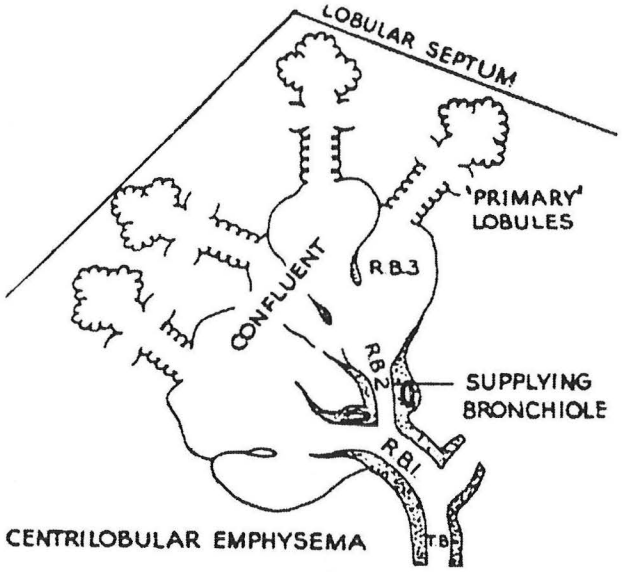
Respiratory Bronchiolitis-Emphysema:

A new name revives an old disease



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Respiratory Bronchiolitis and Emphysema

I. Introduction:

Approximately, 25% of Americans smoke cigarettes[1,2], the major risk factor for the development of emphysema[3,4,5]. In 1991 alone, 86,000 deaths were attributed to emphysema[6,7]. Nevertheless, less than 20% of smokers develop severe or incapacitating airflow obstruction[8,9]. Autopsy studies reveal that emphysema is found in up to 66% of adult patients[10,11]. In part, this gap is due to the fact that approximately 30% of lung parenchyma must be involved before the onset of symptoms or functional abnormalities[1,12].

The initial lesion of emphysema is still debatable[13]. Since its original description by Leopold and Gough in 1957[14], respiratory bronchiolitis (RB) or "smokers' bronchiolitis"[15] has been proposed as emphysema's initial lesion[13,16,17]. Bronchiolitis refers to inflammation of the bronchioles, which bridge bronchi and alveoli[15]. In respiratory bronchiolitis, an accumulation of atypical macrophages is associated with pathologic changes of inflammation, fibrosis, and distal airspace enlargement[13,14,16]. These changes precede the development of airflow limitation and clinical evidence of emphysema[6,16,17,18,19,20,21].

Respiratory bronchiolitis has gained increased recognition after recent descriptions of young smokers with interstitial lung disease in association with respiratory bronchiolitis, RB-ILD[22,23]. This Grand Round will discuss the pathogenesis of emphysema focusing on its initial lesion, respiratory bronchiolitis, thus the term Respiratory Bronchiolitis-Emphysema.

II. Clinical Case Presentation:

A recent clinicopathological exercise, published in the New England Journal of Medicine[24], presented the case of a 35 y/o woman with a 12-year history of cigarette smoking and a three-year history of dyspnea on exertion, sputum production, and weight loss. She had no significant past medical history. Medications included dexamethasone, ipratropium and pirbuterol inhalers, cromolyn, fluconazole, ranitidine, a leukotriene-receptor agonist, conjugated estrogens, and vitamin B12. Her family history was significant for COPD and cystic fibrosis in her father and cousin, respectively.

Physical examination revealed a temperature of 39°C, pulse of 80 bpm, and respiratory rate of 20. Coarse rhonchi and wheezing were heard in both lungs. A chest radiograph was normal. Further testing revealed a negative PPD and normal sweat chloride test, alpha-1 antitrypsin levels and immunoglobulins.

Pulmonary function tests revealed combined restrictive and obstructive defects (Table 1). A high-resolution CT scan of the chest revealed cyst-like areas of low attenuation. She was admitted for evaluation "cystic lung disease" presumed to be lymphangiomyomatosis. Lung transplantation was being considered.

Open lung biopsy revealed "stuffing of terminal and respiratory bronchioles and some alveoli with histiocytes, which contained large amounts of brown, finely granular intracytoplasmic pigment as a result of smoking...The findings are consistent with respiratory bronchiolitis...The dilated airspaces adjacent to the scarred small airways in this patient are consistent with the presence of emphysema."

Symptoms and lung function improved significantly with smoking cessation. This case presents symptomatic and functionally significant respiratory bronchiolitis in association with premature emphysema.

TABLE 1. RESULTS OF PULMONARY-FUNCTION TESTS AND ARTERIAL-BLOOD GAS MEASUREMENTS.

VARIABLE	12 DAYS BEFORE ADMISSION		ON ADMISSION	
	VALUE	% OF PREDICTED VALUE	VALUE	% OF PREDICTED VALUE
Vital capacity (liters)	1.43	39	3.27	84
Forced expiratory volume in 1 sec (liters)	0.78	25	2.30	70
Forced expiratory volume in 1 sec/vital capacity	0.55	65	0.70*	83
Peak expiratory flow (liters/sec)			4.75*	73
Flow at 50% of vital capacity (liters/sec)			2.03*	46
Peak inspiratory flow (liters/sec)			4.64*	102
Maximal breathing capacity (liters/min)			82*	71
Total lung capacity (liters)			5.66	103
Residual volume (liters)			2.39	134
Residual volume/total lung capacity			0.42	130
Airway resistance (cm of water/liter/sec)			1.27†	
Specific airway conductance $\left(\frac{1}{\text{cm of water} \cdot \text{sec}}\right)$			0.210‡	
Carbon monoxide diffusing capacity (ml/min/mm Hg)			14.9	59
Arterial oxygen saturation (%)§			99	
Arterial-blood gases				
Partial pressure of oxygen (mm Hg)§	71			
Partial pressure of carbon dioxide (mm Hg)	21			
pH	7.59			

III. The anatomy of the respiratory bronchiole:

Bronchiolar anatomy must be reviewed in order to understand its relationship to the development of emphysema. The lung has over 300,000 airways that branch through 23 generations[25]. The small airways of the lungs are those with a diameter less than 2-3 mm and are divided into small bronchi and bronchioles (1mm or less)[15]. The bronchioles can be distinguished from bronchi by the lack of supporting cartilage (Figure 2). The first branching bronchioles are the membranous bronchioles. The membranous bronchioles then branch 5-10 times and end in the lobular bronchiole[18], which are the feeding airway of the **secondary pulmonary lobule,** or lobule of Miller. This structure is the smallest discrete unit of lung that is bound by visible connective tissue septae, the interlobular septae (Figure 1). In the secondary lobule, the lobular bronchiole divides into three or more terminal bronchioles that feed an acinus. The terminal bronchiole further subdivides into two to five generations of respiratory bronchioles, alveolar ducts and alveoli[26]. Thus, the secondary pulmonary lobule may contain 3 to 24 acini.

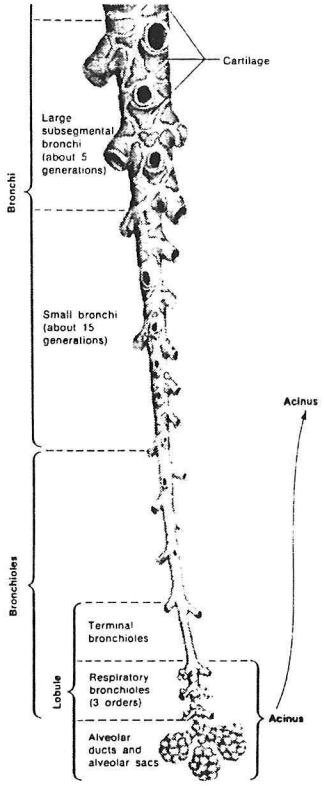


Figure 2: Anatomy of the bronchi and bronchioles. The CIBA Collection 7- Respiratory System. F. Netter

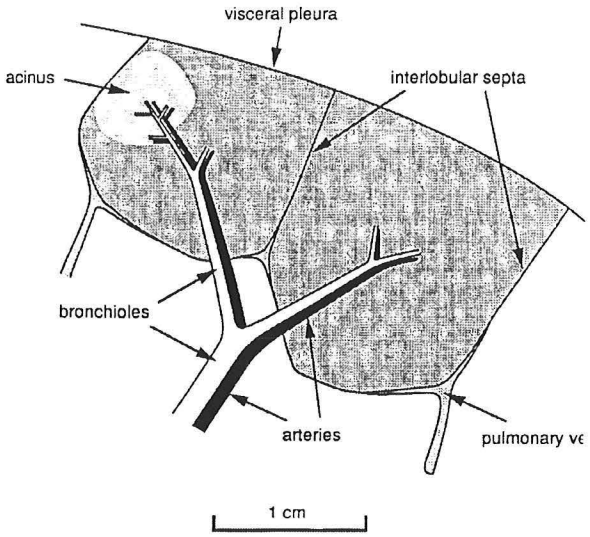


Figure 1: The secondary pulmonary lobule of Miller. From Webb WR, Muller NL, Naidich DP. High-Resolution CT of the Lung, Lippincott-Raven, 1996.

