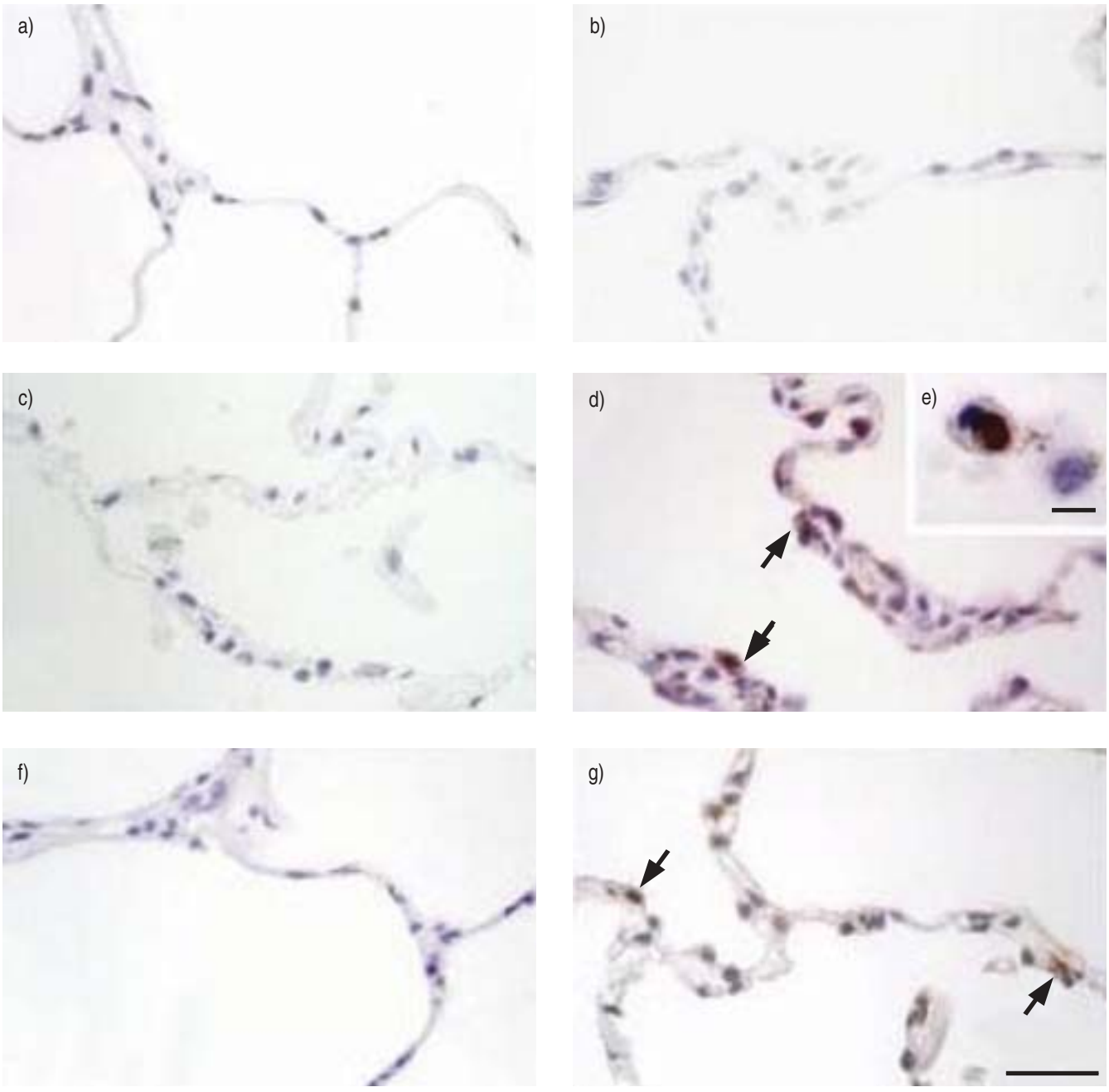

FIGURE 4. Immunohistochemical staining for Bcl-2, Bax and Bad in human lung tissue sections. Bcl-2 was not detected in either normal (a) or emphysematous (b) lung tissue. Bax and Bad expression was not detected in normal lung tissues (c, f), but both proteins were present in emphysematous lung samples (d, g). In emphysema samples, Bax staining was detected in alveolar epithelial cells (d, arrows), whereas Bad staining was distributed among epithelial and mesenchymal cells (g, arrows). In addition, several macrophage-like cells demonstrated inclusion of Bax-positive material in their cytoplasm (e). Scale bars=20 μm (e) and 60 μm (g).

