Cellular and Molecular Mechanisms of Alveolar Destruction in Emphysema

An Evolutionary Perspective

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Emphysema consists of a unique pattern of alveolar destruction, resulting in marked airspace enlargement with reduction of alveolar capillary exchange area. Classical concepts of the pathogenesis of emphysema have relied on the paradigm set by the inflammation and protease/antiprotease imbalance. We propose herein that cigarette smoke constitutes an environmental hazard that causes alveolar destruction by the interaction of apoptosis, oxidative stress, and protease/antiprotease imbalance. We draw a parallel between organismal aging, organ structural maintenance, and the damage resulting from chronic cigarette smoke inhalation. The stochastic interaction between environmental hazards and the effort of an organism or a particular organ to fend off these hazards results in the accumulation of cellular damage and features characteristic of aging. Inflammation follows as the result of the multiplication of injuries. We highlight the importance of understanding the biology of the interaction of alveolar cells in homeostasis and in alveolar destruction, and the potential role of novel processes related to senescence and stress response. An evolutionary perspective of emphysema that incorporates mechanisms related to aging may lead to important advances in the understanding and therapeutic targeting of chronic obstructive pulmonary disease.

Keywords: aging; apoptosis; emphysema; inflammation; oxidative stress

We are in the midst of an epidemic of cigarette smoke-induced diseases. In the United States alone, there are about 4 to 5 million people with emphysema, a characteristic lung-destructive process that is part of the spectrum of chronic obstructive lung diseases. The understanding of the underlying mechanisms of emphysema has evolved significantly in the past 6 years, as was apparent in the talks at this conference. However, a significant challenge remains in that there are no novel or even currently effective treatments aimed at this irreversibly fatal disease. This presentation discusses the involvement of inflammation, apoptosis, and oxidative stress as mechanisms intimately networked in

the process of alveolar destruction in emphysema (Figure 1). The authors discuss the relative importance and interaction of these pathobiological processes in the context of organismal aging, and frame them more broadly within the evolutionary context of species selection and aging. This alternative approach is justified because complex and unique molecular and cellular processes interact to create a phenotypically unique diseased lung. We hope that this expanded conceptual framework stimulates further discussion and triggers future studies aimed at revealing unknown pathobiological elements related to the disease.

THE INFLAMMATION-PROTEASE/ANTIPROTEASE IMBALANCE HYPOTHESIS

In the past 30 years, the prevailing concept to explain emphysema development has relied on lung inflammation caused by cigarette smoke, environmental pollutants, or bacterial products, leading to a protease/antiprotease imbalance (1), which ultimately would cause alveolar destruction in emphysema. A close examination of the scientific literature between 1949 and June 2005 revealed that of the 40,309 papers on chronic obstructive pulmonary disease (COPD), 1,637 involved the word "inflammation" and 187 involved the keyword "protease" or "matrix." Forty-six papers dealt directly with inflammation and protease imbalance. The relative importance of inflammation was recently highlighted by the link between enhanced histone acetylation of inflammatory genes and inflammation in COPD (2), leading to the suggestion that histone acetylation inhibitors be used to treat the disease (3).

However, because inflammation is equated with cigarette smoke-induced lung disease, it is imperative to analyze to what extent the organismal protection against cigarette smoke fulfills the evolutionary (and biological) needs of inflammation in the survival and establishment of species. In other words, we are interrogating whether, teleologically, cigarette smoke-induced inflammation fits with the evolutionary requirement of inflammation in the evolution of the human species. The human species underwent a progressive selection that entailed resistance against dangers imposed by other organisms, particularly parasites, bacteria, and viruses. Accordingly, inflammation has formed the basis of both a broader and first-response innate immunity and a more selective and target-directed acquired immunity, both acting to shield against pathogens. Broadly, neutrophils and macrophages are the pillars against bacterial invaders, lymphocytes, plasma cells, viral diseases, eosinophils, and parasites. Complex cytokine and chemokine networks drive the progressive specialization of these cells in their particular protective function, and the intercellular cross-talks aimed at enhanced host protection. Those pathogens that imparted the strongest selection pressure