Since respiratory bronchioles contain sporadic alveoli in their walls, they serve both conducting and gas exchange functions. It is at this critical junction between respiratory bronchiole and alveoli where centrilobular emphysema develops [14,18].

IV. The pathology of emphysema: Centrilobular and panlobular.

Emphysema is a **pathologic** diagnosis and is defined as irreversible airspace enlargement distal to the terminal bronchiole with absence of gross fibrosis[10,27]. The hallmark of emphysema is the rupture of alveolar wall attachments, [10,30,28]. This leads to large communicating airspaces with reduced alveolo-capillary surface area for gas exchange. Emphysema is not a specific lesion for cigarette exposure and can be a secondary response to a variety of insults. Table 2 presents the pathologic classification of emphysema. For the purposes of this Talk, centrilobular and panlobular emphysema will be discussed.

Centrilobular emphysema (CLE) is characterized by selective airspace dilatation and disruption of the respiratory bronchioles and surrounding acini. The destruction is primarily located at the center of the pulmonary lobules. This emphysema is associated to extrinsic factors such as, cigarette smoking and occupational dust exposure (Figure 3) [29,30].

Table 2
Classification of pulmonary emphysema[30]

I. Related to extrinsic factors (centrilobular or centriacinar)
1. Centrilobular emphysema associated to smoking and environmental agents
II. Related to mainly intrinsic factors (panlobular or panacinar)
1. Emphysema of senescence
2. Emphysema with severe alpha$_1$ antitrypsin deficiency
III. Irregular ("scar") emphysema
IV. Paraseptal emphysema
V. Obstructive emphysema
VI. Compensatory emphysema
VII. Congenital lobar emphysema

Adapted from Anderson AE and Foraker AG. Pathology of Disruptive Pulmonary Emphysema. Charles C. Thomas, 1976; P. 5.

Panlobular emphysema is diffuse and involves all airspaces of the acinus and the secondary pulmonary lobule. This emphysema is primarily found in patients with alpha$_1$- antiprotease deficiency. Recent pathological studies of smokers with normal alpha$_1$ antitrypsin levels have demonstrated the presence of panlobular emphysema in association with centrilobular emphysema[31,32]. It remains to be proven if the panlobular involvement seen in smokers represents a progression of centrilobular emphysema with involvement of all acini.

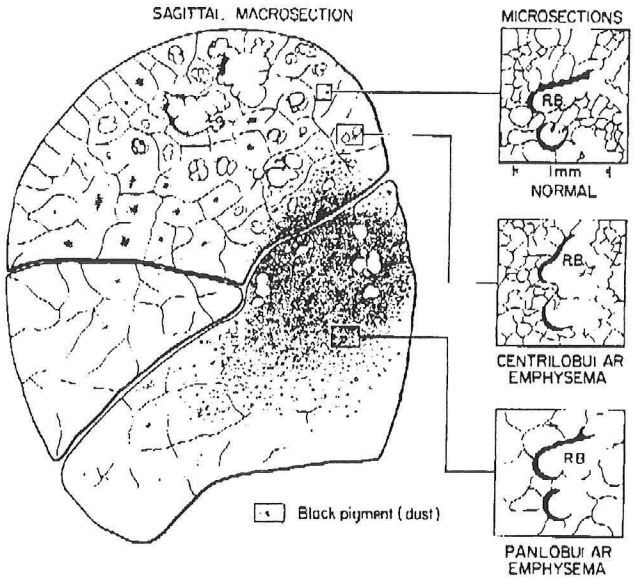


Figure 3: Diagram of gross appearance of centrilobular and panlobular patterns of emphysema.

V. Pathogenesis of emphysema: Historical Introduction

Different hypotheses have been proposed for the development of emphysema[33]. Before 1963, the two major hypotheses were based on mechanical and ischemic influences. Laennec and later, Mendelssohn (reviewed in reference 33) suggested that bronchial obstruction, caused by secretions or mucosal wall thickening, led to expiratory air trapping followed with subsequent alveolar dilatation and rupture of alveolar attachments. The ischemic hypothesis was based on observations of reduced capillary surface area in emphysematous lungs. It was hypothesized that air trapping with increase in alveolar pressure led to compression and thrombosis of capillaries causing alveolar wall ischemia and degeneration. Attempts at reproducing emphysema in animal models exposed to mechanical or ischemic stress were unsuccessful[34].

In 1963, Laurel and Ericksson reported the association of alpha$_1$-antitrypsin deficiency and emphysema[35]. With reinforcement by a multitude of animal studies[34,36], the elastase-antiprotease

theory became the focus of animal and human studies into the pathogenesis of emphysema. This hypothesis will be studied later in the Protocol.

VI. Pathogenesis of emphysema: Role of Respiratory Bronchiolitis

The great majority (>90%) of cigarette smokers who develop emphysema have normal alpha$_1$-antitrypsin levels[37]. The emphysema seen in cigarette smokers is usually centrilobular in location suggesting an extrinsic or inhalational injury, i.e. cigarette smoke[14,29]. The direct relationship between cigarette smoke and emphysema has been supported by numerous clinicopathologic and epidemiologic studies and summarized in various reviews[29,38,39,40,41].

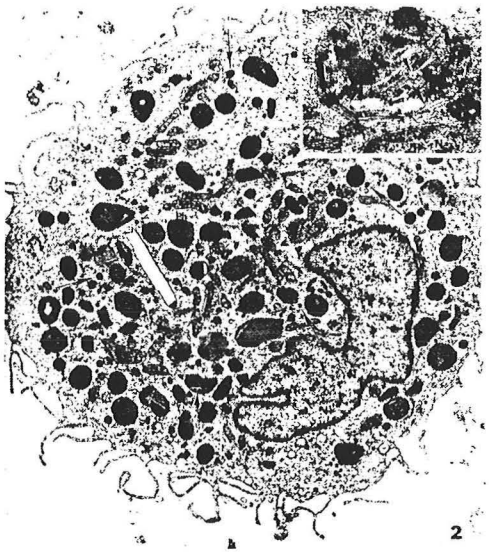


Figure 4: EM of alveolar macrophage of an asymptomatic smoker. Note crystalline, needle-like inclusions specific for cigarette smokers.

Less than 20% of smokers develop evidence of severe airflow limitation or symptoms. These patients usually have extensive emphysema[42]. In contrast, practically all cigarette smokers develop a respiratory bronchiolitis that is usually asymptomatic[16]. Respiratory bronchiolitis (RB) is characterized by an accumulation of pulmonary macrophages at the branching of respiratory bronchioles and alveolar ducts. These macrophages contain fine granular, brown-pigmented cytoplasmic inclusions, "cigarette inclusions", that are specific to smokers (Figure 4)[16,23]. It is associated with squamous metaplasia of the respiratory epithelium, chronic inflammatory infiltrate, and increased connective tissue deposits in the airway walls[14,17]. These findings have been reproduced in beagles and rodents exposed to chronic cigarette smoke[43,44].

Anatomical studies support the temporal and anatomical association of respiratory bronchiolitis with development of emphysema. These studies have been performed in autopsy cases or in patients undergoing lung resection for solitary pulmonary nodules or lung cancer. The initial description of the association between RB and emphysema was made by Leopold and Gough in 1957[14]. They studied 140 autopsy cases with "hypertrophic" emphysema. Seventy-five of these patients had centrilobular emphysema. Pathology specimens reveal the presence of inflammation and cellular exudate in respiratory bronchioles and alveolar ducts in association with proximal dilatation of airspaces and formation of emphysema (Figure 5).

