Bringing Light to Chronic Obstructive Pulmonary Disease Pathogenesis and Resilience

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Abstract

The pathogenesis of chronic obstructive pulmonary disease remains elusive; investigators in the field have struggled to decipher the cellular and molecular processes underlying chronic bronchitis and emphysema. Studies in the past 20 years have underscored that the tissue destruction, notably in emphysema, involves a multitude of injurious stresses, with progressive engagement of endogenous destructive processes triggered by

decades of exposure to cigarette smoke and/or pollutants. These lead to an aged lung, with evidence of macromolecular damage that is unlikely to repair. Here we discuss these key pathogenetic elements in the context of organismal evolution as this concept may best capture the challenges facing chronic obstructive pulmonary disease.

Keywords: cigarette smoke; aging; senescence; stress; oxidative stress

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Resilience 1: the capability of a strained body to recover its size and shape after deformation caused especially by compressive stress. 2: an ability to recover from or adjust easily to misfortune or change. (Merriam-Webster 2011 edition. Available from: <https://www.merriam-webster.com> [accessed 2018 Aug 21]).

The understanding of the pathogenesis of the chronic obstructive pulmonary diseases (COPDs) has continuously evolved for the past six decades. This progression is particularly evident regarding emphysema (1, 2). The recognized progressive enlargement of alveolar spaces (unaccompanied by fibrosis as defined in 1989 [3]) owing to irreversible loss of tissue represents a paradox for investigators in the field. The present author recently reviewed key structural parameters of the normal human lung and those that characterize small airways in COPD and alveolar tissue in emphysema (4) (Table 1). In summary, the lung volume increases by almost 30% in

emphysema, with a reduction of up to 50% of alveolar units; the mean linear intercept, a useful measure of human emphysema (as opposed to its limitations in the milder experimental disease as compared with the pronounced phenotype in humans) increases in excess of twofold versus control values. The internal surface area is reduced by 40%. There is a reduction of 30–40% of airways versus normal lungs (*see below*). This disappearance of cells and structural components, carrying with it the signatures of the pathogenetic process that led to this loss, has imposed significant difficulties in the elucidation of how these processes occur during the course of disease.

Starting with the clinical evidence that patients deficient in alpha-1 antitrypsin developed early and more widespread (so-called pan-lobular) emphysema (5), experimental investigations focused on the use of potent elastolytic agents to model the disease in animals, notably rodents (6). In fact, the model of experimental emphysema

with pancreatic elastase is based largely on these earlier observations. However, this disease model has fallen short of providing key insights into the complexity of cellular and molecular events underlying emphysema caused by cigarette smoke and pollutants. As is apparent in this article, COPD involves complex genetic, epigenetic, molecular signaling, and age-related facets, many of which have been uncovered more recently.

The more recent studies in the field, dating back to 1997, have provided richer yet still somewhat limited insights into the pathogenesis of emphysema when contrasted with the earlier descriptions of the disease in the preceding half century. This renewed approach to uncovering novel pathogenetic mechanisms of COPD started with the use of the first transgenic mouse model with deletion of matrix metalloprotease 12, resulting in protection against emphysema (7). This was followed by the breakthroughs of the identification of

Table 1. Key morphometric parameters of normal and chronic obstructive pulmonary disease lungs

Feature	Normal	COPD
Lung volume	~4.9 L	~6.3 L
Surface area	~80 m ²	~52 m ²
MLI	~289 μ m	~590 μ m
Number of alveoli	480×10^6	218×10^6
Number of airways	~0.86/cm ² lung	~0.63/cm ² lung

Definition of abbreviations: COPD = chronic obstructive pulmonary disease; MLI = mean linear intercept.

Data are based on Reference 4. Values are approximations or based on mean/medium values.

alveolar cell apoptosis (8–11), autophagy (12), and necroptosis (13) as mechanisms central to alveolar destruction. Many of these cellular destructive processes, under control of an ever more complex molecular signaling, can be documented in diseased human samples and appropriately modeled in animals. These discoveries led to studies that focused on the interplay between relevant pathogenetic molecular signaling, including those leading to excessive extracellular matrix proteolysis (14), with molecular processes that underscore the destruction of alveolar components. More recently, concepts centered on aging and senescence, their molecular underpinnings, including DNA damage response (DDR) and telomere attrition, have been unraveled, as outlined below.