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Figure 1. Conceptual framework for emphysema. Cigarette smoke causes a progressive disruption of alveolar maintenance and variable degrees of inflammation, driven by the cigarette smoke itself, oxidative stress, or alveolar cell damage. After years of relentless attack by this underlying process counterbalanced by potential repair, susceptible individuals would activate molecular and cellular processes involved in alveolar destruction, namely protease/antiprotease imbalance, apoptosis, and oxidative stress. These processes are mutually interactive, and promote amplifying feed-forward loops. Endogenous mediators such as ceramide would promote the amplification of these processes, increasing inflammatory cell injury and hampering repair processes. Several of these elements are also present in organismal aging.

over the span of human species evolution led to more effective organismal protective mechanisms (and more efficient vaccines), whereas the pathogens that are of more recent origin, such as the human immunodeficiency virus, still represent a significant challenge to the therapeutic stimulation of protective immunity. A COPD lung presents with increased numbers of neutrophils, macrophages, and lymphocytes, but are these inflammatory elements targeting directly elements of cigarette smoke or, rather, is inflammation a secondary response to cellular and molecular alterations induced by chronic cigarette smoke inhalation? It is presently unclear the extent to which thousands of pathogenetic molecules present in cigarette smoke recapitulate the injury resulting from pathogens, the main target of inflammation. As compared with the 5 million years of selective pressure imparted by pathogens on human evolution, it is unlikely that the 500 years of cigarette smoke or particulate exposure have exerted a selection pressure on the human species sufficiently strong to stimulate inflammation as an adaptive mechanism of survival. The recent evidence of association of airway and alveolar inflammation with more advanced COPD suggests that inflammation may represent a response to a damaged organ, leading to selection of subpopulations of T lymphocytes (4, 5).

The challenge of ascribing inflammation as the earliest and most important mechanism of alveolar destruction in emphysema is even more daunting as one examines the pathology of lung injury caused by pathogens compared with that in emphysematous lungs. Viral or bacterial pneumonias are clearly distinct from the classical simplification of alveolar destruction in emphysema (Figure 2).

EMPHYSEMA AS THE RESULT OF A LUNG INJURY DUE TO CIGARETTE SMOKE

As outlined above, it is imperative that we take into consideration the molecular alterations caused by chronic cigarette smoke on lung cells. Each puff of cigarette smoke contains approximately 5,000 toxic compounds, with 10^{15} free radicals in the gas phase and 10^{18} free radicals per gram of tar, and includes potent oxidants such as hydrogen peroxide, hydroxyl anion, and organic radicals (6). Recent studies demonstrated that the alveo-

lus is composed of a virtual syncytial network among alveolar type I and type II, endothelial, and fibroblastic cells (7). Three-dimensional reconstruction of cell-cell contacts and interactions highlighted the potential cellular pathways used to relay molecular signals originating in endothelial cells and targeting epithelial cells or originating within the alveolus and reaching endothelial cells (8, 9). This unique structural networking provides a potential mechanism for the pathogenetic dysfunction compromising all alveolar septal cells as the result of cigarette smoke exposure. Alveolar destruction requires that type II, type I, endothelial, and myofibroblastic cells be destroyed concordantly. Conversely, proper function of all septal cells is required for preservation of alveolar structural integrity.

Emphysema consists of a prototypic injurious response of the alveolar cells in that structural elements disappear, leaving simplified septa, with a progressive decrease in capillary exchange area. We propose that the mechanisms leading to this destruction relate to critical molecular signals involved in lung development, maturation, and aging. The only other diseases with pathologic evidence of alveolar destruction and increased airspace size similar to emphysema are bronchopulmonary dysplasia (due to prematurity and hyperoxia during the perinatal life) and the aged lung. The prevailing concept underlying bronchopulmonary dysplasia underscores the role of growth factors, such as vascular endothelial growth factor (VEGF), and collapse of the pulmonary circulation (10) in the maintenance of the neonatal lung structure. On the other extreme of life, emphysema bears a remarkable resemblance to the aged lung, where alveolar enlargement is associated with increased airflow resistance (11). With advancing age, the pressure-volume curve shifts to the left (i.e., increased compliance) when compared with the nonaged adult profile. This shift is accounted for by a fragmentation of elastin fibers in alveoli. Morphologically, aged lungs have expanded air ducts and shallow alveolar tissue. Although these changes are more uniform than those seen in cigarette smoke-induced emphysema, they are associated with an increase in mean linear intercept (a measurement of interalveolar septal distance) and a decrease in the surface to volume ratio, indicative of a decrease in air-capillary exchange area (11). In contrast to