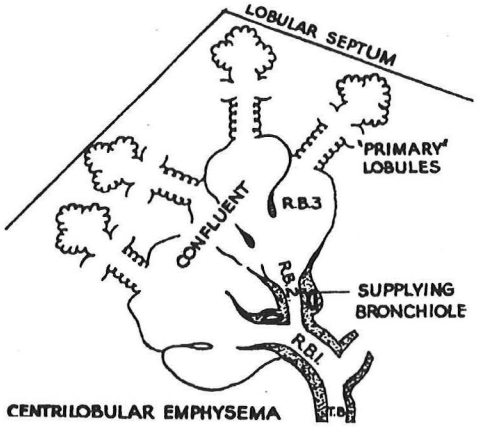


Figure 5: Initial airspace dilatation and destruction occurs in the distal respiratory bronchiole and adjacent alveoli (14).

For the next three decades, investigators focused on pathological correlations between the degree of small airway cellular inflammation and the presence of airflow

limitation and emphysema. These studies are summarized in Table 2.

Table 2:

Small airways disease, respiratory bronchiolitis, and emphysema:

Author (Year)	n=	Autopsy or Resection	Non-smokers (NS), Smokers-Healthy (S-H), Smokers-COPD (S-COPD)	Findings correlating (⇨⇨) with Emphysema or Airflow Limitation
Leopold (1957)[14]	140	Autopsy	Unknown	Small airway inflammation ⇨⇨ emphysema
Cosio (1977)[17]	36	Lobectomy	S-H, S-COPD	Membranous bronchiolar changes ⇨⇨ degree of emphysema and airflow limitation
Berend (1979)[19]	22	Lobectomy	S-H, S-COPD	Small airway narrowing ⇨⇨ airflow limitation
Cosio (1980)[45]	39	Autopsy	All groups	Severity of membranous and respiratory bronchiolitis ⇨⇨ degree of emphysema
Wright (1983)[46]	97	Lobectomy	All groups	**No correlations** between small airways ⇨⇨ emphysema and airflow limitation
Wright (1994)[47]	96	Lobectomy	S-H, S-COPD	Respiratory bronchiolitis ⇨⇨ airflow limitation (small airways)
Paré (1985)[48]	110	Lobectomy	S-H, S-COPD	Membranous and respiratory bronchiolitis ⇨⇨ airflow limitation
Matsuba (1987)[49]	37	Autopsy	S-H	Internal diameter of membranous and respiratory bronchioles ⇨⇨ FEV_1
Willems (1989)[50]	27	Lobectomy	All groups	Small airways disease in membranous and respiratory bronchioles ⇨⇨ degree of emphysema
Eidelman (1990)[51]	36	Both	All groups	Alveolar cellularity ⇨⇨ emphysema

These studies demonstrate the important link between inflammation of the small airways (membranous and respiratory bronchiolitis) and the extent of emphysema and airflow limitation. Respiratory bronchiolitis, the initial lesion observed in smokers, seems to be anatomically and temporally related to the presence of emphysema.

In "cartoon" format, Figure 6 highlights the progression from cigarette smoking to emphysema. Note the black boxes found along the way. The path from cigarette smoking to development of emphysema appears to initiate and possibly require small airway inflammation. The role of respiratory bronchiolitis in the pathogenesis of emphysema will be further discussed by focusing on:

A. Cigarette smoke and the pulmonary macrophage
B. Pulmonary macrophage: Friend or foe?
C. The elastase and antiprotease hypothesis
D. Respiratory bronchiolitis directing the airspace destruction

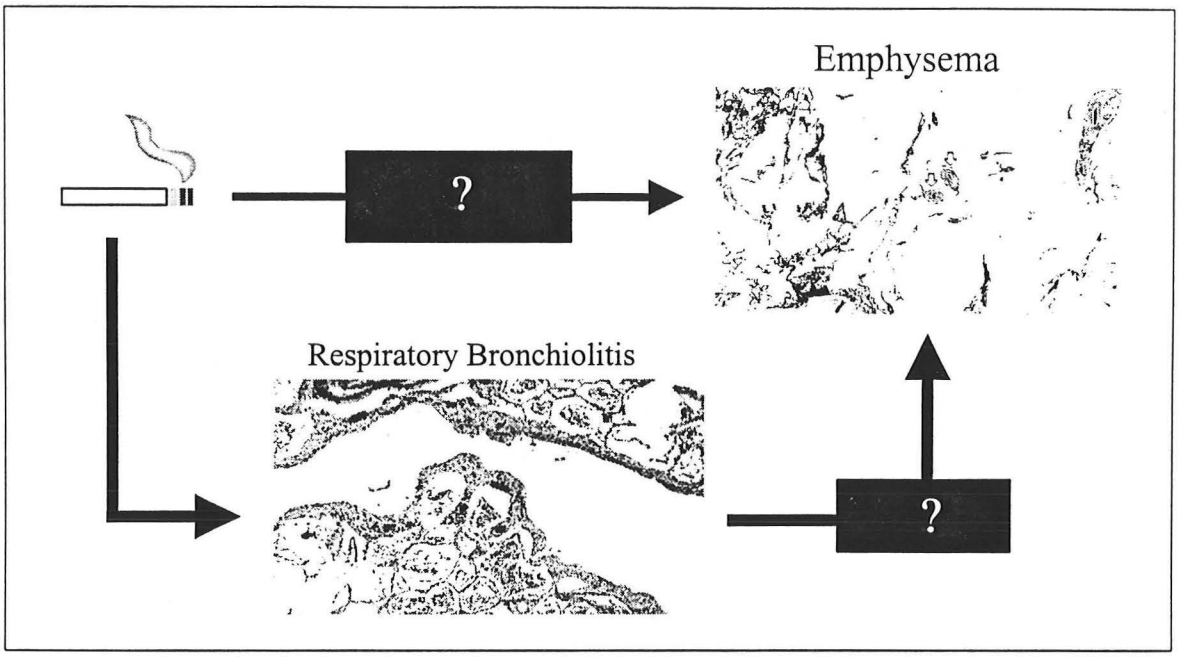


Figure 6: Diagram demonstrating possible pathways for the development of emphysema.

A. Cigarette smoke and the pulmonary macrophage:

Lung macrophages constitute > 90% of the cells recovered in bronchoalveolar lavage (BAL)[6]. They derive from circulating monocytes that originated in the bone marrow[52]. In humans, cigarette smoking leads to a four- to five- fold increase in pulmonary macrophages recovered by BAL[6,53]. These macrophages accumulate at the junction of terminal airways and alveoli where particulates from cigarette smoke come in contact with the respiratory epithelium. In response, pulmonary epithelial cells are known to secrete potent chemoattractants for pulmonary macrophages: RANTES, GM-CSF, and MCP-1 (macrophage chemotactic protein)[54].

The atypical macrophages seen in smokers have characteristic brown-pigmented cytoplasmic inclusions believed to be by-products of cigarette smoke. The structure of the particulate included in these cytoplasmic inclusions has eluded many researchers[13]. Brody et al. (1971) performed electron microscopy of pulmonary macrophages isolated from smokers. The "needle-shaped" inclusions seen by light microscopy were hexagonal-shaped crystalline structures. By x-ray spectrometry, the macrophage inclusions were found to contain **kaolinite**[55]. Kaolinite is aluminum silicate, a common component of clay in agricultural soils. It is postulated that the tobacco plants absorb this component from clay present in agricultural soils and deposit kaolinite in their leaves. The kaolinite is carried into the lung in tobacco smoke. To further prove that kaolinite was the major component of the macrophage inclusions, tobacco cigarettes from different brands were digested and submitted to x-ray spectrometry with detection of aluminum silicate. In addition, *in vitro* studies of isolated guinea pig pulmonary macrophages exposed to kaolinite develop the characteristic inclusions by electron microscopy[55]. Early studies of animals exposed to kaolinite have demonstrated development of pulmonary fibrosis, but not emphysema[56].

Could kaolinite represent the offending particulate in cigarette smoke that leads to macrophage recruitment and phagocytosis? Could an inefficient phagocytic response lead to release of injurious products extracellularly potentially degrading connective tissue and elastin? A parallel could be drawn to the fibrotic response seen in patients exposed to silica.

B. The pulmonary macrophage: Friend or Foe?

Pulmonary macrophages are important during lung injury and repair. Lung injury results in an inflammatory reaction led by macrophages and lymphocytes[57]. Lung repair is also mediated by the pulmonary macrophage through stimulation of fibroblast proliferation and collagen deposition resulting in pulmonary fibrosis. Their key role in these processes is mediated by the secretion of hundreds of different molecules including chemoattractants, chemokines, cytokines, arachidonic acid derivatives, growth factors, and reactive oxygen species[54]. Some important factors in the inflammatory and fibrotic response include IL-1, TNF-alpha, PDGF, epithelial growth factor/TGF-alpha, TGF-ß, and insulin-like growth factor [54,57,58].