to observations of KASAHARA and coworkers [10, 11], who demonstrated apoptosis of both alveolar septal and endothelial cells. Relative specificity of apoptosis for pulmonary emphysema was suggested by the absence of TUNEL reactivity in patients with diffuse interstitial lung diseases. These results are also in agreement with the observations of KASAHARA and colleagues [10, 11], who found no apoptosis in the lungs of patients with primary pulmonary hypertension. The finding of TUNEL-positive material within alveolar macrophages suggests a role for macrophages as scavengers of apoptotic cells, as shown for other cell systems [21]. The current authors have extended previous findings by demonstrating biochemical evidence of apoptosis and caspase activation in emphysematous lung tissue. The sequence of caspase activation is an indispensable process in the apoptosis pathway [22]. Caspase-3 functions downstream of cell damage in apoptosis, playing a pivotal role in targeting molecules for proteolysis [23]. Proteolysis of PARP into an 85-kDa fragment, as shown in the emphysema samples in the current study, is a specific event mediated by caspase-3 [24].

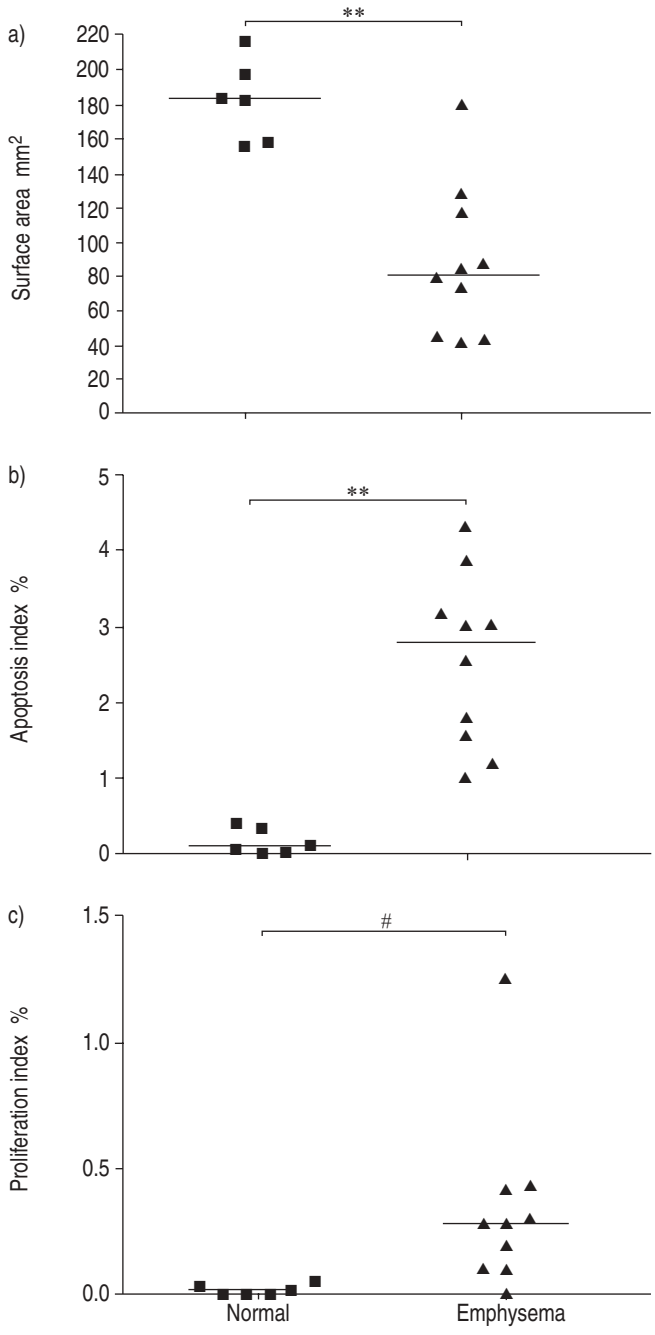

FIGURE 5. Scatter plots of individual lung samples. Lines represent the median value. Surface area measures (a) and apoptosis by fluorescein isothiocyanate terminal deoxynucleotide transferase-mediated dUTP nick-end labelling (b) were significantly different from normal samples. c) The average proliferation rate in the emphysema samples was higher than that for normal lungs. #: p=0.049; **: p<0.01.