Figure 2. Comparison between human lung pathology caused by infectious agents and that caused by cigarette smoke. Morphologic comparison of lung inflammation caused by infectious agents (*a–c*), showing acute bacterial pneumonia (*a*), a gross lung section with a cavitary fungal lesion (*b*), and microscopic highlight of cytomegalovirus pneumonia (*c*). The respective infectious agents that cause these pathologies are highlighted in *d–f*. On the other hand, cigarette smoke (*h*) causes the characteristic alveolar loss (*i*) and the characteristic simplification of alveolar septa with airspace enlargement (*j*).

emphysema, these changes are not considered to be destructive and are not generally believed to be associated with inflammation. Despite these considerations and given the striking similarities between senile and cigarette smoke-induced emphysema, studies aimed at establishing significant parallels among the molecular events underlying both types of emphysema are warranted. The discussion that follows is an attempt to highlight the potential interface between aging and cigarette smoke-induced lung destruction.

AGING AS A RESPONSE TO INJURY AND REPAIR

There are clear parallels between the pathophysiologic responses to aging and those involved in COPD, particularly emphysema (readers are referred to an outstanding set of articles on aging published by *Cell* in 2005 [12]). The survival of a species results from its ability to ultimately procreate, which occurs as the result of a concerted process of organismal development, growth, and ability to fend off environmental attacks. To this end, individuals have to optimize resource utilization, and protect their progeny. Once this goal is accomplished, the nature of the evolutionary determinants of post-procreation life expectancy is unclear. The observed difference in life expectancy among species suggests that these determinants might exist but may not be driven by genetic events. Kirkwood (12) advanced the concept of “disposable soma” in which aging, rather than being programmed and determined by selected genes, results from the stochastic interaction between injury and repair, as the result of the energy devoted by an individual to maintain organ integrity and protect against DNA and oxidative injury. The biological price of the survival effort would be the wear and tear of organs, which is characteristic of the aging process. In this model, the failure to maintain and repair cells and organs results from the integrated action among genes, environment, and intrinsic organismal defects. This failure of this organ maintenance and repair would ultimately account for aging (Figure 3). Emphysema may therefore recapitulate one of the events underlying accelerated lung aging process resulting from a failure of lung maintenance and repair due to significant and sustained lung injury imposed

by cigarette smoke, exposure to air particulates, or exposure to pollutants (13). As will become apparent subsequently, inflammation might represent the response to unrepaired organ injury, rather than the first line of response to environmental stresses. The engagement of several pathobiological processes leading to active destruction of alveolar structures might constitute the difference between the pathobiology and pathology of emphysema and the milder lung alterations present in aging.

DISRUPTION OF THE LUNG'S CELLULAR AND MAINTENANCE PROGRAM IN EMPHYSEMA: ROLE OF VEGF

We propose that common molecular mechanisms control lung developmental and postnatal growth programs, which, if severed, cause the characteristic pattern of emphysematous lung injury. There is compelling evidence that VEGF is an obligatory lung maintenance factor throughout lung development (14–16),

Figure 3. Kirkwood model of aging based on the concept of “disposable soma.” Reprinted by permission from Reference 12.

including infancy (17), adulthood (18, 19), and old age. VEGF represents a family of ligands (A–D), which bind to three sets of receptors (VEGF-R1, VEGF-R2, and VEGF-R3) (20). VEGF-A (herein called VEGF) stimulates endothelial cell and type II cell growth and survival by binding VEGF receptor 2. Disruption of VEGF signals results in arrested lung development and death, simplification of alveolar structure in the neonate (recapitulating bronchopulmonary dysplasia), and emphysema in the adult. More importantly, there is growing evidence that human emphysema is associated with decreased VEGF gene expression (21, 22). As discussed in the following sections and in other reports in this issue, the finding of the requirement of VEGF in lung maintenance and its role in emphysema has allowed for the identification of important signaling processes involved in alveolar maintenance and destruction, particularly those related to oxidative stress and apoptosis.

