PULMONARY EMPHYSEMA: CURRENT CONCEPTS OF PATHOGENESIS

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Introduction

Emphysema is a Greek word meaning "to blow into" or "air-containing." Pulmonary emphysema is today a major public health problem, affecting in some form almost 10 million individuals in the United States alone.

Although many theories have been advanced concerning the etiology of this disease, large scale clinical and epidemiologic studies have established that cigarette smoking is responsible for the majority of cases of emphysema with urban air pollution playing only a minor role (1). This review will therefore concentrate on the pathogenesis of emphysema in smokers, though it will rely on many experimental models where cigarette smoke was in itself not the culprit in the development of the disease. This reflects the likelihood that the pathogenesis of emphysema is multifactorial.

The last two decades of research in this area has yielded many important scientific discoveries, some of which are now being put to clinical use. In particular, the hypothesis that emphysema is the result of a local imbalance between elastase and anti-elastase in the lung with resultant destruction of lung elastic fibers has led to the development of therapeutic modalities which aim to alter the protease-antiprotease balance in the lung. This hypothesis is attractive, yet other factors such as abnormal tissue response to injury, direct damage of lung tissue by oxidants and cigarette smoke constituents and the role of phagocytes in the lung in digesting lung tissue may be of equal importance. Before reviewing the data concerning pathogenesis, it would be helpful to briefly review the histology and pathophysiology of pulmonary emphysema.

Histopathology

An early attempt at defining the gross morphologic lesion in emphysema can be found in a monograph on diseases of the chest by Laennec in 1826 (2). Laennec appreciated that the lung undergoes a profound change in appearance upon deflation and removal from the thorax. This led to the development of a technique which allowed for excised lungs to be prepared for examination in an inflated state. Laennec noted that when this was done some specimens contained markedly enlarged alveoli, a process he called "vesicular emphysema." Laennec speculated that the abnormal enlargement of alveoli would result in a loss of lung elastic recoil and that this would cause severe airflow obstruction. He also noted that emphysema was associated in life with symptoms of persistent cough, purulent sputa and chronic dyspnea.

The modern pathologic description of emphysema was developed by the British pathologist Gough in 1952, and is based on the pattern of airway involvement (3). The unit of the lung where gas exchange occurs is called an acinus. The acinus is composed of the conducting airways distal to the terminal bronchiole and consists of respiratory bronchioles which end in alveolar ducts, alveolar sacs or alveoli. There are approximately 28,000 acini in the human lung.

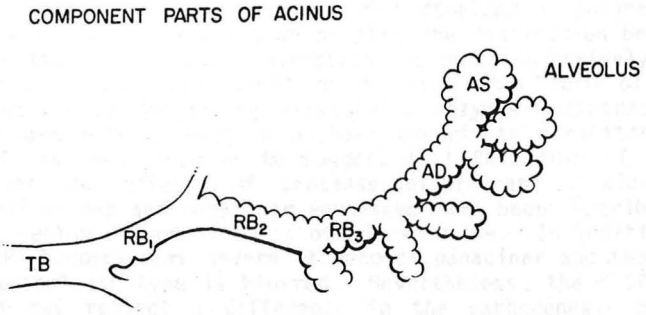

Figure 1. Schematic representation of an acinus showing terminal bronchiole (TB), first (RB_1), second (RB_2) and third order (RB_3) respiratory bronchioles, alveolar duct (AD), alveolar sac (AS) and alveolus.

Emphysema is defined as a condition of the lung characterized by abnormal, permanent enlargement of air spaces distal to the terminal bronchiole accompanied by destruction of their walls and without obvious fibrosis. Several types of emphysema can be described based on the pattern of airway involvement.

Central acinar (or centrilobular) emphysema is the most common type of emphysema and is almost exclusively a disease of smokers (4,5). It is characterized by a predominant involvement of the respiratory bronchioles early in the disease, with a subsequent extension of the process to the walls of adjacent alveoli.

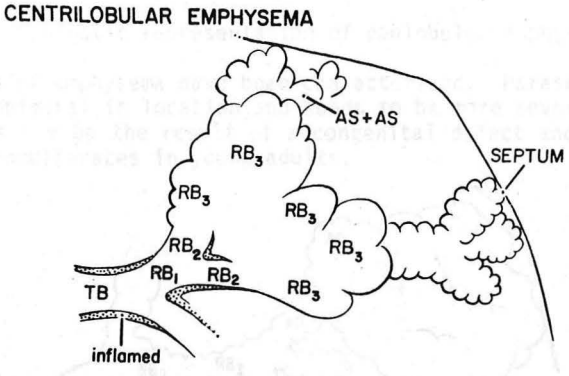

Figure 2. Schematic representation of centrilobular emphysema.

Centrilobular emphysema is remarkable for its inhomogeneity within a given lung. The posterior and apical segments of the upper lobes are most frequently affected, though the process varies greatly in extent and severity within the same lung. This pathologic observation is important as most studies of lung function in emphysema patients thus reflect an average of lung areas with varying degrees of disease. This is true regardless of whether the study in question measures airflow or the amount of elastase in respiratory secretions.

Panacinar or panlobular emphysema is characterized by progressive enlargement of all the alveoli in the acinus so that the distinction between alveolar ducts and alveoli is lost (6). Airspaces become progressively enlarged and gross simplification of lung structure occurs. This form of emphysema is produced in animals by instilling elastolytic enzymes intratracheally and is observed in humans with homozygous alpha-1 proteinase inhibitor (α1Pi) deficiency (7). It is thus tempting to suggest that this form of emphysema does indeed represent the effects of protease-antiprotease imbalance. However, although central acinar and panacinar emphysema have been described as separate entities, the overlap between them is often extensive. In addition, as central acinar emphysema becomes more severe it becomes panacinar and thus the distinction amongst pathologic type is blurred. Nevertheless, the different patterns of involvement may reflect a difference in the pathogenesis of central and panacinar emphysema.