The insights gained from these studies provide a framework to examine why COPD may be a disease of failed resilience for coping with environmental and endogenously generated pathogenetic events. The present author still believes that an evolutionary perspective on the nature of COPD provides a useful conceptual framework that can guide the interpretation of prior studies in the field while providing a much-needed background for moving forward. This perspective, although unable to comprehensively review the extensive literature of the pathogenesis of COPD, seeks to underscore the significant challenges in the field; their recognition provides a needed step in guiding future studies, with the ultimate goal of uncovering disease-modifying therapies.

Why an Evolutionary Perspective?

The recognition that COPD may involve processes akin to aging has relied on the

evidence of the impact of age on disease presentation (15), development of models of emphysema based on age-related paradigms (16), morphological resemblance of alveolar simplification in both aged and COPD human lungs (17), and the role of telomerase (a key molecular determinant of cellular senescence; *see below*) (18).

Moreover, there are additional observations that support this evolutionary concept of COPD. On the basis of evidence that the current presentation of bronchopulmonary dysplasia (triggered by high oxygen, prematurity, or lung injury during perinatal life) is of alveolar destruction and an emphysematous pattern, my colleagues and I proposed that emphysema may represent a default lung phenotype when critical molecular and cellular processes involved in lung development and maintenance of homeostasis (including repair after injury) are compromised (19). Disruption of proper alveolar development and maturation, such as that induced by excessive collagenase type I expression (20), or lack of the protective klotho protein (21), or inhibition of vascular endothelial growth factor (VEGF) (22, 23), leads to developmental airspace simplification, with shared features with cigarette smoke-induced emphysema. On the other extreme of the lifespan, senile emphysema is the prototypic lung phenotype related to advanced age. Notwithstanding that the pathogenic processes of bronchopulmonary dysplasia, COPD-related emphysema, and senile emphysema may differ, the end result of all these diseases is largely superimposable with a reduction of alveolar blood/gas exchange area.

A key factor driving the evolution of living species is the action of evolutionary selection factors, which have shaped, over millions of years, organismal adaptation

with the ultimate goal of improving species' overall survival (24). Key selection factors consist of environmental hazards and infections. It is in the context of environmental hazards that cigarette smoke and pollution appear to act on how living organisms react to stresses. Ultimately, these hazards trigger molecular damage and inflammation, imposing important strains in an individual contribution to survival of the species, particularly procreation. Aging can therefore be interpreted as the end result of the positive versus negative effects of specific molecular adaptation to stresses: Specific genes are protective, with the goal of favoring procreation, whereas their continued activation may lead to accelerated aging. This concept underscores the evolutionary concept of "antagonistic pleiotropy" (19, 25). A complementary evolutionary concept in aging is that of the "disposable soma," in which large cellular and molecular resources are allocated to optimize somatic maintenance and procreation, ultimately leading to "exhaustion," with molecular and structural scars that characterize aging. This brief synopsis provides an important context in which to frame how to best interpret lung resilience and its failure in COPD.

Stage 1 of the Pathogenesis of COPD: Coping with Early Stress Responses

My colleagues and I have proposed that COPD evolves in three pathogenetic stages (Figure 1), which include immediate responses to COPD-causing triggers (e.g., cigarette smoke and/or environmental pollution), a progressive stage in which there is activation of an endogenous process leading to alveolar destruction and remodeling, and finally a consolidated stage with an aging molecular and phenotypic signature (2).