MITOCHONDRIA, OXIDATIVE STRESS, AGING, AND EMPHYSEMA

As previously mentioned, there are important similarities between the aged and the emphysematous lung due to chronic cigarette smoking. A common molecular mechanism to both conditions is oxidative stress.

The free radical theory of aging was originally proposed by Harman in 1956, focusing on the mitochondria as a potential source of free radicals (23). This theory complemented the one proposed by Pearl, that the metabolic rate was directly linked with the life span of an organism (23). The link between free radicals and the mitochondria is based on the finding that the mitochondria (mostly complexes I and III) are the source for 90% of free radicals produced *in vivo*. Because approximately 0.2% of consumed oxygen leads to formation of reactive oxygen species, a set of antioxidant defenses, including glutathione and enzymes such as superoxide dismutase, glutathione peroxidase, and peroxiredoxins, maintains homeostasis by reducing free radicals.

With aging, there is evidence of mitochondrial dysfunction and accumulation of macromolecular imprints of oxidative injury, such as formation of 8-oxo-2'-deoxyguanosine, which results from hydroperoxide-induced DNA damage. Mitochondrial function decreases with aging, possibly mediated by decreased transcription of electron transport proteins. Increase in free radicals induced by exposure to hyperoxia leads to molecular changes common to aging. Conversely, increases in antioxidants (e.g., overexpression of superoxide dismutase) increase the life span of cultured cells and decrease the rate of telomere shortening. Interestingly, sir-2, a histone deacetylase that increases the life span of worms, appears to direct the accumulation of oxidatively modified proteins in daughter worm cells during cell division (23) and increases antioxidant defenses. The mammalian ortholog SIRT1 has an equivalent protective action in mammalian cells (23).

There is ample evidence that oxidative stress plays a major role in COPD (24), with increased expression of markers of oxidative stress in patients with COPD systemically and in diseased lung (25, 26). Although there is evidence that enhanced expression of superoxide dismutase attenuates cigarette smoke-induced injury in cell culture systems (27), the first clear demonstration that antioxidant defenses determine susceptibility to emphysema was reported in mice deficient in the master transcription factor NRF2, which regulates multiple critical antioxidant enzymes (28) and which was described in detail in this conference.

It is increasingly evident that oxidative stress represents a "hub" pathobiological process in emphysema, because it is inter-

linked with inflammation, activation of proteases, inactivation of antiproteases, and apoptosis, all of which have a major role in COPD (13). The interaction between oxidative stress and apoptosis in experimental emphysema was illustrated in an earlier study that showed that, in the rodent, emphysema caused by VEGF receptor blockade, markers of oxidative stress and apoptosis (i.e., active caspase-3), colocalized in the central portion of the alveolar lobule and that inhibition of oxidative stress by means of administration of a superoxide dismutase mimetic abrogated alveolar cell apoptosis and emphysema (29). Because blockade of apoptosis prevented oxidative stress and emphysema in this model, we proposed that oxidative stress and apoptosis mutually interact in the process of alveolar cell destruction (29). As stated by Balaban and colleagues in their review on the role of mitochondria and oxidative stress in aging: "In the end, the seeds of both our vitality and our ultimate mortality would seem to be intertwined in the combustible combination of nutrients and oxygen that continuously occurs in our mitochondria. Economists often warn us that there is no such thing as a free lunch!" (23) Indeed, there is a price to pay, either for the biological effort of supporting the soma through the procreation stage of an organism, or, by extension, for the effort to repair the damage caused by cigarette smoke inhalation over several decades of exposure.

APOPTOSIS AND SENESCENCE IN AGING AND EMPHYSEMA

The cell responses to stress are directed toward cell arrest, or, if the damage is beyond repair, toward cell death. Perhaps paradigmatic of this "end of life" dilemma is the dual effect of p53 in promoting either cell death or cell arrest when injury affects the well-being of a cell. In the past 6 years, there has been increasing awareness of alveolar cell apoptosis as a critical "hub" in the pathogenesis of emphysema (30). The identification of alveolar cell apoptosis in emphysema was initially documented in human lungs (31) and in the rodent model of apoptosis-dependent emphysema in the VEGF receptor blockade model (19). These findings were followed by the documentation of an expanded paradigm that linked apoptosis to oxidative stress (28, 29) and, more recently, as part of a feedback loop involving lung IFN- γ increases, activation of cathepsin-S, and alveolar inflammation in the genesis of mouse emphysema (32). However, the balance between alveolar cell repair and apoptosis would account for the disappearance of alveolar structures in emphysema. Because there is evidence of ongoing cell proliferation in emphysematous lungs in association with apoptosis (33), it is apparent that additional cellular events intimately related to aging may also contribute to generate the emphysematous phenotype.