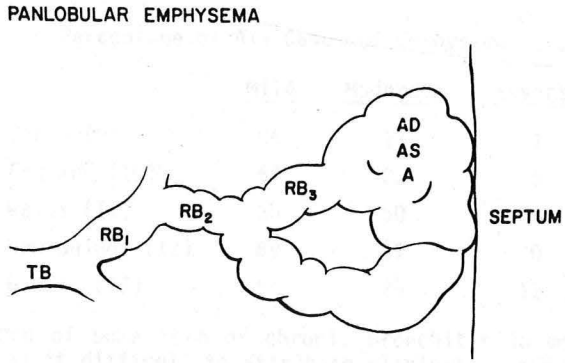

Figure 3. Schematic representation of panlobular emphysema.

Two other types of emphysema have been characterized. Paraseptal emphysema is predominantly subpleural in location and tends to be more severe in the upper lung zone (8). This may be the result of a congenital defect and is associated with spontaneous pneumothoraces in young adults.

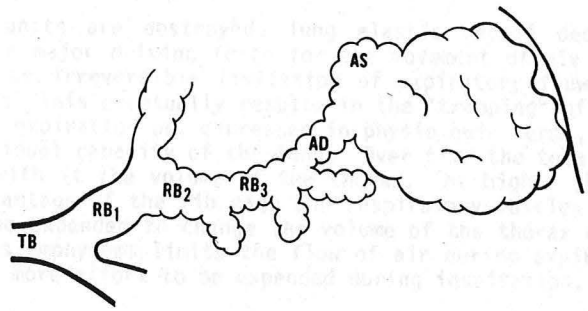

Figure 4. Schematic representation of paraseptal emphysema.

Irregular or paracicatricial emphysema is usually found adjacent to areas of scar tissue and because of the presence of fibrous tissue does not conform to the current definition of emphysema.

Emphysema is a common disease, yet the finding of severe emphysema in lung specimens is rare. Regardless of geographic location, as shown in Table 1, the severity of emphysema in most studies is graded mild to moderate. Emphysema severe enough to cause death itself is rare and is less frequent than one would suspect if emphysema alone were responsible for all of the morbidity of chronic obstructive pulmonary disease (COPD).

Table 1

Severity of Emphysema by Geographic Location

Percentage of All Cases of Emphysema

	Mild	Moderate	Severe
Japan (9)	86	13	1
England (10)	67	28	5
Wales (11)	30	50	20
New Guinea (12)	89	11	0
U.S.A. (13)	64	25	11

The coexistence of some form of chronic bronchitis in most patients with emphysema (14) makes it difficult to attribute clinical symptoms and physiologic derangements to emphysema alone. Nevertheless, the clinical complex associated with emphysema can be related in part to the consequences of destruction of acinar units.

Pathophysiology

As alveolar units are destroyed, lung elastic recoil decreases. Since elastic recoil is a major driving force for the movement of air from the lungs during expiration an irreversible limitation of expiratory flow occurs in the emphysematous lung. This eventually results in the "trapping" of air within the lung at the end of expiration or, expressed in physiologic terms, an increase in the functional residual capacity of the lung. Over time the total lung capacity may increase and with it the volume of the thorax. At higher thoracic volumes the mechanical advantage of the rib cage and respiratory muscles diminishes and more effort must be expended to change the volume of the thorax during inspiration (16,17). Thus emphysema limits the flow of air during expiration and also secondarily causes more effort to be expended during inspiration.

Destruction of acinar units also results in the loss of pulmonary capillaries. This in turn has several effects on gas exchange within the lung. Loss of capillaries within emphysematous airspaces which are still being ventilated results in an increase in physiologic dead space. In order to maintain a normal pCO_2, minute ventilation must be increased resulting in an even higher work of

breathing. Mismatching of ventilation and perfusion can also contribute to arterial hypoxemia, though most patients with emphysema do not become significantly hypoxemic at rest (18). The major consequence of a loss of capillary units on gas exchange is that the surface area across which gas can be exchanged is reduced. This is manifested by a decrease in the diffusion capacity of the lung ($D_L CO$). Although this does not cause hypoxemia at rest, it is associated with desaturation of arterial blood during exercise and is likely a major factor responsible for the severe limitation of physical activity in patients with emphysema.

Table 2

Physiologic Consequences of Emphysema

1. Limitation of expiratory flow.
2. Increased thoracic volume with diminishment of rib cage mechanical advantage.
3. Increased dead space.
4. Ventilation-perfusion mismatch.
5. Lowered diffusion capacity.

Attempts to correlate the severity of physiologic abnormality with the pathological severity of emphysema have been difficult. This may be due in part to the coexistence of other forms of lung disease in emphysema patients. In general, the best correlations between extent of emphysema and physiologic derangement has been with diffusion capacity, especially when expressed in ratio to alveolar volume, expiratory flow rates and lung elastic recoil (7). It should be remembered, however, that emphysema is a pathologic diagnosis. Thus clinical studies employing patients with emphysema diagnosed on the basis of aberrant pulmonary functions alone must be interpreted with some caution concerning the contribution of other coexisting lung diseases to the patients clinical state.

Pathogenesis

Although many physicians advanced theories on the pathogenesis of emphysema, the modern study of this problem did not begin until relatively recently.

In 1963 Laurell and Eriksson described an increased incidence of emphysema in individuals with low levels of serum alpha-1 globulins (19). The subsequent finding that this reflected a decrease in alpha-1-proteinase inhibitor ($\alpha 1Pi$) suggested that a diminishment of anti-protease activity could be related to the development of emphysema. Almost simultaneously Gross and colleagues produced an animal model of emphysema by instilling papain into the lungs of rats (20). This effect of papain was subsequently shown to be the result of elastolytic activity in papain extracts.