The early stage involves the lung's reaction to cigarette smoke as a stress response. Given that the use of tobacco dates back approximately 500 years (a time frame unlikely to shape evolutionary adaptation vs. millions of years of organismal selection), my colleagues and I have hypothesized that cigarette smoke "borrows" from molecular pathways (i.e., molecular sensors), which respond to environmental stressors that have shaped organismal evolution over millennia. These largely involve nutrient

Figure 1. The proposed three stages of the evolution of chronic obstructive pulmonary disease (COPD), which include lung responses driven by environmental stresses (stage 1), progressive amplification of injury allowing for the progression of the disease (stage 2), and a later stage of consolidation (stage 3). The multiple areas of investigation in COPD are outlined in the lower half of the figure. Dashed lines are meant to represent that each stage may prolong over time with the potential for intersections among all stages.

and oxygen availability; indeed, depletion of the amino acid leucine (as an index of nutrient supply) and/or hypoxia activate key sensor-like molecules, ultimately converging in the control of signaling hubs such as the mammalian target of rapamycin complex (mTOR). This multidomain, serine-threonine-containing kinase controls several key molecular processes, such as metabolism, protein synthesis, and lysosomal activity, ultimately directing cell growth (26). In the setting of nutrient deprivation (leading to inactivation of mTOR in the lysosomal nutrient-sensing machinery) or hypoxia-driven negative signals, mTOR signaling is decreased, whereas mTOR signaling is activated when the overall conditions are favorable for cell growth.

Our work uncovered that baseline lung mTOR has a protective role against acute and chronic cigarette smoke exposures (27). Cigarette smoke leads to oxidative stress-mediated increased expression of Rtp801, also known as Redd1 (regulated in DNA damage and development 1) or DDIT (DNA damage-induced transcript 4). Rtp801/Redd1 is a negative regulator of mTOR by binding to 14-3-3 adaptor protein and thus releasing Tsc1/2 (tuberous sclerosis 1/2) to block this pathway (28). Rtp801/Redd1 was

initially identified in a screen of hypoxia-inducible factor 1 α -dependent gene expression and found to be triggered by oxidants (29). We determined that the upregulation of Rtp801/Redd1 by cigarette smoke is fast, detectable within 1 day of exposure, and is both necessary and sufficient to cause lung inflammation (27). Importantly, acute or even subacute exposures to cigarette smoke do not suffice to cause emphysema, which in C57BL/6 mice occurs only after 6 months of exposure; the phenotype is mild, with about a 10% increase in mean linear intercept when compared with air-exposed littermates. Our data were supported by the independent findings of Rtp801/Redd1 upregulation by cigarette smoke (30, 31). Further evidence that mice with conditional airway cell deletion (driven by the CC10 [club cell protein 10] promoter) of mTOR show enhanced inflammation, apoptotic and necroptotic cell death due to cigarette smoke supports the role of the Rtp801/Redd1-mTOR axis in cigarette smoke-induced lung injury (including inflammation, cell death, and emphysema) (32). Moreover, these data suggest that as the lung responds and copes with acute stresses (e.g., cigarette smoke), it progressively develops pathology, as outlined below. Whether repetitive acute

stresses are sufficient to cause disease remains unknown. It is conceivable that epigenetic and genetic factors control whether the lung has the needed resilience to cope with these stresses. The present author has postulated that acute exogenous (i.e., environmental) injuries progressively overwhelm protective mechanisms, leading to activation or recruitment of endogenous maladaptive injurious processes that act in concert or in lieu of cigarette smoke.

Stage 2 of the Pathogenesis of COPD: Progression

As the result of multiple stress responses and/or owing to progressive imbalance in lung repair and molecular and structural maintenance in the setting of genetic predisposition, COPD may progress in approximately 20–25% of long-term smokers. On the basis of longitudinal studies of patients with COPD, the disease can become progressively independent of continual inhalation of tobacco smoke (33). It is conceivable that the progressive state occurs when endogenous mediators of alveolar injury are inappropriately activated and/or protective mechanisms are worn out. These may represent key insights that were not available to earlier investigators when COPD was considered as mostly a syndrome due to excessive protease-antiprotease imbalance.