Lung injury and repair have their price. The pulmonary macrophage has the potential to contribute directly and indirectly to lung damage and lung fibrosis[57]. Smoker's macrophages demonstrate a 4-5-fold increase in metabolism and are active in phagocytosis of bacteria and inhaled particles[59]. Besides these functional changes, recent investigators have demonstrated an altered phenotype in the pulmonary macrophages of smokers. These macrophages demonstrate an increase in membrane glycoproteins essential for cell adhesion and phagocytosis. Meanwhile, cell-surface proteins essential for antigen-presentation, CD11a (LFA-1) and its ligand CD54 (ICAM-1), are reduced[60].

This phagocytic role is accompanied by detrimental release of proteolytic substances. Secretion of oxygen radical species, hydrogen peroxide and superoxide anion during phagocytosis is increased in healthy smokers when compared to nonsmokers[58,61]. Indirectly, the macrophage of smokers increases the inflammatory response by influencing neutrophil influx into the lung. *In vitro* exposure of macrophages to smoke leads to secretion of potent chemoattractants contributing to an increase in neutrophils recovered from smoker's lungs[62]. The neutrophil contributes to further injury by secreting matrix-degrading substances and oxygen radical species[62].

This evidence suggests that the accumulation of atypical, pigmented, and activated macrophages in the respiratory bronchioles of smokers cannot be overlooked. These macrophages may be playing an essential role in the development of emphysema. A review of the elastase-antiprotease hypothesis focusing on the role of the macrophage will be explored next.

C) The elastase and antiprotease hypothesis:

As reviewed earlier, hypotheses on the pathogenesis of emphysema focused on mechanical or ischemic stress on airspaces[13,33,63]. Further pathologic studies of emphysema reported the

presence of thickened and degraded connective tissue of bronchioles and alveoli followed by loss of alveolar attachments and dilatation[14,16].

In 1963, the elastase-antiprotease hypothesis was born. Laurel and Eriksson[35] reported the absence of serum alpha$_1$ globulin in a number of patients with chronic bronchitis, bronchiectasis, and emphysema. The deficient protein was identified as alpha$_1$-antitrypsin. Because of its broad antiprotease activity, alpha$_1$-antitrypsin has been named alpha-1 protease inhibitor (API). Absence of antiprotease activity leads to unchecked elastin degradation with subsequent development of diffuse emphysema, identified as panacinar or panlobular[33]. Animal studies confirmed the importance of elastase in the development of emphysema by reproducing airspace dilatation and destruction with intratracheal administration of papain, an elastolytic enzyme from plants. This was later confirmed by the administration of purified human neutrophil elastase[64].

The emphysema seen in smokers cannot be explained by alpha$_1$-antitrypsin deficiency since greater than ninety percent have normal alpha$_1$-protease inhibitor levels[65]. Recent research has focused on the theoretical imbalance between proteases (elastase) and antiproteases altered by

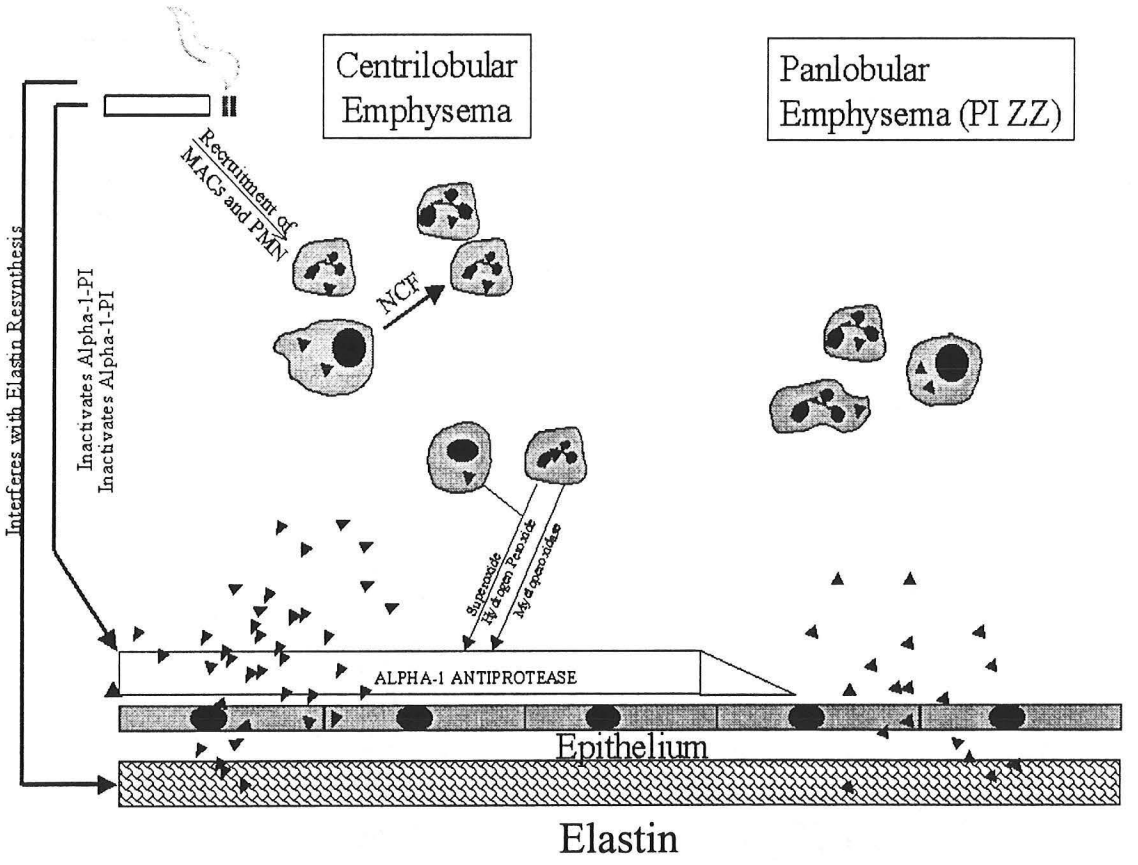


Figure 7: Summary of mechanisms involved in the pathogenesis of emphysema (centrilobular and panlobular) as suggested by the Protease-Antiprotease hypothesis. Adapted from Luisetti et al. Respiration 1992; 59(suppl 1): Page 25.

cigarette smoke[63]. Evidence to support the elastase-antiprotease hypothesis in smokers is extensive and best summarized by dividing into 1) effects of cigarette smoke, 2) role of recruited PMNs and pulmonary macrophages, and 3) detection of elastin degradation. These mechanisms are summarized in Figure 7.

1) Cigarette smoke has been demonstrated to inhibit by 50% the activity of alpha$_1$-protease inhibitor (API) [62]. Therefore, investigators have introduced the concept of an "acquired" API deficiency in smokers [63]. Cigarette smoke also directs the influx of neutrophils and pulmonary macrophages into the lung with subsequent release of elastase, serine proteinase, and superoxide radicals[66]. Finally, cigarette smoke delays the repair of degraded elastin by inhibiting lysyl oxidase, an enzyme essential in cross-linking[63].

2) Controversy exists between which cell, the neutrophil or the macrophage, contributes the most proteolytic (elastase) activity. As reviewed earlier, the macrophage is attracted by smoke particles to release potent chemoattractants for neutrophils. The neutrophil was originally thought to be the essential proteolytic cell in the lung. First, it has a short life span in the lung and at the time of cell death, bursts with release of highly concentrated neutrophil elastase, a serine proteinase[62]. Second, the instillation of purified neutrophil elastase (NE) in animal models has led to the development of elastin degradation and emphysema[64].

Macrophages are not innocent bystanders. They internalize neutrophil elastase for later secretion[67,68] and secrete macrophage elastase (MME), a metalloenzyme **not** inhibited by the alpha-1 protease inhibitor (API). In fact, MME actually inactivates API[69,70]. The pulmonary macrophages of smokers demonstrate increased secretion of MME[71]. Interestingly, the alveolar macrophages recovered from smokers with COPD have higher macrophage elastase secretion than healthy smokers[72]. Further studies have demonstrated that macrophages release other important metalloproteinases with elastolytic activity, such as gelatinases[73].