These findings led to the development of a theory that unrestrained elastolytic activity in the lung was the major pathogenetic mechanism responsible for the development of pulmonary emphysema. This was later modified to

suggest that a local disruption of elastase-antielastase balance was key to the development of emphysema.

The data concerning the role of proteases in emphysema will be reviewed with regard to the pattern of injury, factors affecting the extent of injury, mechanisms which exist in the lung for the prevention of injury by proteases and how these mechanisms might be subverted. Particular attention will be paid to how these factors might contribute to the development of emphysema in smokers.

Lung Injury by Elastase

The major method of inducing emphysema in animals relies on the intratracheal instillation of enzymes with elastolytic activity. A variety of enzymes have been used, some with the ability to digest proteins other than elastin. In general it is clear that the production of emphysema in these models is the result of an enzyme's ability to degrade elastin and not some other component of lung connective tissue (21). However, the destruction of other connective tissue elements besides elastin may contribute to the severity of the lesion (22).

Intratracheal instillation of elastase produces a widespread and fairly uniform lesion 3 weeks later. Histologically this lesion resembles human panlobular emphysema (23). Two aspects of this model bear consideration. First, the lesion evolves in a much shorter time span than human emphysema. The incidence of clinically relevant emphysema peaks after an average of 30 years of smoking (24). Even in nonsmokers who have a α1Pi deficiency, the onset of emphysema is measured in decades. Secondly, the degree of lung damage is dose related (25-28). This would suggest that the greater the imbalance between proteases and antiproteases in the lung, the more prevalent emphysema would be.

Most of the original animal models of emphysema utilized pancreatic elastase. Although there are a few studies which suggest that during acute illnesses the level of pancreatic elastase in serum rises, there is little evidence that suggests pancreatic elastase would have an important role in producing emphysema. The anti-elastase content of serum is much greater than that found in pulmonary secretions and is capable of rapidly inactivating large amounts of pancreatic elastase. Indeed, the production of emphysema in animals by intravenous injection of elastase is virtually impossible (29,30).

A more relevant source of elastase is likely to be found in neutrophils. Neutrophils have continuous access to the lung through the pulmonary circulation. In the absence of inflammation, neutrophils enter the lung at low rates through the interstitium beneath Type II alveolar cells and then migrate through the alveolar basement membrane into the alveoli (31). It is rare to find neutrophils in bronchoalveolar lavage (BAL) obtained from normal nonsmoking individuals. However, the absolute number of neutrophils in the lungs of smokers is markedly increased, as is the number of alveolar macrophages (AM) (32). The factors responsible for this recruitment of neutrophils into the lungs of smokers have not been fully elucidated. AM produce peptides and prostaglandin metabolites, most notably leukotriene D_4 which are chemotactic for neutrophils (31). In addition, the chemotactic peptide produced by AM is capable of stimulating neutrophils to release elastase (33). Thus the "burden" of leukocyte elastase would be increased in cigarette smokers.

Table 3

Recovery of cells by Bronchoalveolar Lavage [$X10^6$ and by percent ( )]

	AM	PMN	Lymphocytes	Total Cells
Nonsmoker	14 (93)	.03 (1)	1.0 (6)	15
Smoker	54 (90)	2.6 (4.4)	3.4 (5.6)	60

Highly purified preparations of human neutrophil elastase have been utilized to induce emphysema in animals (34-36). Although not as potent as pancreatic elastase, neutrophil elastase is capable of producing histologic and physiologic abnormalities similar to that found in human panlobular emphysema. Thus neutrophil elastase may be a major mediator of elastin degradation in the lung.

Human neutrophil elastase is a single chain polypeptide with a serine residue in position 195 of the enzymes primary amino acid sequence, and is thus a serine protease. It has a molecular weight of 33,000 daltons, a strongly basic isoelectric point (pH 10 to 11) and is active at a neutral pH (37). It is synthesized primarily in promyelocytes and stored in azurophilic granules of mature neutrophils. Neutrophil elastase is released during PMN phagocytosis or after cell death (38). Several connective tissue elements are subject to attack by elastase. Core proteins of proteoglycan molecules in connective tissue ground substance (39,40), Type III and IV collagen, and fibronectin are all components of pulmonary connective tissue which can be degraded by elastase.

Relatively few studies have attempted a direct measurement of leukocyte elastase in the lung secretions of patients with emphysema. Elastase-like activity was detected in the BAL of coal workers with pneumoconiosis (41). In addition, elastase has been reported to be increased in lavage samples collected from healthy cigarette smokers (42,43) when compared to nonsmokers. These studies are of interest because some of the elastase activity was inhibitable by chelating agents, suggesting that a metalloenzyme secreted by macrophages was present. This observation is important as metallo-elastases, unlike serine elastases, are not inhibited by α1Pi. The overall amount of elastase activity found in these studies was quite low and the significance of these findings is unclear.

The sequence of events which follow the instillation of elastase intratracheally has been elucidated using a variety of techniques. Edema, hemorrhage and a modest infiltration of neutrophils and mononuclear phagocytes occurs within the first few hours (26,44). Enlargement of the air spaces is noted within hours following this, although total lung volume itself is not increased, in part because of the presence of inflammatory cells and edema. By 24 hours up to two-thirds of the elastin in lung tissue is lost, along with a reduction in lung collagen (45). Most animal models show that histologic lesions identical to human pulmonary emphysema have stabilized at 21 days following a single instillation of elastase. This might suggest that once injury occurs the progression to emphysema cannot be modified. Several lines of evidence suggest that this is not the case.