The concept of lung structural and molecular maintenance arose from the finding that VEGF receptor blockade could induce an emphysema phenotype in rats, dependent on alveolar cell apoptosis with no participation of infiltrating inflammatory cells (8, 34). In fact, my colleagues and I have highlighted that the paradigm of lung structural and molecular maintenance can account for the findings related to a multitude of molecules that function to protect the lung; many of these promote cell regeneration, such as the Wnt family and their receptors (35), or act as antiinflammatory or antiapoptotic agents, such as adiponectin (36), or act to scavenge oxidants, such as Nrf2 (nuclear erythroid 2-related factor 2) or SOD3 (superoxide dismutase 3) (37, 38).

The interdependence of key pathogenetic factors involved in alveolar tissue destruction is a key element to understand in order to appreciate the complexity of the pathogenesis of COPD

(19). My colleagues and I have proposed this interdependence on the basis of observations that the interaction between apoptotic alveolar cell death and oxidative stress is central in the model of emphysema caused by VEGF receptor blockade (39); further interconnectivity among pathogenetic process was uncovered by studies that revealed the interdependence of cigarette smoke-induced alveolar epithelial cell death and activation of extracellular matrix proteases, such as cathepsin S (40). These investigations brought forward the fundamental question of which cell orchestrates the destruction of alveolar septa. In addition to the potential role of inflammatory cells, notably of neutrophils (41, 42) and macrophages (7, 43), the roles of alveolar type II cells and capillary endothelial cells in driving the process of destruction have remained unclear. There is a strong rationale that these cells, in addition to interstitial fibroblasts and alveolar type I cells, act in concert to preserve alveolar septal structure (44). It is therefore anticipated that triggers of alveolar type II cell (11) or endothelial cell death (45) lead to an emphysema phenotype. How each cell deals with injury in the setting of chronic cigarette smoke exposure and the determinants of septal disappearance versus repair are central questions that need to be addressed. The answer may provide key pathogenetic insights into molecular drivers of lung resilience and reveal important targets for drug discovery.

There are recent insights that the vascular dysfunction in COPD may be systemic, with a dysfunction of endothelial cells underlying the renal impairment in patients with COPD (46). It is possible that as cigarette smoke severely compromises lung and systemic endothelial cells, the disease becomes progressive and potentially irreversible. This is supported by evidence of increased concentrations of endothelial cell microparticles indicative of apoptosis in the circulation of patients with emphysema (47, 48).

It is apparent that with continuous exposure to cigarette smoke and environmental pollution, there is progressive engagement of detrimental signaling processes, which are pathogenetic determinants of or important contributors to alveolar injury in COPD. Many of these pathogenetic molecules include damage-associated molecular patterns (49), including fragments of collagen (50), elastin

(51), and the receptor for advanced glycation end products (RAGE) (52, 53). RAGE has received substantial focus as a potential mediator of inflammation in the setting of cigarette smoke exposure. RAGE is expressed in the basolateral membrane of type I cells and in several other cells in the lung. Membranous RAGE is increased in cigarette smoke-exposed cells and in human COPD lungs (54), whereas concentrations of soluble RAGE (which antagonizes signaling via membranous RAGE) are decreased in plasma of patients with COPD (55, 56). Furthermore, although mice with knockdown of RAGE had an increased measure of interseptal distance (mean linear intercept) at baseline, they showed a decreased response to chronic cigarette smoke exposure with emphysema (vs. wild-type mice) (57). This protection was recapitulated in decreased pulmonary inflammatory infiltrate, notably of alveolar neutrophils during acute exposure, and reduced alveolar cell death in chronically exposed mice (vs. wild-type littermates).

Ceramide is a potent second-messenger lipid involved in apoptosis, oxidative stress, and inflammation (58). Moreover, it can trigger feedforward mechanisms leading to its own persistent synthesis (59) due to cigarette smoke, further amplifying lung injury. Of note, on one hand, intermediates in the ceramide synthetic pathway can enhance inflammation, such as sphingosine-mediated impairment of efferocytosis (60); on the other hand, the downstream intermediate sphingosine 1 phosphate enhances cell survival (61). Also consistent with its amplifying effects regarding cell death, oxidative stress, and inflammation, ceramide can be induced by targeting endothelial cells to apoptotic cell death (45) and has a mutual positive feedback with Rtp801 (62).