Senescence is a process defined in cultured cells, in which cellular stresses converge to promote cell cycle arrest. Interestingly, in addition to replicative senescence in which progressive erosion of the telomere ends of chromosomes leads to senescence, oxidative stress-induced DNA damage can similarly promote a cell growth arrest (34). There is correlative evidence linking cellular senescence with organismal aging (35), most importantly with expression of p16^{Ink4} and p19^{Arf}, two critical cyclin kinase inhibitors and retinoblastoma gene product activators. It is noteworthy, however, that there is no definitive evidence showing a causal role of cellular senescence in aging. Nevertheless, the coexpression of p16 and senescence-associated β -galactosidase activity in atherosclerotic lesions, skin ulcers, and arthritic joints argues for such a mechanistic relationship (36). Senescent cells may impact organ repair (via blockade of cell renewal), structure (by enhanced alterations of extracellular

matrix by secretion of matrix metalloproteases), and localized inflammation (via release of inflammatory cytokines) (36). There is scant evidence that chronic cigarette smoke inhalation causes lung cellular senescence, with the only evidence presented in abstract forms (37). Telomere length and protein DNA capping dictate replicative senescence in cell cultures and shortened telomeres can be produced by oxidative stress (34). There is also evidence that COPD lungs have shortened telomeres as compared with age-matched nonsmokers' lungs (36). The presence of cellular senescence in emphysema could explain several features of the disease, including absent or reduced repair potential by a reduced population of progenitor cells, alveolar inflammation, and release of matrix proteases. Furthermore, cellular senescence could also explain the significantly increased rate of lung cancers in emphysematous patients, as documented for the role of mammary gland senescence and the increased risk of breast cancer (36).

INTERACTION OF APOPTOSIS, MATRIX PROTEOLYSIS, AND OXIDATIVE STRESS IN THE PATHOGENESIS OF EMPHYSEMA: ROLE OF CERAMIDE

We have proposed that the interactions of apoptosis, matrix proteolysis, and oxidative stress represent the final conduit of alveolar destruction in emphysema (13). It is also known that ex-smokers may experience progression of alveolar destruction and emphysema years after quitting and that ex-smokers' lungs may persist with significant inflammation (38). It is therefore intuitive that endogenous molecular signaling may generate feed-forward amplification loops involving these destructive processes despite the absence of ongoing cigarette smoke injury. Again, on the basis of the emphysema model of VEGF receptor blockade, we documented that early up-regulation of the sphingolipid ceramide mediates alveolar cell apoptosis, oxidative stress, matrix proteolysis, and alveolar destruction in the model (39) (Figure 4). Most importantly, this paradigm reveals novel (endogenous) molecular switches that integrate alveolar destructive processes with cigarette smoke. Because endothelial cells are probably the most sensitive cells to the proapoptotic effects of ceramide, and a source of its synthesis, these results underscore the importance of endothelial cells in the preservation of alveolar structure, in line with the concept that alveolar destruction may follow endothelial cell collapse, also known as the vascular hypothesis of emphysema (40). Of note, other vascular diseases worsened by cigarette smoking, such as arteriosclerosis, have evidence of endothelial cell senescence, and induction of senescence in cultured endothelial cells triggers a proinflammatory phenotype similar to that found *in vivo* (41). Furthermore, ceramide has been implicated in cellular senescence and aging as well (42).

Given the close interaction among alveolar septal cells as outlined by Sirianni and coworkers (7), it is conceivable that they coordinate their response to injury or stresses, and that coordinated cell death and extracellular matrix degradation have to occur to produce an emphysematous lung. It is presently unclear whether a hierarchy exists in the cross-talk among alveolar septal cells. To start addressing this critical question, one has to target specifically alveolar septal cells to destructive processes present in cigarette smoke-induced emphysema, such as apoptosis.