Twenty-four hours after the instillation of elastase, the majority of free elastase has been cleared from the alveolar space through the interstitium and into the pulmonary circulation where it presumably becomes rapidly inactivated (46). However, a small portion of elastase is now localized within alveolar macrophages and can be found in AM up to a week following the original instillation. Human AM have receptors for neutrophil elastase and the elastase which they ingest remains enzymatically active (47,48). This might allow for a "pool" of neutrophil elastase within AM which could be slowly released over time and contribute to elastin degradation. Indeed, experimental evidence suggests that elastin degradation continues after free elastase has been cleared from the alveolar space.

Desmosine, which is the major cross-linking protein of mature elastase, can be used as a marker of ongoing elastin degradation (49,50) and correlates well with the extent of emphysema. Desmosine appears in the urine of hamsters for 3 days following initial enzyme instillation. More sensitive enzyme-linked immunoassays have detected products of elastin breakdown up to 2 weeks following initial instillation, suggesting that ongoing elastin degradation continues in the absence of free elastase in the alveolus (51,52).

The damage produced by a given dose of elastase can also be modified by host factors, including the level of antiprotease in the respiratory tract. In addition, the presence of cigarette smoke increases the severity of emphysema produced by elastase (53). To understand how these factors affect the severity of emphysema it is necessary to review the repair process which occurs following elastin degradation in the lung.

Repair and Resynthesis of Elastin

As mentioned previously, 24 hours after the instillation of elastase, total lung elastin is markedly reduced. At this point there is only minimal reduction of lung collagen. In the next few days a dramatic increase in synthesis of new elastin and collagen occurs. Several months later, the amount of elastin in the lung has returned to normal (45), though the presence of emphysema persists.

The three dimensional structure of elastin is highly stabilized by covalent cross-links between adjacent elastin peptide chains (54). As new elastin is synthesized it is released as a soluble precursor known as tropoelastin. Lysine residues on tropoelastin are oxidized by the copper dependent enzyme lysyl oxidase to form lysinal residues. These lysinal residues then become the site for cross-linking of elastin fibers by desmosine.

Lysyl oxidase is increased in lung homogenates following the instillation of elastase (55). Substances which interfere with the function of lysyl oxidase greatly exacerbate the degree of emphysema produced by elastase. Beta-amino-propionitrile (BAPN) inhibits lysyl oxidase. When BAPN is given to hamsters treated with intratracheal elastase, significantly worse emphysema results (Table 4).

Table 4

Effect of BAPN on Emphysema Induced by Elastase [56]

	Lm(u)
Control	60±1.6
BAPN	57±2.6
Elastase	118±21*
Elastase + BAPN	173±33†

Lm = mean linear intercept (a measure of airspace volume)
*p<.005 compared to control
†p<.005 to control and p<.01 to elastase done

Since lysyl oxidase requires copper, copper malnutrition can also worsen the extent of elastase injury (57). This may have clinical significance as copper-chelators are known to be present in the gas phase of cigarette smoke. Indeed cigarette smoke itself has been shown to have an effect on elastin synthesis.

Several investigators have demonstrated that cigarette smoke worsens the emphysema produced by intratracheal elastase (53). A variety of explanations have been advanced, including the effect of oxidants in cigarette smoke on lung tissue. The role of oxidants in emphysema will be discussed in a later section. There appears to be good evidence that cigarette smoking interferes with the function of lysyl oxidase.

The level of lysyl oxidase in lung homogenates of hamsters treated with intratracheal elastase is reduced in hamsters chronically exposed to cigarette smoke (58). In vitro experiments have shown that cigarette smoke blocks the cross linking of elastin (Table 5).

Table 5 [59]

Inhibition of Desmosine Cross-Link Formation by Smoke

Smoke Fraction	Desmosine Recovery (% Control)
None	100
Whole	18*
Gas Phase	23*
Acidic	17*
Basic	86

*p<.001

These findings would suggest that longterm exposure to cigarette smoke could contribute to a decrease in the development of elastin-cross links in areas of the lung undergoing repair of elastase induced damage. Another effect of smoke on elastin synthesis may be related to the observation that acidic, but

not basic, smoke constituents inhibit the formation of elastin cross-links. Lysyl oxidase is a negatively charged molecule (60) and the binding of polyanions to elastin are known to decrease the binding of lysyl oxidase (61). Conversely, polyanions bound to elastin would promote the attraction and binding of the positively charged elastase. Thus cigarette smoke could theoretically affect lung elastin synthesis by a) decreasing the activity of lysyl oxidase; b) decreasing the binding of lysyl oxidase to elastin; and c) increasing the binding of elastase to elastin. The host response to elastase injury may therefore be modified by defective repair processes and could contribute to the difference in severity of emphysema amongst smokers with similar cigarette consumption.

The wide range of target substrates for neutrophil elastase suggests that rapid inactivation of elastase would be crucial to maintaining the structural integrity of connective tissue. The role of antiproteases in the lung has thus received much attention in the literature. The next section will discuss the nature of antiproteases in the lung and the consequences of lowered antiprotease levels on the development of emphysema.

Elastase Inhibition in the Lung

Alpha-1-proteinase inhibitor (α1Pi) is the major anti-elastase in serum and pulmonary secretions. α1Pi is a glycoprotein with a molecular weight of 52,000, which is produced primarily in the liver (62-66). Normal concentration of α1Pi is 136-225 mg/dl and the protein is transuded into the alveolar milieu.

Structural studies have demonstrated that the active site of α1Pi contains a methionine-serine peptide bond at position 358 (67,68). This methionine-serine bond is recognized by a proteolytic enzyme and covalent bonding between the protease and inhibitor occurs. α1Pi thus acts as a false substrate for the protease and inhibition of elastase by α1Pi depends on the integrity of the methionyl residue in the active site (68).