Elucidation of the pathogenetic interaction among key molecular mediators of emphysema is key to starting to unravel how the disease may progress. The complexity of interacting signaling hubs may explain the progressive nature of the disease, its evolution despite smoking cessation (albeit at a slower rate), and the difficulty of therapeutic control. This is particularly pertinent to enzymes that generate oxidants directly or as by-products, such as Nox4 (NADPH oxidase 4). In fact, Nox4 may represent another example of antagonistic pleiotropy, because it has been uncovered in studies addressing its role in

interstitial fibrosis (63). Nox4 is activated by Rtp801 and leads to cytosolic production of superoxide and hydrogen peroxide, thus potentially amplifying the oxidative stress caused by cigarette smoke (64).

On the basis of the preceding discussion, it is apparent that oxidative stress due to reactive oxygen species/reactive nitrosative species (ROS/RNS) provides a unifying mechanistic theme between cigarette smoke-induced lung stress responses and the progressive disruption of cellular and molecular maintenance. Early in the disease, oxidants present in tobacco smoke can initiate inflammatory and stress responses; this is supported by the recent observation that Rtp801/Redd1 activation, inflammation (including nuclear factor- κ B activation), and alveolar enlargement rely on cigarette smoke oxidants acting proximally to potential amplifiers of RNS, such as inducible nitric oxide synthase (31). Importantly, ROS/RNS provide a mechanistic disease progression leading to engagement of processes (such as DDR and telomere erosion) that ultimately lead to aging/senescence, as discussed in the next section of the text.

As these endogenous processes involved in the potential progression of COPD are engaged, they promote and enhance inflammatory processes. My colleagues and I have proposed that inflammation, involving both innate and acquired responses, may change in the course of cigarette smoke exposure and progressive damage to the structure of the lung (2). In contrast to their beneficial and reparative function, it is conceivable that these components of inflammation become pathogenic, further enhancing tissue destruction. With progression of the disease, the lung becomes permanently damaged. These alterations may underscore the state of consolidation of lung impairment in COPD.

Stage 3 of the Pathogenesis of COPD: Consolidation of Tissue Injury

The failure of retinoic acid supplementation to treat established disease (65) can be interpreted as the COPD lung having undergone permanent damage, and, in this state, it has lost the potential for repair. Most of this preclinical work was performed in rodents, which develop a mild disease-like phenotype and retain the ability to grow

alveoli. In humans, the growth of the lung, including formation of alveoli, is largely complete by the age of 9 years (4), but evidence shows that the lung can continue forming new alveoli into the second decade of life (66). Thus, one might predict that the lung's resilience to injury based on formation of new alveoli is restricted to the first two decades of human life. However, it is unclear whether the lung can form new alveoli after being persistently damaged or if the injuries have occurred during key stages of lung development; that bronchopulmonary dysplasia manifests with severe alveolar simplification into the second decade of life is consistent with the concept that the COPD lung has exhausted its reparative potential. My colleagues and I have highlighted the extent of "scars" of persistent injury in COPD, including increases in collagen type I in alveolar structures; persistent macromolecular oxidant damage, including DNA and protein modifications; and progressive senescence (2). The overall failure of progenitor or stem cell populations in COPD includes the bone marrow, as demonstrated by DNA- and senescence-related abnormalities in endothelial progenitor cells of patients with COPD (67).

The concept that COPD involves a premature aging process has been supported by the evidence of telomere attrition, including abnormalities in telomerase and its associated proteins in this stage of the disease (18, 68). The genetic lack of telomerase in mice predisposes them to enhanced lung injury and emphysema when compared with wild-type control animals (18), and it depletes alveolar progenitors (68). These findings suggest that signatures of aging/senescence, and all its antireparative implications, may indeed manifest earlier in the disease process as and telomerase associated proteins expression and activity decrease.