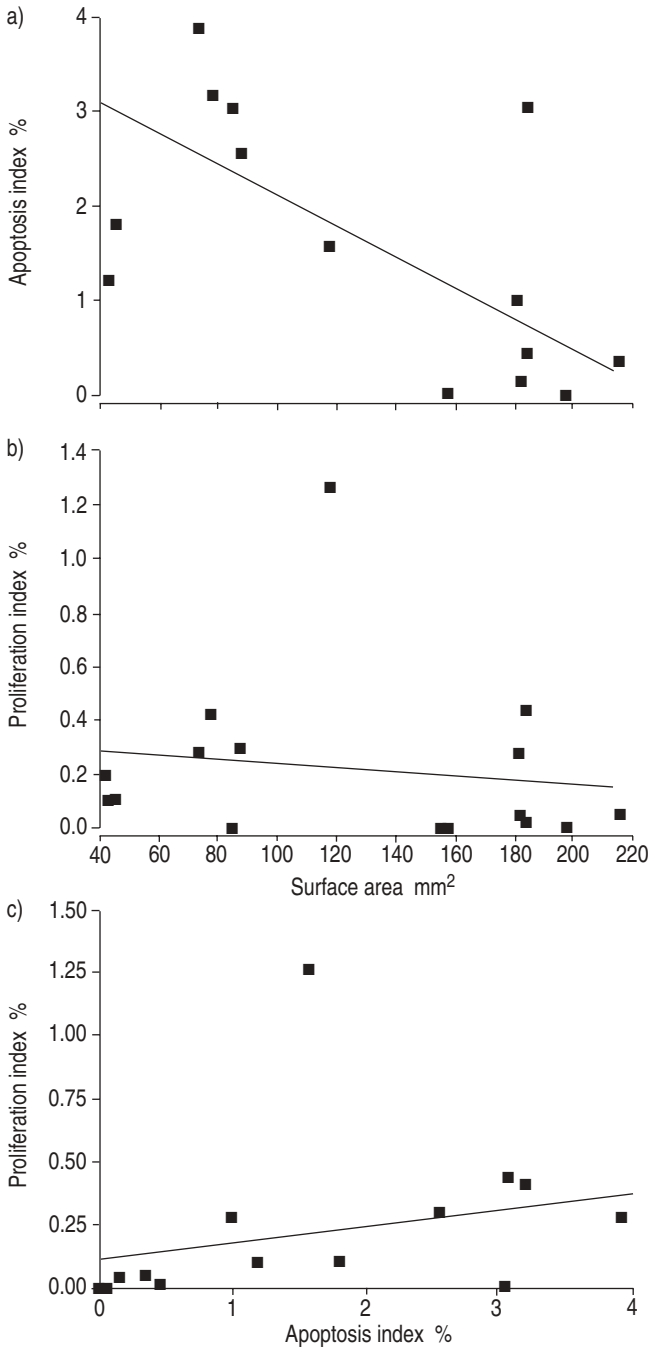

FIGURE 6. Relationships between surface area, apoptosis (fluorescein isothiocyanate terminal deoxynucleotide transferase-mediated dUTP nick-end labelling) and cell proliferation. Individual patient values are shown. a) An inverse correlation between surface area and apoptotic index is observed by linear regression (p=0.001). There is no significant correlation between surface area and proliferation (b), or between apoptosis and proliferation (c), even though emphysema samples had higher levels of both of these cellular indices. However, the positive slope of the regression line suggests that apoptosis and proliferation increase together.

An important finding of the current study was the elevation of both apoptosis and cell proliferation in the emphysema samples (fig. 5b and c, respectively). Interestingly, correlation analyses revealed a significant inverse relationship between surface area and apoptosis (fig. 6a), with no correlation between surface area and cell proliferation (fig. 6b). Together, these data suggest that, although both cell parameters are increased in emphysema, apoptosis and proliferation may not have the same relationship to lung surface area. This mechanism could explain the accelerated, destructive nature of the emphysematous process that exceeds the rate predicted

by traditional Fletcher curves, even after patients stop smoking [8, 9]. Interestingly, the increase in cell proliferation may explain why the elevated rate of apoptosis does not cause total loss of parenchyma.