Several proteolytic enzymes can be inactivated by α1Pi including elastase, trypsin, collagenase and thrombin (69). The inactivation of elastase by α1Pi occurs in less than 1 millisecond, several logs faster than other potential substrates, suggesting that the major physiologic role of α1Pi is the inactivation of elastase. Removal of α1Pi from normal lung lavage fluid by immunoprecipitation decreases neutrophil elastase inhibitory activity ten-fold (70).

Although α1Pi is the major anti-elastase in the lung, other antiproteases may play a significant role. Alpha-2 macroglobulin is a high molecular weight protein which can bind elastase (71). This protein is markedly increased in the serum of patients with α1Pi deficiency and may be important as a "backup" to α1Pi (72). Low molecular weight (MW) antiproteases such as bronchial mucus inhibitor can be demonstrated in epithelial cells lining the small airways of human lung (73). It has been suggested that low MW inhibitors and α1Pi may have independent physiologic functions. α1Pi can dissociate neutrophil elastase which has complexed with low MW inhibitors. The free low MW inhibitors appear to be more effective than α1Pi in inactivating neutrophil elastase which has already bound to insoluble elastin (74). Thus α1Pi may bind free elastase and keep low MW inhibitors uncomplexed with elastase allowing low MW inhibitors to interact with elastin-bound elastase.

Ironically, a satisfactory animal model of α1Pi deficiency does not exist. However, the clinical syndrome of α1Pi deficiency has provided important insights into the role of antiproteases in the pathogenesis of emphysema.

Several genetic variants of α1Pi have been described. (A detailed discussion of this subject can be found in the Grand Rounds on α1Pi deficiency by Dr. Goldstein.) The major alleles of the protease (Pi) locus are M, Z, S and null. The alleles correspond with varying levels of serum α1Pi (Table 7). The most frequent Pi phenotype is MM, accounting for 95% of all Pi types in the United States. The ZZ phenotype is associated with a 90% reduction in α1Pi levels. The frequency of this phenotype is approximately 1 in 2500. MZ individuals have a moderate reduction in α1Pi levels and account for 4% of the U.S. population. SS and SZ individuals also have reduced levels of α1Pi.

Table 6 (75)

Frequency of Pi Phenotypes

	MM	MZ	SS	SZ	ZZ
% normal α1Pi	100	60	50	35	10
Frequency	95%	4%	1/250	1/800	1/2500

The finding of low levels of α1Pi in non-MM individuals provides a human model for the relevance of antiproteases in the pathogenesis of emphysema. A review of 246 Swedish patients with ZZ phenotype disclosed evidence of emphysema in the majority (76). The pattern of emphysema was remarkable for an early age of onset. The onset of dyspnea occurred at a median age of 40 in ZZ smokers, while nonsmokers had onset at age 53. This data has been criticized as the patient sample contained a large percentage of smokers. Many of the nonsmokers came to medical attention because of respiratory complaints or because they were family members of other ZZ subjects. Therefore, an unknown number of asymptomatic nonsmoking ZZ individuals would have escaped medical attention. Indeed, not all individuals with ZZ phenotype develop emphysema and the true incidence of emphysema in ZZ individuals is unknown.

In ZZ individuals who develop emphysema, smoking history appears to play a major role. However, there is good evidence to suggest that the deficiency of α1Pi results in an increased elastase load in the lungs. Neutrophil elastase has been found in the BAL of nonsmoking α1 Pi-deficient individuals with severe emphysema (77). Replacement therapy with intravenous α1Pi, which will be discussed subsequently, resulted in a disappearance of elastase from subsequent BAL specimens. Once emphysema develops it proceeds at a relatively rapid rate. The rate of decline of FEV_1 in ZZ individuals is nearly twice that reported in patients with normal α1Pi levels and COPD (78,79).

We have previously seen that the amount of emphysema produced by elastase is dose related. If one extrapolates this finding to reflect an elastase/antielastase imbalance, a reduced level of α1Pi should predispose individuals with MZ phenotypes to an increased incidence of emphysema. Several studies (79-85) have attempted to determine the prevalence of emphysema amongst MZ heterozygotes. Many of these studies have serious methodologic problems which make interpretation difficult. However, two large scale studies (86,87) could find no relationship between an intermediate α1Pi level and evidence of airways

obstruction. In addition, measurements of antigenic levels of α1Pi in the BAL fluid of cigarette smokers do not differ from nonsmoking controls (54).

The relationship between α1Pi levels and the development of emphysema in smokers is thus uncertain. Most studies would suggest that moderately decreased levels of α1Pi do not play a major role in the development of emphysema. Contrasted with animal models which show a strong correlation between the dose of elastase and the development of emphysema, a similar dose-response curve cannot be drawn for the level of α1Pi. This has important implications for α1Pi replacement therapy and will be discussed subsequently.

Inactivation of Elastase Inhibitors

Although the data regarding the significance of absolute levels of α1Pi in the pathogenesis of smokers emphysema is inconclusive, considerable evidence suggests that local inactivation of α1Pi may be a major contributor to the development of emphysema. Oxidation of α1Pi under conditions favoring the oxidation of methionine side chains results in a loss of elastase inhibitory activity (68). Oxidation of the inhibitor decreases the rate of association of α1Pi and elastase by a factor of 2,000 and the α1 Pi-elastase complexes formed appear to be relatively unstable (88). Low MW elastase inhibitors also contain methionine residues which can be oxidized and thus α1Pi is not the only antiprotease in the lung affected by this process.

An excellent source of oxidizing agents can be found in the gas phase of cigarette smoke (89). In vitro studies have shown that aqueous extracts of freshly generated cigarette smoke can inactivate α1Pi and that this is prevented using oxygen-radical scavengers (90).

Several studies have examined the level of α1Pi activity in lavage fluid obtained from smokers. The initial studies suggested that chronic human smokers had decreased activity of α1 Pi/μg protein compared to nonsmokers (91,92). However, subsequent studies disputed these findings (93,94). Differences in the methods used in these studies made comparison difficult. Recently a carefully controlled study evaluated the time course of α1Pi inactivation by cigarette smoke (95).