Markers of DNA damage (i.e., expression of DDR markers γ -H2AX [γ -histone 2AX] and 53BP1 [p53-binding protein 1]) have been documented in COPD lungs (67, 69–72), possibly caused by ROS and RNS. Oxidative and nitrosative stresses cause DNA damage and promote telomere erosion (73), ultimately contributing to cellular senescence. Furthermore, telomere ends that are unprotected by telomerase and its associated proteins trigger DDR and senescence. These processes not only involve lung (airway and septal) cells but

also affect bone marrow progenitors, including endothelial progenitor cells (67). Epigenetic alterations, because the COPD-related aging process is linked to decreased expression of the deacetylase SIRT1 (Sirtuin 1), also impair DNA repair (67) and HDAC2 (histone deacetylase 2) (74). HDAC2 is downregulated in COPD lungs (possible owing to ROS/RNS), which can lead to enhanced inflammatory gene expression, enhancement of DDR, and accelerated senescence (74). Moreover, microRNAs may underlie DDR; decreased expression of miR126 in endothelial cell progenitor cells leads to increased expression of the DDR mediator ATM (ataxia-telangiectasia mutated) protein and the VEGF inhibitor SPRED-1 (Sprouty-related EVH1 domain-containing 1) (75). These processes may have far-reaching consequences, not only affecting lung cells but also underscoring the systemic vascular abnormalities characteristic of COPD.

In this scenario, early treatment aimed at minimizing lung stresses and curbing progression might be critical to impacting the late-stage phenotype. Approaches designed to delay or counteract senescence may be beneficial in COPD; however, interventions based on targeting mTOR (such as with its pharmacological inhibitor rapamycin [76]) may have potentially detrimental results, given the protective functions of mTOR in stress responses.

The importance of these pathogenetic concepts may extend to airways, the site of airflow limitation in COPD. It is becoming apparent not only that the loss of structural components involves the alveolar compartment in emphysema but also that large and small airways are depleted in COPD lung (77, 78); whether the destruction of these different compartments conforms to similar pathogenetic paradigms of alveolar tissue destruction will require further investigation. In line with this prediction, airway cells of COPD lungs have evidence of telomere shortening and DDR (79), previously shown in alveolar septal cells (80).

With progressive depletion of a progenitor cell population (by apoptotic/necroapoptotic processes or replacement by senescent cells), the blueprint for lung regeneration is severely compromised. It is in the setting of a deeply injured organ that inflammation may be driven by autoimmune processes (81), including the formation of autoantibodies against adducts of proteins and DNA (82). Exhaustion of

antioxidant defenses allows for formation of ROS- and RNS-modified lipids, proteins, and/or DNA; these may act as neoantigens, driving persistent inflammatory responses despite smoking cessation (33). This is the stage at which lung resilience might be the lowest or even absent in COPD.

Conclusions

Collectively, the field of COPD pathogenesis has evolved on the basis of a rich collection of paradigms that build on the protease–antiprotease imbalance. These insights have allowed better comprehension of why lung resilience may falter in this disease. These novel advances, though significant because they allow better understanding of COPD and planning of future therapeutic development, are limited by their somewhat reductionist nature. It is still a struggle to integrate the recent insights into the alterations of mitochondria and iron metabolism into understanding the pathogenesis of the disease (83), which further underscores the extent and complexity of the pathogenesis of the disease. The same limitations pertain to the role of innate and acquired immunity. The need for integration of these multidimensional components with the concepts of stress responses, lung structural and molecular maintenance, aging, and macromolecular damage is pressing (Figure 1). This complexity may indicate that potential therapies need to target diverse pathogenetic processes in multiple cellular compartments simultaneously to achieve meaningful benefits for patients with COPD. Moreover, moving ahead, it is vital that investigators address how healthy smokers resist the development of COPD, patients' genetic susceptibility to succumb to the disease, genetic and epigenetic determinants of the multiple disease phenotypes (84), how different lung compartments react to the stresses imposed by cigarette smoke, and the contribution of each cell to COPD disease phenotypes. It is possible that in the not-distant future, reestablishing lung resilience by targeting the first two pathogenetic stages might be an achievable goal in the management of COPD, thereby preventing or delaying the consolidation of lung destruction. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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