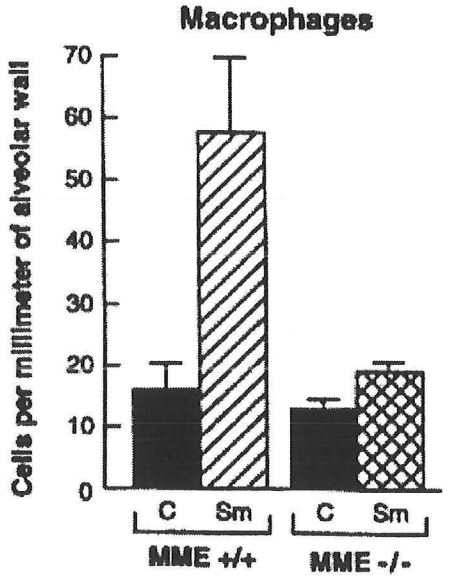


Figure 8: Pulmonary macrophage accumulation in the lung depends on macrophage elastase. From Hautamaki RD. Science 1997 (Ref. 74).

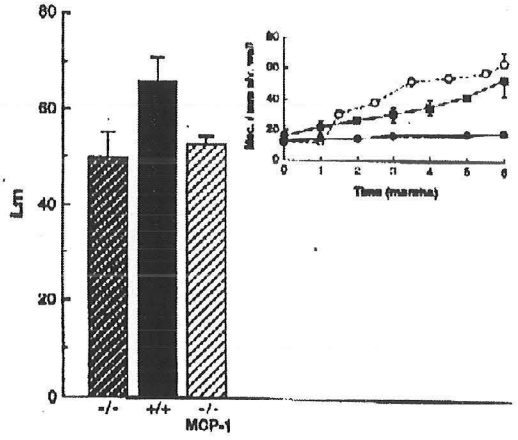


Figure 9: Intratracheal instillation of MCP-1, chemotactic agent for macrophage, leads to macrophage influx (insert) but no emphysema formation. Lm is the mean linear intercept between opposing walls of alveoli, a morphometric measure of emphysema. Hautamaki RD. Science 1997 (Ref. 74).

An important study demonstrating the macrophage role in the pathogenesis of emphysema was recently published in *Science*[74]. This study utilized transgenic knockout mice with absent macrophage elastase (MME-/-). These macrophage-deficient mice (MME-/-) and their wild-type littermates (MME+/+) were exposed to cigarette smoke for 6 months. The MME deficient mice did not accumulate macrophages in the lung or develop emphysema (Figure 8). In contrast, the wild-type mice with active MME did both. Presence of macrophage elastase rather than the macrophage accumulation seemed to be the essential component in the development of emphysema. This was demonstrated by the absence of emphysema formation in MME deficient mice after the induction of macrophage influx with intratracheal instillation of macrophage chemotactic factor-1 (Figure 9). This model supports the elastase-antiprotease hypothesis and stresses the essential role of macrophage elastase in the development of emphysema.

3) Further proof of the importance of elastase-antiprotease balance in smokers comes from evidence of increased elastin degradation[6]. Plasma and urine levels of elastin peptides are higher in smokers with COPD than in healthy smokers, who in turn have higher levels than nonsmokers[75,76]. Sensitive detection methods that measure urinary levels of desmosine, an aminoacid product of elastin degradation, confirm that there is active and increased elastin degradation in smokers, particularly those with airflow obstruction[6].

The above evidence supports the elastase-antiprotease imbalance hypothesis as the current leading hypothesis explaining the pathogenesis of emphysema in susceptible smokers. The respiratory bronchiolitis is primarily composed of macrophage influx and inflammation. Secretion of macrophage elastase by these macrophages could directly alter the elastase-antiprotease balance at a key anatomical location for the development of centrilobular emphysema.

D. Respiratory bronchiolitis directing the airspace destruction:

The evidence delineated above suggests a destructive role for the **respiratory bronchiolitis** in smokers. Review of the literature and scientific evidence demonstrates that the respiratory bronchiolitis is the initial lesion for emphysema[14,16]. It can be speculated that the respiratory bronchiolitis initializes and sustains the necessary cellular and molecular inflammatory background for the development of emphysema in susceptible smokers.

Cigarette smoke directs the macrophage to the respiratory bronchiole and adjacent alveoli, the active site for the development of early centrilobular emphysema[14,16]. These macrophages are atypical with kaolinite (aluminum silicate) inclusions likely persisting due to inadequate phagocytosis and clearance[55]. The macrophages express a phenotype consistent with phagocytic function[60] leading to secretion of proteolytic substances that degrade the extracellular matrix and elastin. As documented above, chemotaxis of macrophages and development of emphysema seems to be dependent on macrophage elastase[74]. The macrophage also secretes chemoattractants for neutrophils leading to further inflammation response and proteolysis. This excess of proteolytic activity could overwhelm the antiprotease system leading to digestion and weakening of alveolar structures with loss of alveolar attachments[50].

VI. Smoker's susceptibility in relation to respiratory bronchiolitis:

Which factors may be leading to the development of emphysema in smokers? Genetic and environmental influences have been the focus of recent reviews[42,77,78]. Some risk factors for smokers' susceptibility to COPD include male gender, amount and duration of smoking, caucasian race, first-degree relatives with COPD, low socioeconomic status, atopy, childhood infections, and occupational dust exposure[77].

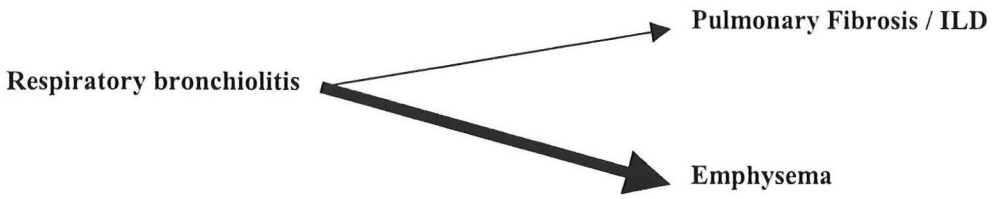
If respiratory bronchiolitis is found in the majority of smokers, then why do most smokers (>70%) not develop significant emphysema or airway obstruction? Studies have demonstrated that symptoms of emphysema and airflow limitation may be more common than previously believed[79,80,81]. Early emphysema and small airway disease are underestimated by our available insensitive techniques, such as chest radiographs and pulmonary function tests (PFTs)[16,81].

The role of respiratory and membranous bronchiolitis in association with smokers' susceptibility has focused on correlating the **severity** of small airway inflammation with the presence of alveolar destruction and/or degree of airflow limitation and emphysema (Table 2). Two studies compared the severity of small airway inflammation between resistant and susceptible smokers. Hale (1984) demonstrated that susceptible smokers had the same type of small airway abnormalities than resistant smokers. The major difference was in the extent of small airways involvement [82]. Bosken (1990) evaluated 200 different airways (90% membranous bronchioles) of resistant and susceptible smokers. Susceptible smokers had statistically significant narrower and inflamed airways with muscular, connective tissue, and epithelial hypertrophy. These and other studies have led to the speculation that individual host differences in the inflammatory response and connective deposition of the small airways, including respiratory bronchioles, may be an important factor in the development of chronic airflow obstruction[83].

VI. The clinical presentation of respiratory bronchiolitis:

Increased interest in respiratory bronchiolitis has been sparked by recent reports of a rare but fascinating clinical presentation of respiratory bronchiolitis with clinical features of interstitial lung disease (RB-ILD)[22,23,85,86]. These patients are young smokers with dyspnea and cough, restrictive pulmonary function tests, low DLCO, and hypoxemia. Open lung biopsies have demonstrated the accumulation of brown-pigmented macrophages in respiratory bronchioles and proximal alveoli with accompanied interstitial fibrosis. Some manifest premature emphysema, but this is not believed to be causing symptoms. This presentation appears to be distinct and less prevalent than the previously described asymptomatic respiratory bronchiolitis of smokers.

Respiratory bronchiolitis appears to progress through two distinct clinical pathways, fibrosis and emphysema, as diagrammed below. The difference in arrow weights suggests the different prevalence for both syndromes. For the purpose of this Talk, RB-ILD will be discussed first, followed by the evaluation, diagnosis, and treatment of RB-emphysema.



A- Respiratory Bronchiolitis- Interstitial Lung Disease:

The initial description of RB-ILD was reported by Myers in 1987. He described six young smokers with progressive respiratory deterioration accompanied by restrictive PFTs, decreased DLCO, and hypoxemia[22]. This original description was followed by a report by Yousem (1989) of 18 smokers with similar clinical presentation and biopsy-proven RB-ILD[23].