VASCULAR HYPOTHESIS OF EMPHYSEMA: ROLE FOR ENDOTHELIAL CELLS IN ALVEOLAR ORGANIZATION

To address the specific role of alveolar endothelial cells in the genesis of emphysema, one has to consider the following chal-

lenges: (1) to determine whether lung capillary endothelial cells have unique phenotypic cell surface characteristics as compared with nonlung endothelial cells (these cell surface determinants of lung endothelial cells define a characteristic lung vascular address [43]), (2) to find out whether this lung endothelial cell surface molecule allows for peptide internalization, and, (3) to deliver a proapoptotic stimulus specifically to endothelial cells. To accomplish these goals, we allied the strengths of our research group in Baltimore (R.M.T. and I.P.) with those of the group in Houston (R.P. and W.A.), who developed the bacteriophage display methodology to assign vascular or so-called zip addresses to endothelial cells *in vivo* to probe into fundamental mechanisms involved in cancer angiogenesis and obesity.

Filamentous phages, such as M-13, were introduced in the mid-1980s by George Smith to convey libraries of genetic information, by means of polypeptide expression encoded by exogenous DNA sequences cloned and integrated into the phage's own genome. The coat protein pIII has been used as the phage protein domain of choice to display efficiently a large family (10^7 to 10^{11}) of peptides. Ideally, up to 10-residue peptides can be efficiently expressed by pIII libraries (44). The physical link between a peptide displayed on the surface of the phage and its cloned genetic coding information provides a critical methodologic advance afforded by the filamentous phage (45). A vast peptide array, now displayed on the surface of the phage coat, can be used to interrogate binding to target ligands without prior knowledge of identity or structural characteristics of the peptide or its target ligand. Due to their versatility, phage-displayed peptide libraries are being increasingly applied to identify binding peptides for diagnostics, receptor antagonists or agonists,

Figure 4. Ceramide causes emphysema-like changes. Intratracheal ceramide instillation causes airspace enlargement as described in Reference 39 with evidence of alveolar cell apoptosis highlighted by terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) on Day 1 postinstillation (*top right panel*). +ctl = positive control with endonuclease DNA cleavage and TUNEL; -ctl = negative control with TUNEL lacking DNA polymerase. *Bottom panel* highlights alveolar cell apoptosis with double fluorescence for apoptotic cells (*green*) and nuclei (4,6 diamidino-2 phenylindole [DAPI], *blue*).

enzyme inhibitors, mimics of epitopes or natural ligands, reagents for protease substrate specificity, or the creation of vaccines (45). Selection of unique sequences can be performed using negative selection with unrelated cells or tissues and then followed by positive selection in cell cultures or *in vivo* after tail vein inoculation and a 5-minute circulation time. Unique peptide sequences that recognize lung, skin, pancreas, brain, or kidney endothelial cells *in vivo* were identified. A chimera peptide with a homing domain (NGR motif made cyclic with two flanking cysteines or a double-cyclic RGD-containing motif [4RGD-4C]) linked to a synthetic proapoptotic (PAP) 14-amino acid (KLAKLAK)₂, when internalized by cultured endothelial cells, caused mitochondrial swelling and disruption, followed by apoptosis. The endothelial cell killing is more pronounced under conditions leading to angiogenesis, both *in vitro* and *in vivo*, in mouse tumor xenografts (46). More recently, our authors' (R.P. and W.A.) lab demonstrated that an adipose tissue binding peptide coupled to PAP led to reduction of adipose tissue in an obese mouse model (47).

We identified a unique lung endothelial cell peptide (called LHP) using cultured mouse endothelial cells for positive selection. This LHP bound specifically to lung endothelial cells, but not to prostate, brain, bone marrow, and kidney endothelial cells. Importantly, fluorochrome-labeled LHP bound and was internalized by cultured endothelial cells. *In vivo* LHP-containing phage homed to lung but not brain. We then confirmed the ability of LHP-PAP to kill lung endothelial cells *in vitro* and to promote lung alveolar cell apoptosis and emphysema *in vivo*. LHP-PAP caused alveolar cell apoptosis and alveolar enlargement 48 hours after tail vein inoculation, as compared with a scrambled peptide linked to PAP. After 3 weeks, LHP-PAP resulted in persistence of alveolar cell apoptosis, associated with increased lung ceramide levels, increase in markers of oxidative stress, and influx of macrophages when compared with untreated, or LHP unlinked to PAP controls. In aggregate, these data demonstrate that endothelial cell apoptosis suffices to trigger several of the critical events associated with experimental emphysema. This work provides us with the framework to test whether specific targeting of epithelial or endothelial lung cells have differential impact on alveolar enlargement and reversibility of alveolar destruction, a feature not reproduced by any of the experimental models, including the cigarette smoke model.