Table 7 (95)

Effect of Smoking on α1Pi Activity in BAL

	Nonsmoking Controls	Smoking 2 Cigarettes		
Time Interval	10 min	10 min	30 min	60 min
No. subjects	8	6	6	6
Age	31.2±6.9	26.8±3.4	26.0±9.0	26.8±5.9
Smoking (cigs/day)	0	19.2±9.2	22.5±10.8	25.0±4.5
Initial α1Pi activity (μg PE/μg α1Pi)	.470±.041	.484±.043	.497±.051	.521±.033
α1Pi activity $\bar{p}$ smoking (% initial)	100.5±6.7	95.6±4.4	92.5±9.8	90.0±10.6

$p<.05$

This study was performed after overnight abstinence from smoking. Smokers, or nonsmoking controls, were bronchoscoped and lavaged and the activity of α1Pi determined. The bronchoscope was then withdrawn and the subjects smoked 2 cigarettes. The bronchoscope was then reinserted at a later time point and the procedure repeated.

Following smoking, the activity of α1Pi decreased with a statistically significant decrease found 1 hour after smoking. This effect was transitory and α1Pi activity was normal 2 hours after smoking. Analysis of individual data points showed that in some individuals the reduction in α1Pi activity was more severe than reflected in the overall mean. These findings are striking in light of the fact that the lavage process obtains samples from areas of the lung where cigarette smoke if inhomogenously distributed. Other studies in animal models have also found a transient drop in α1Pi activity 30-60 minutes after smoking (96). This inactivation has been shown to be due to specific oxidation of the methionyl residue at the active site of α1Pi.

Several antioxidants are present in the lower respiratory tract which could combat the oxidation of α1Pi. Ceruloplasmin is a plasma copper transport protein which is an effective antioxidant. Ceruloplasmin is present in BAL fluid and can block the inactivation of α1Pi by cigarette smoke (97,98). One recent report suggests that ceruloplasmin levels are increased in smokers but that the specific antioxidant activity is decreased (99). This report is intriguing but needs further confirmation. Catalase and glutathione are also important antioxidants and are found in erythrocytes and phagocytes in the lung (100,101). These enzymes could also contribute to antioxidant defense.

Another antioxidant present in human lung is methionine sulfoxide reductase (102). This enzyme is capable of reducing oxidized α1Pi and restoring activity (103). The development of antioxidants capable of reactivating α1Pi is currently a topic of great interest and may be put to clinical use in the next few years.

The presence of oxidants in cigarette smoke thus may be important in the pathogenesis of emphysema. Of equal importance, however, may be the alteration in the phagocyte population of the lung induced by cigarette smoke.

Role of Lung Phagocytes

The use of cigarette smoke to induce emphysema in animal models has not been effective (104-108). This may be related to the relatively short periods of time (2-4 months) which have been used in these studies. However, although emphysema itself was not produced in these studies, alterations in the phagocyte population in the lung occurred in response to smoke. Increased numbers of macrophages were found in the airways of these animals. AM accumulated in the respiratory bronchioles of these animals and pathologic changes consistent with a respiratory bronchiolitis were observed (106-109). Similar findings have been observed in the lungs of young, asymptomatic human cigarette smokers (110).

The role of AM in recruiting neutrophils to the lung has been discussed previously. Much of the focus of research in pulmonary emphysema has been on the way neutrophils may contribute to the pathogenesis of the disease. Little data exists in the literature on the role that AM might play in the development

of emphysema. This is surprising for several reasons. First, although the number of PMN are increased in the lungs of smokers, this increase is far less dramatic than the absolute increase in the number of AM. Secondly, AM accumulate in the respiratory bronchioles of smokers, the precise location of the lesion in central acinar emphysema. Third, AM are capable of releasing substances, such as oxidants and proteases, which could directly injure lung parenchyma. It would be important therefore to review some of the data concerning the possible role of AM in the pathogenesis of emphysema.

Human AM are derived from bone marrow produced precursors, most likely blood monocytes (Mo) (111,112). Monocytes contain an elastase which is antigenically and biochemically closely related to neutrophil elastase (113). Although on a cell for cell basis Mo contain only 3% as much elastase as PMN, the location of the elastase on Mo is of particular interest. Rather than being contained intracellulary (as in PMN), monocyte elastase is found on the cell surface (114,115). It is possible therefore that as the mononuclear phagocyte population of the lung increases in response to cigarette smoke, an increased flux of Mo into the lung occurs. The surface bound elastase on Mo could cause damage to connective tissue as Mo move from the blood into the interstitium.

As Mo mature into macrophages in vitro, their elastase changes (116). The serine-protease disappears and a metalloenzyme with a zinc atom at its catalytic site appears. The significance of this from a practical standpoint is that metalloenzymes are not inhibited by α1Pi. This protease is neither stored in macrophages nor bound to the cell surface but is secreted into the extracellular milieu. Human AM have been shown to produce this metalloprotease in culture (117) though the ability of this enzyme to degrade elastin is unclear.

Murine macrophages secrete a metalloenzyme in culture. In a 24-hour period 200 mouse macrophages secrete as much enzyme as that contained in 1 human neutrophil (118,119). Although this is a small amount, a potentially important effect of this macrophage protease is that it digests α1Pi (120). The possibility that such an interaction occurs in humans is supported by a study showing that a metalloenzyme can be found in BAL obtained from smokers and that the activity of α1Pi in lavage samples is inversely correlated with the amount of metalloenzyme (43). Thus, even if the metalloprotease produced by human AM cannot degrade elastin, it could play an important role in lowering the activity of α1Pi in respiratory bronchioles.