The patients are usually young in presentation with age ranging from 22-53 y/o. It is a disease exclusively of smokers. No specific sex or ethnic predilection has been described. The reported amount of cigarettes consumed ranged from 7-75 pack-years. Chronic symptoms consist of dyspnea (67%), sputum production (44%), cough (50%), and chest pain persisting for approximately five years prior to presentation. Rare asymptomatic patients have been reported in each series.

The physical examination can reveal late inspiratory bibasilar crackles, coarser than the "velcro-like" crackles of idiopathic pulmonary fibrosis. Laboratory data is unremarkable except for some cases with hypoxemia at rest or with exercise[87]. The pulmonary function tests can present with normal flows or more commonly, demonstrate a mixed obstructive and restrictive pattern with characteristic low DLCO. An interesting feature reported in the literature is a case of respiratory bronchiolitis with dramatic reversal of a restrictive and obstructive pattern after bronchodilator administration. This phenomenon called "reversible restriction" is believed to be secondary to bronchodilation of small airways and alveolar ducts[88].

The chest radiographs can be normal or present with bilateral and diffuse reticulonodular infiltrates. High-resolution CT scan findings range from normal to ground-glass opacification, linear opacities, atelectasis, and even emphysema[86,89].

The diagnosis should be suspected in a young smoker with unexplained dyspnea, cough, significant restrictive or mixed physiology, and low DLCO. Open lung biopsy specimens reveal an inflammatory lesion composed of mononuclear cells in the submucosa of membranous and respiratory bronchioles (Figure 10). The key feature that distinguishes this disease

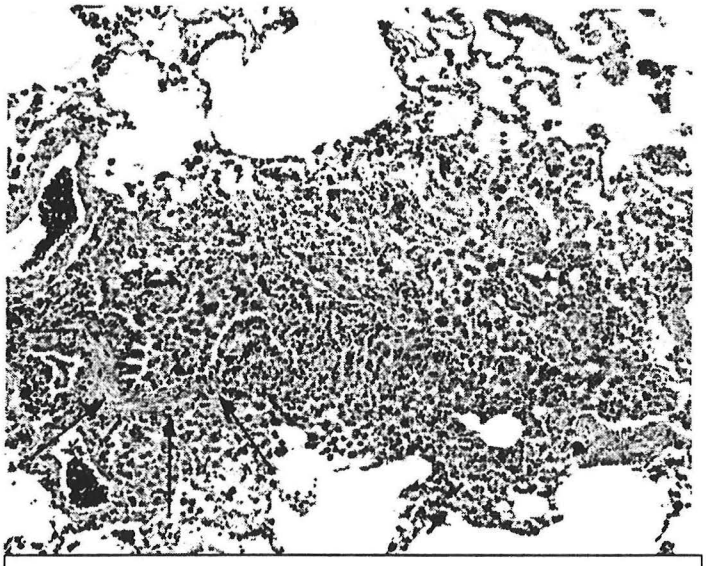


Figure 10: RB-ILD pathology demonstrating a respiratory bronchiole obliterated by macrophages and involving adjacent alveolar structures. From Reference 22.

from the RB of smokers is the predominance of associated fibrosis extending in "stellate fashion." from the respiratory bronchioles to the surrounding alveolar walls [85].

The small number of reported patients makes generalizations on treatment options impossible. Yet, most patients appear to respond to smoking cessation. Anecdotal response to corticosteroids has been published[23].

B- Respiratory Bronchiolitis-Emphysema:

1) Epidemiology:

Cigarette use has declined in the recent years (Figure 11)[90]. However, 25.7 % of the general population over the age of twenty smoke cigarettes[6,90]. In the U.S., an estimated 10 million people suffer from emphysema and COPD. An estimated 85,944 deaths were reported in 1991[77].

In 1974, Niewoehner[16] demonstrated the prevalence of respiratory bronchiolitis in virtually all smokers studied. Yet, most smokers (>70%), do **not** develop incapacitation or death from emphysema. Autopsy studies of smokers dying non-pulmonary deaths reveal the presence of emphysema in approximately 40-62% of patients[13,17,79,80]. Thus, the prevalence of respiratory bronchiolitis in association with clinically silent emphysema may be this high.

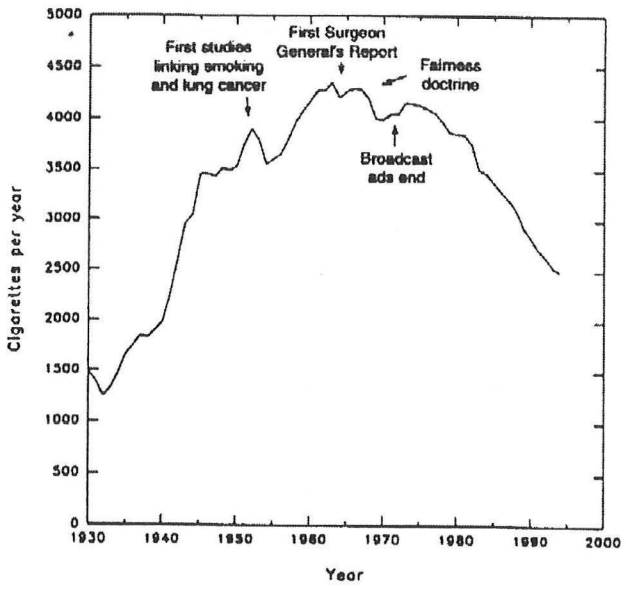


Figure 11: Historical plot of cigarette consumption. Adapted from reference 90

2) Clinical presentation:

Symptoms: Most smokers demonstrate an inflammation of the respiratory bronchioles consistent with respiratory bronchiolitis[16,45]. Since these studies were performed in autopsies of young victims of "sudden death", little is known about the symptoms and signs associated with this respiratory bronchiolitis. When smokers with normal PFTs are specifically asked about pulmonary symptoms, they report significantly more complaints than age-matched, non-smoking controls[40,41,81]. In one study, smokers had significantly more complaints of morning cough (30% vs. 6%), all-day cough (14% vs. 6%), sputum production (23% vs. 4%), dyspnea (41% vs. 16%), and wheezing (33% vs. 6%) than nonsmokers. The presence of these symptoms correlate with increased bronchiolar abnormalities and emphysematous changes detected with high-resolution chest CT scan[81]. It remains to be proven whether these symptoms are directly related to the presence of respiratory bronchiolitis.

Signs: A study correlating the physical examination to the presence of pathology-proven respiratory bronchiolitis in conjunction with premature emphysema has not been done. A normal lung examination is expected in the absence of airflow limitation.

Chest Radiograph: The term "dirty lungs" or "smokers lungs" refer to the well-described chest x-ray abnormalities of smokers. These abnormalities consist of subtle linear and nodular densities seen diffusely in both lungs. In 1971, a study revealed that two radiologists unaware of the smoking history of the female patients studied, could recognize heavy smokers from nonsmokers by identifying these radiographic abnormalities[92]. In autopsy studies, chronic cigarette use has been associated with an increased incidence of non-specific and diffuse pulmonary fibrosis that is suggested to be the cause of these linear and nodular densities. Radiographic and pathologic correlation studies are not available[95].

3) The diagnosis of RB-emphysema:

Conventional CT and High-resolution CT scans:

The conventional chest CT scan has increased sensitivity in the detection of emphysema over the chest radiograph (96% vs. 68%, respectively). Its role in the examination of small, peripheral airway inflammation in smokers has not been evaluated. This is best suited for the high-resolution CT scan (HRCT), a technique that has revolutionized the study of bronchiolar and interstitial lung diseases. It identifies abnormalities within the secondary pulmonary lobule. The resolution of the HRCT allows detection of structures as small as 0.5 mm in size. The respiratory bronchioles, at 0.3-0.5mm, are below the HRCT sensitivity[86]. Nevertheless, inflammation of the structures of the pulmonary lobule can be detected by this technique[96,97,98].

In 1993, two important studies performed by Remy-Martin et al.[81] support the role of HRCT in detection of symptomatic respiratory bronchiolitis. The first study included 175 age and sex-matched volunteers (98 smokers, 26 ex-smokers, and 51 non-smokers) with absent or minimal pulmonary complaints. These patients were younger than 43 years of age and had mild to absent airflow limitation by PFTs. Careful questionnaire, chest radiograph, PFTs, and HRCT scans were performed. When compared to non-smokers, smokers and ex-smokers reported significantly more respiratory symptoms and had more parenchymal abnormalities by HRCT scan (Table 3). Symptoms of dyspnea, wheezing, AM cough and sputum production correlated with the presence of abnormal HRCT findings such as, emphysema, parenchymal micronodules, ground glass opacification, and bronchial wall thickening[81].