INFLAMMATION AS THE RESULT OF FAILURE OF MAINTENANCE: ROLE IN AGING AND EMPHYSEMA

The concept of aging based on disposable soma proposed by Kirkwood (12) predicates that aging consists of accumulation of cellular injury and molecular damage, caused by random environmental events, stress, and poor nutrition. The rate of aging and life span would rely on genetic control of organismal maintenance. When there is accumulation of cellular defects, inflammation would ensue. These inflammatory reactions can exacerbate cellular injury. The importance of inflammation in limiting life span has been shown in that the increase in the human life span is correlated to better control of systemic inflammation, particularly the control of infectious diseases late in the 19th and into the 20th century (48). It is conceivable that aging-related inflammation may impose further limitations on human life span.

Consistent with the hypothesis that inflammation may supersede age-damaged organs, inflammation in emphysema might result from the organ-specific damage caused by cigarette smoke, or excessive accumulation of endogenous mediators such as ceramide, or pathologic processes such as degradation of extracellular matrix, oxidative stress, and abnormal levels of apoptosis. Indeed, we found that intratracheal instillation of ceramide (39)

or intraperitoneal instillation of LHP-PAP led to influx of macrophages in alveoli, which was associated with alveolar cell apoptosis and oxidative stress. Apoptosis, although antiinflammatory in nature, can lead to persistent inflammation if apoptotic bodies are not removed in a timely manner, possibly initiating autoimmune processes (49), as highlighted in this conference (50, 51). The nonstochastic stimulation of inflammatory cells is perhaps best illustrated by the finding of oligoclonal populations of lymphocytes in emphysematous lungs (52, 53). These findings may provide compelling reasoning to challenge the concept of inflammation as the initiator of the cascade of events related to COPD. Instead, inflammation could represent the result of a long-standing destructive process in the lung and itself a source of additional injury. Early intervention blocking these destructive processes caused by cigarette smoke might represent more appropriate targets for treatments.

CONCLUSIONS

Kirkwood concludes in his review on the underlying concepts behind aging that "cellular defects often cause inflammatory reactions, which themselves exacerbate existing damage" and that "inflammatory and anti-inflammatory factors can play a part in shaping the outcomes of the aging process" (12). These statements are perfectly suited to the understanding of the role of inflammation in COPD. The collapse of protective mechanisms may explain the extensive nature of emphysema present in α_1 -antitrypsin-deficient patients. α_1 -Antitrypsin acts not only as an antiprotease but also binds and inactivates active caspase-3, thus protecting against apoptotic lung destruction (54). Interventions aimed at inflammation may produce beneficial results if linked to the underlying tissue damage promoted by chronic cigarette smoking. Organismal stresses induce potent molecular responses as means to decrease cellular damage. One of these "early" reaction pathways involves the intersection between the growth-promoting PI3 kinase/AKT/mTOR pathway with the tumor suppressive negative regulator pathway involving RTP801 (or REDD1 for REgulated in Development and DNA Damage responses), and the tuber sclerosis complex 1 and/or 2. In this conference, we presented data showing that RTP801, a molecule induced by hypoxia in a hypoxia-inducible factor-1-dependent manner (55), and cellular responses with apoptosis and oxidative stress, is intimately involved with acute lung inflammatory and apoptotic responses caused by experimental cigarette smoke inhalation. This pathway has been extensively investigated in *Drosophila* flies and mammalian cells, directing cellular responses (i.e., cell growth arrest or apoptosis) to an adverse environment such as that due to glucose deprivation or hypoxia. The studies linking the AKT pathway, protein translation, and cell growth with inflammation will provide further evidence that the alveolar injury caused by cigarette smoke should be framed more broadly, where inflammation and protease and antiprotease imbalance are pathobiological elements to be considered within the context of lung cellular injury involving oxidative stress and apoptosis caused by chronic cigarette smoke inhalation.