Human AM possess other proteases capable of injuring lung tissue. Cathepsin B is a proteinase capable of degrading elastin. An enzyme similar to Cathespin B was recently described on the surface membrane of human AM (121,122). Another protease located on the cell membrane of human AM is plasminogen activator (123-125). By converting plasminogen to plasmin, AM could initiate elastinolysis. Plasmin is extremely effective at degrading proteoglycans and fibrin and could thus expose elastin and collagen fibers in connective tissue to further attack by elastases. In addition, as previously mentioned, human AM are capable of storing human neutrophil elastase. The possible role of human AM in altering the protease-antiprotease balance at a site where central acinar emphysema occurs could thus involve the release of stored neutrophil elastase, the delivery of surface bound proteases onto connective tissue and the local inactivation of α1Pi by metalloproteases (126).

Another major contribution of AM, and neutrophils, to protease-antiprotease imbalance and lung injury is likely to be the production of oxidants. Stimulated neutrophils and AM are capable of producing a variety of toxic oxygen species (superoxide anion, hydrogen peroxide, hydroxyl radical). Increased production of toxic oxygen compounds occurs in the AM of asymptomatic smokers (127).

Table 8[127]

Superoxide Anion Release by AM ($nmO_2^-/10^6$ AM)

	Smokers	Nonsmokers	
AM alone	12.8±1.9	7.1±1.2	p<.05
AM + PMA	38.6±3.4	18.9±2.9	p<.05

The amount of these products produced by AM is far less than produced by PMN on a cell-for-cell basis. However, the much greater number of AM in the lung suggests that by mass effect alone AM would be the most important source of cellular oxidants. Indeed, the production of oxidants by AM stimulated with cigarette smoke is maximal at 30 to 60 minutes (128). This is the same time curve at which maximal inactivation of α1Pi occurs in BAL fluid following smoking.

In addition to inactivating α1Pi, oxidants in cigarette smoke and those produced by AM and PMN are capable of directly damaging connective tissue. Stimulated AM produce H_2O_2 capable of killing fibroblasts in vitro (127). This may also contribute to the pathogenesis of emphysema.

A major difference in the production of oxidants by AM and PMN is the ability to use these oxidants optimally. Myeloperoxidase (MPO) is an enzyme found in PMN which is capable of enhancing the activity of cellular-produced oxidants. MPO catalyzes a reaction between hydrogen peroxide (H_2O_2) and halide ion (Cl^-, I^-, etc.) to form hypohalogenous acids (129,130). The products of this reaction are extremely potent oxidants. The ability of H_2O_2 to kill target cells is increased a log power by the presence of MPO in vitro. In addition, MPO greatly accelerates the oxidation of α1Pi by H_2O_2 (31) and is more potent at inactivating α1Pi than is cigarette smoke.

Mature human AM have virtually no MPO (131,132). Although AM are capable of producing H_2O_2, the amplification of its effect through the MPO system is severely limited (133). Human AM have been shown to be capable of ingesting MPO released by PMN (54) resulting in a reconstitution of functional MPO within AM. Since AM produce a peptide which results in the release of PMN enzymes (33), the possibility exists that an increase in MPO within the airspaces in emphysema occurs. This could result in a tremendous increase in the toxicity of oxidants produced by AM. Thus in addition to releasing elastase, neutrophils may interact with AM in the lungs of smokers in ways which could further damage lung connective tissue.

The large amount of research on the pathogenesis of pulmonary emphysema has produced several possible therapeutic strategies. Three recent developments will be reviewed.

Correction of Protease - Antiprotease Imbalance in Humans

The major thrust in this area has been concerned with increasing the levels of antiprotease defense. Because α1Pi appears to be the major inhibitor of neutrophil elastase in serum and PiZZ individuals have an increased incidence of emphysema, most of the efforts have been directed at raising the level of α1Pi in α1 Pi-deficient individuals. Several approaches to this problem have been undertaken.

Danazol Therapy

Danazol is an isoxazole derivative of the synthetic steroid 17 α-ethinyl testosterone. Although structurally similar to testosterone, Danazol has no major androgenic properties. Danazol has been used to increase the level of C1 esterase inhibitor in patients with hereditary angioedema. Because both C1 esterase inhibitor and α1Pi are hepatocyte produced antiproteases, Danazol has been used to increase the level of α1Pi in PiZZ homozygotes (134). A recent report evaluated the response of 43 ZZ individuals to a 30-day trial of 600 mg/day Danazol (135).

Table 9

Response of PiZZ individuals to Danazol[135]

	Number	Pre $\overline{rx}$ α1Pi (mg/dl)	Post $\overline{rx}$ α1Pi	% increase
Responders	23 (53%)	28.6±1.3	43.5±1.6	52%
Nonresponders	20 (47%)	30.2±2.1	30.3±2.4	0

Approximately 50% of the patients in this study had a >20% increase in the serum level of α1Pi. However, the mean post-Danazol level of α1Pi in the responders was only 43.5 mg/dl, or approximately 18% of the normal level as measured by these investigators. From epidemiologic studies an α1Pi level >35% of normal is thought to be necessary to protect the lung against neutrophil elastase. Thus, even in the responding patients the efficacy of Danazol therapy is small. Even higher doses of Danazol could not improve the α1Pi level further.

A more reliable way of increasing serum α1Pi would be the direct administration of purified α1Pi. Two approaches have been undertaken.

Replacement of α1Pi

Recombinant DNA technology offers great promise as a means of producing α1Pi in large quantities. Work in this area has provided many important insights into the molecular biology of α1Pi. The nucleotide sequence for the M, Z, and S alleles has been determined (136-138) and the mutation responsible for the Z phenotype (342 glutamic acid → lysine) identified. The Pi M gene has been cloned in both yeast and E coli (139,140). Several technical problems, however, currently complicate use of α1Pi produced in this fashion. Leader peptide sequences which remain attached to the synthesized α1Pi are immunogenic. In

addition, the carbohydrate content of recombinant-produced α1Pi is small, a fact which apparently results in a very short half-life in vivo. These problems have resulted in current trials being limited to α1Pi purified from blood products.