Table 3: HRCT Findings

Finding	Smokers (n=98)	Ex-smokers (n=26)	Nonsmokers (n-51)	*P* Value
Parenchymal micronodules	26 (27%)	1 (4%)	0	< .001
Areas of ground-glass Attenuation	20 (21%)	1 (4%)	0	.001
Emphysema	20 (21%)	2 (8%)	0	< .001
Bronchial wall thickening	32 (33%)	4 (16%)	9 (18%)	.06
Dependent areas of Attenuation	33 (34%)	11 (43%)	6 (12%)	.003
Septal lines	6 (6%)	2 (8%)	4 (8%)	...
Subpleural micronodules	37 (38%)	10 (39%)	11 (22%)	.11

From Reference 81.

The second investigation by the same group is the only study available correlating the HRCT abnormalities directly to pathology specimens[99]. The study population consisted of 41 heavy smokers (>20 pack years) scheduled for resection of a solitary pulmonary nodule. Approximately 40% of the patients complained pre-operatively of respiratory symptoms and had FEV_1 ranging from 60% to 100%. HRCT scan was performed before surgery and the resected lung underwent targeted HRCT. The following observations were made:

1) Ground-glass opacification correlated with pathology demonstrating accumulation of pigmented macrophages and/or mucus, fibrosis, and inflammation in the alveolus.
2) Parenchymal micronodules correlated with bronchiolectasis and peribronchiolar fibrosis. These nodules were seen predominantly in the upper lobes at sites of centrilobular emphysema. They are believed to represent cigarette-induced bronchiolar wall abnormalities, consistent with respiratory bronchiolitis[99].

In conclusion, HRCT detects significant number of abnormalities in smokers with pathology suggesting the presence of small airway disease, alveolar exudate, and emphysema. HRCT scanning could serve as a tool in identifying patients with significant small airways disease and premature emphysema.

Pulmonary Function Tests:

Can we detect the presence of respiratory bronchiolitis and early emphysema (RB-emphysema) with pulmonary function testing? Increases in small airway resistance are usually not detected by routine pulmonary function testing until significant airflow limitation develops. Thirty percent of the pulmonary parenchyma has to be involved with emphysema before development of functional abnormalities[100]. Standard PFTs, forced spirometry (FEV1, FVC, FEF 25-75%)

and measurement of diffusion capacity (DLCO), are insensitive to early changes in small airway resistance.

A large area of research has focused on the anatomical location of airflow limitation in both normal and emphysematous lung. Early work performed by Hogg[21] successfully measured total peripheral airway resistance by wedging a peripheral catheter in the small airways (2-3 mm) of autopsied or resected lungs from patients with advanced COPD. In normal lung, the peripheral airways resistance (R*p)* contributed only to about one-quarter of the total airway resistance R_L (Figure 11, Graph A). In contrast, patients with COPD, peripheral resistance (R*p)* was the major determinant of total airway resistance (Figure 11, Graph B).

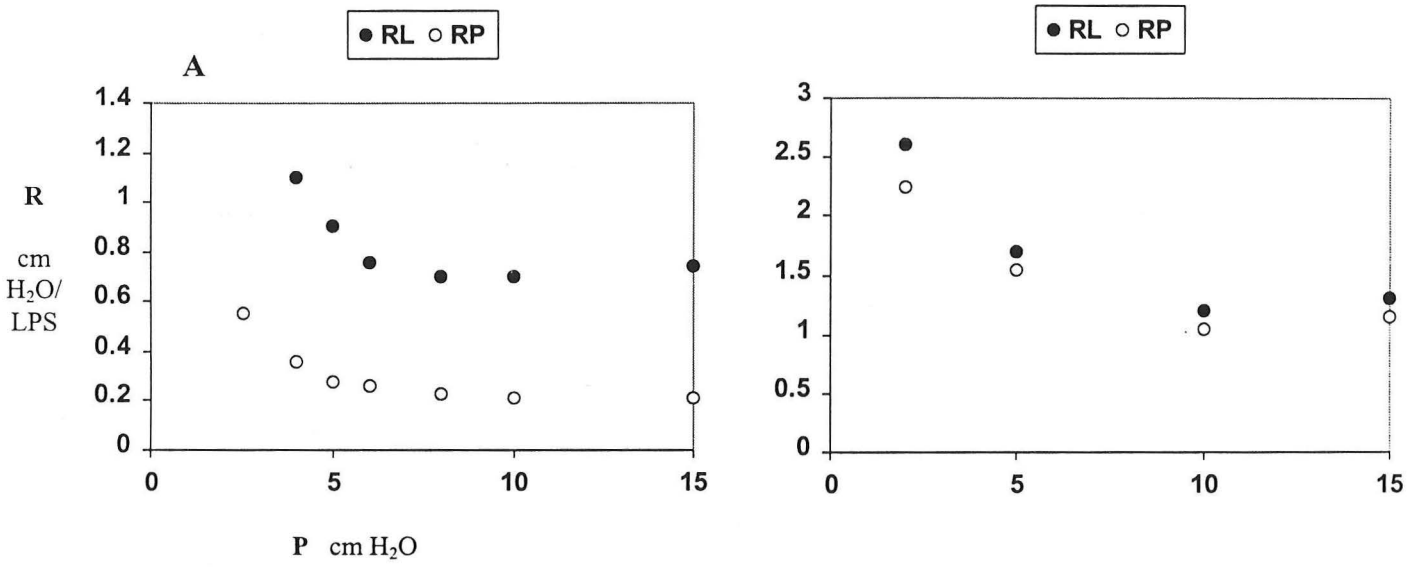


Figure 12: Retrograde catheter data of a normal patient (Graph A) and a patient with centrilobular emphysema (Graph B). Note that in the patient with emphysema (Graph B) there is an increase in total airway resistance, R*L* (dark circles) largely related to an increase in peripheral airway resistance, R*p* (open circles). Adapted from reference 21.

Niewoehner[101] found that measuring frequency-dependent dynamic compliance (CDYN) and maximal expiratory flow at different lung volumes could detect changes in small airway resistance. Increased small airway resistance correlated with the diameter of the membranous bronchioles. Cosio[17] demonstrated that patients with early bronchiolitis had an abnormal slope of phase III of the nitrogen washout curve (Figure 13) and volume at isoflow of helium and oxygen, both measures of small airway resistance. These test are sensitive enough to allow separation of normal patients with uninvolved bronchioles from patients with injured and inflamed small airways yet normal FEV_1 and FVC. Finally, Wright[47] demonstrated the detection of membranous and respiratory bronchiolitis by three different specialized tests for small airway disease.

These studies strengthen the concept of early detection of respiratory bronchiolitis before the detection of emphysema and reduced FEV_1. These tests could be utilized to identify susceptible smokers before the development of severe emphysema.

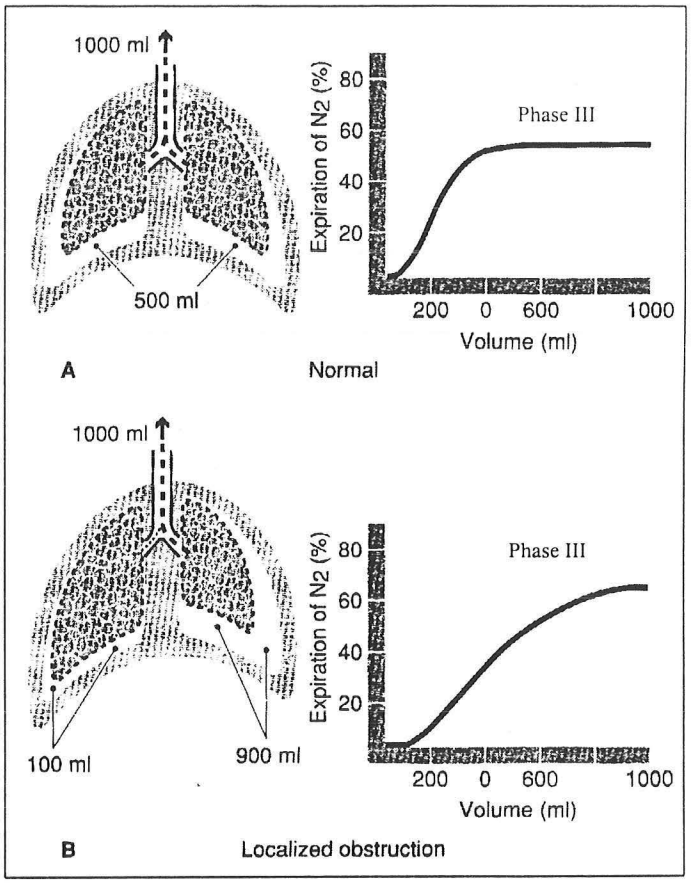


Figure 13: Small airway obstruction leads to inhomogeneity of gas mixing and upward slope of Phase III of the nitrogen-washout test. From Cherniack R: Pulmonary Function Testing. Philadelphia, W.B. Saunders Co., 1992.