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Ceramide Causes Pulmonary Cell Apoptosis and Emphysema

A Role for Sphingolipid Homeostasis in the Maintenance of Alveolar Cells

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Alveolar cell apoptosis is a critical element of experimental and human emphysema, in that it accounts for alveolar destruction and interacts with oxidative stress, inflammation, and protease activation, all contributing to the unique nature of the disease. Prompted by our finding of up-regulated ceramide levels in lungs of patients with smoking-induced emphysema (1), we demonstrate that ceramide, a second messenger lipid, is a critical mediator and amplifier of alveolar cell apoptosis in experimental emphysema. Inhibition of enzymes of the *de novo* ceramide synthesis prevented lung ceramide increases, alveolar cell apoptosis, oxidative stress, and emphysema caused by blockade of vascular endothelial growth factor receptor (VEGFR) with SU5416 in both rats and mice. The lung alterations seen with VEGFR blockade were reproduced with intratracheal instillation of ceramide in naive mice. Furthermore, reduction of lung levels of very long ceramides after administration of a neutralizing ceramide antibody *in vivo* and the inability of acid sphingomyelinase-deficient fibroblasts to augment endogenous ceramide synthesis in response to exogenous ceramide indicated a feed-forward mechanism of ceramide regulation mediated via the secretory acid sphingomyelinase. Finally, the finding that supplementation of sphingosine-1-phosphate (S1-P) prevented lung cell apoptosis due to VEGFR blockade suggests that ceramide-S1-P balance might be critical in the maintenance of alveolar septal integrity. We have therefore identified ceramide as a critical pathogenetic element in emphysema and a promising target for pharmacologic intervention in a disease with no available therapies.

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Epithelial Cell PPAR γ Is an Endogenous Regulator of Normal Lung Maturation and Maintenance

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Peroxisome proliferator-activated receptor- γ (PPAR γ) is a nuclear hormone receptor that regulates gene expression, cellular differentiation, and tissue inflammation. PPAR γ is expressed in the murine lung in a spatio-temporally restricted pattern and is prominent within the airway epithelium. To study the physiologic role of this transcription factor in regulating lung development and the response to inflammatory stimuli, we have generated mice with conditional PPAR γ deficiency within the conducting airway epithelium. Isolated airway epithelial cells from conditionally targeted mice showed a 75% reduction in PPAR γ mRNA expression by real-time polymerase chain reaction and a 50% reduction in PPAR γ -expressing cells by immunocytochemistry. These mice display airspace enlargement in the absence of overt inflammation. Morphometric analysis revealed a 13% increase in chord length and 33% increase in airspace area (both $p < 0.01$) in targeted animals compared with littermate controls at 8 wk of age. There was no overt evidence of inflammation present. Abnormal airspaces were not present at 2 wk of age and persisted, but did not progress, at 9 mo of age. These data suggest a defect in terminal lung maturation, which occurs throughout the first month of life in rodents. Whole lung genomewide expression profiling revealed a general decrease in extracellular matrix (ECM) gene expression, including elastin and type I collagen. As these proteins are not expressed by the targeted epithelial cells, the data suggest a molecular mechanism for the observed phenotype whereby PPAR γ deficiency disrupts epithelial-mesenchymal interactions, resulting in decreased structural ECM gene expression and abnormal lung structure. Expression profiling of freshly isolated airway epithelial cells identified dysregulation of genes involved in PPAR γ function, lipid metabolism, cellular differentiation, and inflammation in the conditionally targeted animals. To examine the role of epithelial cell PPAR γ in regulating lung inflammation, we challenged adult mice to cigarette smoke for 6 mo. Targeted animals had greater smoke-induced emphysema when compared with age-matched, wild-type littermate controls (28% increase in chord length, 50% increase in alveolar area) and age-matched, unchallenged knock-outs (20% increase in chord length, 29% increase in alveolar area). Targeted mice also showed an approximate twofold increase in macrophages number ($p < 0.01$) after smoke exposure, as quantified by MAC3 immunohistochemistry. We conclude that epithelial PPAR γ is necessary for proper lung maturation and response to injury. Ongoing studies are further defining the regulatory mechanisms involved.

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