Human plasma Cohn Fraction IV-1 is a side product from the production of albumin and immunoglobulin which contains a large amount of α1Pi. A technique for rapidly isolating and purifying α1Pi from this fraction was developed by Cutter Laboratories. This allowed for the first large scale trial of α1Pi replacement in ZZ homozygotes.

The planning for this trial was extensive and several NIH workshops were convened to deal with experimental design. Statisticians determined that a trial of α1Pi therapy with a goal of reducing the rate of decline in in FEV_1 by 30% would require a 5-year study involving more patients than have ever been identified with ZZ phenotype. Thus, a trial with a clearly defined clinical response as the endpoint could not be undertaken. Instead efficacy was defined as the ability of the infused α1Pi to reconstitute α1Pi levels in serum and lung lavage fluid without significant side effects.

Preliminary reports from these trials have been encouraging and have been published in abstract form (141).

Table 10[141]

Effects of α1Pi Infusion in 19 PiZZ Individuals

	Pre-Therapy	Post-Therapy	
Serum α1Pi level (mg/dl)	31±3	130±5	p<0.01
Serum α1Pi activity (μM)	4.9±0.4	15.4±0.8	p<0.01
BAL α1Pi level (μM)	0.3±0.2	1.9±0.2	p<0.01
BAL α1Pi activity (μM)	0.6±0.2	1.4±0.1	p<0.01

Patients were infused with 60 mg/kg of purified α1Pi intravenously once/week for 6 months. Levels of α1Pi plateaued at 6 weeks and were maintained for the duration of the study. The incidence of adverse reactions was minimal.

This study supports the practicality of administering α1Pi intravenously to ZZ individuals. However, the impact of this therapy on the patients clinical course is not known at present, and may never be known. The use of this modality in ZZ individuals does appear reasonable, and may also have utility in preventing the liver disease associated with the PiZZ phenotype.

The use of this therapy to prevent emphysema in smokers is more troublesome. As discussed previously the development of emphysema in smokers has little correlation with the antigenic level of α1Pi. A more plausible consideration would be that the oxidation of α1Pi results in a loss of functional activity in smokers. Although it is possible that infusion of α1Pi in supraphysiologic doses could "saturate" the oxidant system, this approach is probably not practical. Therefore, attempts have been made to develop mutant forms of α1Pi which are more resistant to inactivation by oxidants.

Several investigators have recently cloned a mutant gene for α1Pi which codes for a valine residue rather than a methionine residue at position 358 (142,140). The 358 valine α1Pi is 10 times more resistant to the effect of oxidants than the normal α1Pi (143). This form of α1Pi, however, can still be oxidized and its clinical utility is unclear. In addition, although this form of α1Pi effectively inactivates elastase, its activity against other proteases could conceivably be less than the normal α1Pi. This particular area of research does however offer great promise.

Summary

A variety of mechanisms which could contribute to the pathogenesis of emphysema in smokers has been reviewed. The following is a sequence of events, drawn from the reviewed data, which could contribute to the development of emphysema in smokers.

The critical lesion in smokers is the appearance of large numbers of AM with an increased number of PMN in the respiratory bronchioles. This results in a focal increase in proteases, including neutrophil elastase. As these cells are stimulated by cigarette smoke, oxidants are produced which briefly and locally inactivate α1Pi and in combination with proteases cause direct damage to lung parenchyma. The function of repair processes at this site is altered by the inactivation of lysyl oxidase by cigarette smoke at precisely the time that injury occurs. Over time repetitive low level insults are delivered to the same area of lung resulting in the gradual worsening of central acinar emphysema. This scenario is by no means all-inclusive but does correspond with much of the data reviewed.

The development of emphysema in smokers is a slow and inhomogeneous process. It is likely that tissue injury occurs focally from the pathologic correlation of smoking with central acinar emphysema. As such, pathogenetic factors at the point of injury need to be measured directly. It is unlikely that such measurements are practical in vivo, as most samples of lavage fluid reflect areas of the lung with different degrees of damage. The use of cellular techniques, particularly the development of connective tissue matrices for in vitro use, seems a logical next step.

There is considerable evidence that protease-antiprotease imbalance in the lung contributes to the pathogenesis of emphysema. However, most of this evidence is circumstantial and provides evidence of "what can happen" rather than "what does happen" (144). The role of α1Pi in the pathogenesis of this disease is unclear and the equation of protease-antiprotease imbalance with decreased levels of α1Pi is an oversimplification.

Our knowledge of the molecular biology, genetics and biochemistry of α1Pi is far greater than our understanding of the role α1Pi might play in the pathogenesis of emphysema. The likelihood that purified α1Pi will soon be available for use in patients with emphysema is potentially both a triumph and a tragedy. The economic consequences of inappropriate use of α1Pi in patients with COPD could be staggering if this drug is aggressively marketed.

Conclusion

The study of emphysema has provided many new insights into mechanisms of lung injury. Many of these discoveries have importance far beyond their relevance to the pathogenesis of emphysema. It is likely that continued research in this field will provide clinically useful tools.

A sense of perspective must be maintained however. Unlike most major biomedical research efforts which seek to define the mechanisms of a disease in hope of providing a cure, knowledge already exists which, if properly utilized, would prevent emphysema. The cessation of cigarette smoking by the majority of smokers in this country would have a far greater impact on the morbidity and mortality of emphysema than any foreseeable scientific discovery. Continued efforts at educating the public about the dangers of smoking remains the major weapon against the development of emphysema.

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