4) Pathology of RB- Emphysema:

Most of the data available on the pathology of respiratory bronchiolitis and early emphysema is derived from autopsy studies and lobectomy specimens. This is due to the fact that smokers with respiratory bronchiolitis rarely require tissue diagnosis and when symptoms of airflow limitation develop, emphysema is usually diagnosed.

Autopsy studies reveal that young smokers have statistically significant abnormalities in the small airways and respiratory bronchioles (Figure 14)[16,103]. When compared to age-matched nonsmokers, the features consistently seen in the peripheral airways of young, asymptomatic smokers are:

In membranous bronchioles,

1) Denuded epithelium
2) Mural inflammatory cells

In respiratory bronchiolitis,

1) Luminal brown-pigmented macrophages
2) Mural edema
3) Peribronchiolar fibrosis
4) Epithelial cell hyperplasia

Studies performed on lobectomy specimens reveal that patients can be divided into groups by severity of inflammation, connective tissue proliferation, and muscular hypertrophy. The different stages of inflammation correlated with

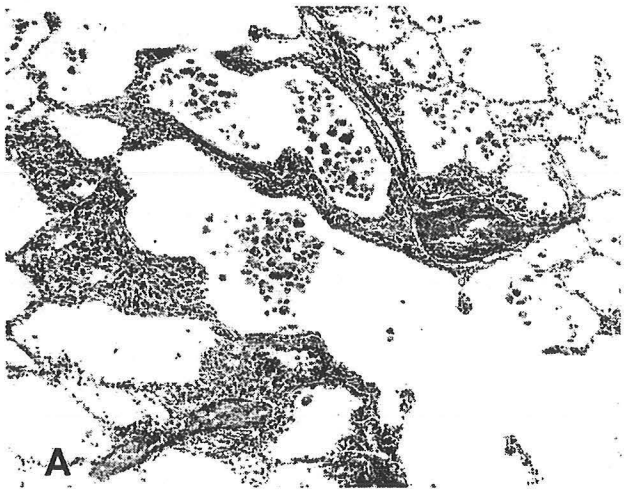


Figure 14: Autopsied lung of a 24-year-old smoker demonstrating peribronchiolar inflammation and fibrosis, intraluminal pigmented macrophages, and respiratory bronchiolitis. From reference 103.

deterioration in airflow limitation of the small airways measured by closing volume, slope of phase III of the nitrogen washout curve, and volume at isoflow of helium and oxygen[17].

5) Treatment and prevention of RB-emphysema:

The evidence supporting the role of respiratory bronchiolitis as the initial lesion of emphysema has been introduced. The name RB-emphysema introduces the concept of an early lesion that has the potential of reversibility if smoking cessation or novel forms of therapy are instituted. Very few therapeutic options are available for the prevention and treatment of respiratory bronchiolitis and early emphysema. Experimental therapy has focused on the development of elastase inhibitors.

Conventional therapy: Conventional therapy is mainly focused on smoking cessation. Physicians and patients are aware of the difficulties encountered in achieving this goal. Nicotine is highly addictive and associated with physical and psychological dependence. Thus, less than 30% of smokers quit before the age of sixty[104]. The study of nicotine addiction and smoking cessation is reviewed elsewhere.

Because of unavailable lung tissue, pathological studies documenting reversal of the bronchiolitis with smoking cessation are lacking. Some studies have indirectly measured reversibility of small airway disease with smoking cessation by detecting changes in small airway obstruction with the use of specialized pulmonary function tests[105,106]. Indirectly, Rennard (1990) utilized bronchoalveolar lavage (BAL) in smokers to evaluate for bronchial and alveolar inflammation. Fifteen healthy but heavy smokers were submitted to BAL before and after two months of smoking reduction from an average of 50 cigarettes per day to 20 per day. These patients were compared to nonsmoking controls but not to a control group consisting of heavy smokers without reduction in smoking habit. The number of recovered neutrophils and macrophages and levels of neutrophil elastase in BAL were significantly reduced after smoking reduction[104].

Experimental therapy: The Elastase-Antiprotease hypothesis proposes that smokers have a functional deficiency of antiprotease activity. Most of the work with early emphysema has focused on detection of susceptible smokers and consideration of replacement of antiprotease activity. This has been a topic of a recent review[6].

Research has concentrated on animal models of emphysema with administration of **elastase inhibitors**. One of these inhibitors, BOROVAL (methoxy succinyl-L-alanyl-L-alanyl-L-prolyl-ambo-boro-val pinacol) was tested in a hamster model of emphysema. Intratracheal administration of BOROVAL before and at the time of intratracheal administration of neutrophil elastase led to no improvement and even, paradoxical worsening of emphysema[107]. It is postulated that these inhibitors may actually bind and transport elastase to the interstitium followed by complex dissociation and activation. Similar results were obtained with a second inhibitor, PCI[6]. Studies in humans have been suggested[6].

Recent interest has sparked with the potential use of **retinoic acid** for prevention and treatment of early emphysema. Massaro[108] administered retinoic acid to postnatal rats demonstrating an

increase in the number of alveoli in postnatal rats after administration of retinoic acid. This group also studied the effects of retinoic acid in a rat model of experimental emphysema induced by elastase administration. The retinoic acid-treated rats had reversal of airspace dilatation and actually demonstrated an increase in the number of alveoli. This is one of the first studies demonstrating the "plasticity" of adult lung with neogenesis of alveolar structures. It is believed that retinoic acid induces regression to gene expression seen during early lung development[110]. During a recent visit to our Medical Center, Dr. Massaro communicated the interest of the National Institutes of Health in initiating a study with retinoic acid as a potential treatment of emphysema. The role of retinoic acid on respiratory bronchiolitis is unknown.

VI- Conclusions and Future Direction:

Respiratory Bronchiolitis-Emphysema is proposed as a new name for the emphysema associated with cigarette smoking, predominantly centrilobular emphysema. This name suggests the early identification of a potentially reversible lesion in young smokers. This lesion perhaps is the most common histological lesion of human lung. The evidence delineated above suggests a destructive role for the **respiratory bronchiolitis** in smokers. This evidence also suggests that the respiratory bronchiolitis provides a source of inflammation primarily consisting of alveolar macrophages, a cell known to secrete macrophage elastase and other proteases known to be important in the development of emphysema.

In susceptible smokers, a natural progression from a respiratory bronchiolitis to centrilobular emphysema can be proposed. Notwithstanding, we must answer the following question. Is RB pathogenic of emphysema or just a marker of smoking?

This question addresses the concept of smoking susceptibility. Clearly, multiple genetic, environmental, and unaccounted factors lead to the development of a susceptible smoker. Respiratory bronchiolitis is present in both susceptible and resistant smokers thus suggesting that it is not the presence of the bronchiolitis that makes the smoker susceptible. Nevertheless, is it the background of bronchiolar inflammation in conjunction with genetic, intrinsic, and environmental influences directly contributing to the development of emphysema? Could inhibition of the development of small airway inflammation protect even the susceptible smoker?

Evidence suggests that early small airway disease can be detected by pulmonary function testing and sensitive radiological techniques. If this lesion could be modulated, or better yet, prevented, could the role played by genetic, environmental, host factors, etc. be lessened? Or, could a patient faced with the fact that there is direct cigarette smoking damage to his or her lung work harder towards smoking cessation?

Many of you may be thinking that the argument over therapeutic options is irrelevant. That emphysema could be prevented by one simple act, smoking cessation. Many, including myself, believe that human nature is such that many smoking will continue forever. Even today, human beings continue to rationalize its use despite insurmountable evidence demonstrating its harm. Yet, there is hope that this self-inflicted damage will continue to be studied, understood and prevented.

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