Recently, YOKOHORI *et al*. [12] also analysed apoptosis and cell proliferation in emphysema lungs. They detected much higher levels of PCNA staining (median values were ~2.5% for emphysema lungs and 0.1% for normal) and lower biotin TUNEL staining (~0.9% for emphysema lungs and 0.1% for normal) than the current authors. The reason for the differences between the current authors' results and those of YOKOHORI *et al*. [12] may rest in the sample population. First, the average age of the sample population in the current study was younger and the emphysema samples came from patients with a shorter smoking history, averaging 40.9 pack-yrs compared with an average of 95 pack-yrs for the samples in the study by YOKOHORI *et al*. [12]. Samples in the current study were collected from emphysema patients who had quit smoking $\geqslant$6 months prior to the collection of the lung tissue. In addition, although no report was made on the morphometric assessment of airspace, it is possible that the samples in the study by YOKOHORI *et al*. [12] had more severe emphysema than those in the current study. Most importantly, the normal samples in the study by YOKOHORI *et al*. [12] were from lung cancer patients, which could have a higher proliferative capacity than the normal lung samples in the control group in the current study.

The identification of Bax expression in emphysema, but not in the normal lung, provides cellular links to apoptosis. Bax proteins are pro-apoptotic molecules, whose expression is increased when cells die by depletion of cell adhesion to the ECM [25]. Bax proteins counteract the protective effect of Bcl-2 proteins and trigger caspase activation [22]. Data from the current study demonstrate no Bcl-2 expression, with elevated Bax and Bad reactivity in emphysematous lung samples. In addition, there were macrophage-like cells containing Bax-positive particles within their cytoplasm. This is an intriguing finding in a disease involving increased levels of proteases that potentially disrupt the matrix to modify cellular adhesion and signalling.

The current study extends observations that cellular apoptosis occurs in emphysema, but does not entirely clarify the mechanisms of apoptosis. Intuitively, apoptosis may be related to the disruption of the ECM. Importantly, the observation of cellular apoptosis does not negate the role of proteases in emphysema pathogenesis. Indeed, it may represent a continuum of the destructive process, in which loss of matrix attachment leads to increased cellular Bax and caspase-3 activity, driving apoptosis of alveolar cells. It is possible that the increased protease burden results in loss of local matrix. Thus, it is conceivable that the cell death occurring in emphysema may be anoikis, programmed cell death due to loss of matrix adhesion [26]. Certainly, the increased protease burden in emphysema could potentiate loss of matrix, cleavage of cell surface receptors, cell detachment, and subsequent loss of signalling necessary for cell survival. In addition, other mechanisms, such as activation of cell surface death receptors or involvement of Fas ligand [27], may play a role in loss of pulmonary cells in emphysema. Alternatively, blockade of VEGF receptor signalling induces cellular apoptosis and emphysema, suggesting that inhibition of endothelial growth factors plays a role in the pathogenesis of disease [10, 11].

Regardless of the initiating cellular insult, the present study demonstrates that apoptosis occurs in emphysema lungs concomitant with disease progression. This is a mechanism that may explain the destruction of the lung during progression of the disease. Numerous studies have demonstrated a role for apoptosis in a variety of chronic human diseases, including neurodegenerative disease, heart failure, atherosclerosis and viral diseases [28]. In several of these diseases, anti-apoptotic agents are expected to treat patients or slow disease progression. Many of those agents are under evaluation and could potentially be applied to disrupt the progressive tissue loss characteristic of pulmonary emphysema.

To the current authors' knowledge, no previous reports have examined the relationship between cellular mechanics and loss of alveolar architecture, as reported here. The increase in cell death as morphometric lung surface area falls suggests that loss of cells contributes to reduced integrity of alveolar septa, resulting in increased pulmonary compliance and reduced elasticity. This hypothesis is supported by studies on emphysema induction following pulmonary caspase delivery in mice [14]. However, considering the events in reverse sequence, it is conceivable that the loss of matrix along the alveolar wall induces cell death without compensatory increases in cell proliferation. Nonetheless, much work remains to be carried out to define the mechanisms involved in apoptosis during the progression of pulmonary emphysema.

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