Age-dependent changes in pulmonary endothelium contribute to worsened clinical outcomes in elderly individuals. Due to altered pulmonary endothelial responses, older participants have increased vulnerability to infection-related sequelae, higher prevalence of pulmonary hypertension, mitigated DNA repair mechanisms, and attenuated parenchymal healing. Aberrant signaling in pulmonary endothelium undergird these clinical processes. In this review, we provide an overview of the work that has elucidated age-related molecular derangements in pulmonary endothelial cells. In particular, we summarize studies describing mishandling of intracellular reactive oxygen species, pathological nitric oxide signaling, and deficient recruitment of endothelial stem cell precursors. We conclude with a summary of potential future avenues of investigation. The signaling pathways associated with pulmonary endothelial senescence reviewed herein suggest a number of putative therapeutic drug targets. Further elucidation of the cellular processes associated with aging in the pulmonary endothelium may provide critical insights into the rational design of therapies that may subvert or even reverse the effects of aging on a molecular level.

Key Words: Endothelium—Aging.

Received September 6, 2011; Accepted December 13, 2011

Decision Editor: Rafael de Cabo, PhD
DYSFUNCTION IN SENESCENT PULMONARY ENDOTHELIUM

Reciprocally, murine transgenic hosts with selective overexpression of SOD3 in the pulmonary endothelium had a 30% decrease in mortality compared with wild-type hosts when subjected to normobaric hyperoxia (7). Increased survival in the transgenic hosts was associated with decreased pulmonary polymorphonuclear and mononuclear cell infiltration, pulmonary edema, and morphologic lung damage. Therefore, it is believed that the age-associated reduction in enzymatic activity of SOD3 could in part be responsible for the observed increased susceptibility of aged mice to lethal sepsis.

Conversely, mishandling of intracellular reactive oxygen species (ROS) in the senescent pulmonary vasculature likely contributes to worsened clinical outcomes in elderly patients with pulmonary sepsis. Elderly patients exhibit reduced tolerance to systemic inflammation with an increased mortality rate compared with younger septic individuals (9). This reflects a critical burden to the health care system, where within the United States an estimated 700,000 patients, the majority of whom are older, present with septic shock, resulting in approximately 215,000 deaths (10).

The observed decreased tolerance to infection-related sequelae is thought in part to be related to aberrant responses of pulmonary endothelium to inflammatory mediators. During a systemic inflammatory response, toxin-mediated recruitment of immune cells elicits neutrophil and mononuclear cell activation, resulting in respiratory burst and local elaboration of free radicals. These free radicals like superoxide, when exposed to NO produces highly cytotoxic reactive nitrogen species such as peroxynitrite that promote posttranslational modification of native amino acid residues like tyrosine, generating neo-epitopes in pulmonary parenchymal tissues (11). It was further demonstrated that elderly mice injected with LPS displayed greater pulmonary edema and hemorrhage and concurrent increases in oxidative nitration of pulmonary self-antigens when compared with lungs of young mice. The authors identified proteins that were disproportionately modified via tyrosine nitration in aged hosts relative to young LPS-treated hosts. These protein targets included a broad array of proteins that played a role in cellular oxidative metabolism, ionic transport, and cytoskeletal dynamics. The relative increase in pathological modification of these proteins in aged hosts was postulated by the authors to result in concomitant potentiated compromise of their respective protein functions. In turn, the authors surmise that the greater compromise of protein function could explain the worsened pulmonary sequelae observed in elderly hosts.

The higher burden of acquired neo-epitopes in sepsis models has also been described to result in increased pro-thrombotic responses in elderly hosts (12). Elderly and young murine hosts showed an 80% and 0% mortality rate, respectively, when treated with an identical dose of LPS (2.5 mg/kg). The authors found that, in response to LPS, senescent hosts displayed increased thrombosis and hemorrhage in the pulmonary vasculature. Underlying this response, fibrin formation was found to be increased in elderly hosts along with grossly attenuated expression of thrombomodulin, an endothelial cell membrane receptor that accelerates the conversion of protein C to activated protein C that in turn leads to downregulation of thrombin and fibrin formation. The authors posit that the age-related loss of thrombomodulin expression led to increased pulmonary thrombosis and depletion of coagulant factors, resulting in hemorrhage and worsened outcomes in senescent hosts in response to endotoxemia.

Enzymatic activity of antioxidants was recently examined in senescent pulmonary endothelium. Enzymes implicated in cytoprotective reduction of ROS such as Cu/Zn superoxide dismutase, Mn superoxide dismutase, catalase, glutathione peroxidase, and NADPH oxidase showed a trend to decreased levels in aged pulmonary endothelial cells. In

Figure 1. Mechanisms of dysfunction in senescent pulmonary endothelium.
this study, the downregulated expression of antioxidant enzymes was associated with concomitant age-dependent significant increases in measured ROS, superoxide, and hydrogen peroxide in segments of pulmonary arterial segments ex vivo (13).

The pathologically altered response to oxidative stress during sepsis is further compounded by defective age-related resolution of pulmonary edema by pulmonary endothelium. Interstitial fluid accumulation commonly occurs during septic states due to increased capillary permeability, creating ventilatory mismatch by decreasing oxygen-diffusing capacity into pulmonary capillaries. Clearance of interstitial fluid is mediated by aquaporins, membranous protein channels inducibly inserted into the abluminal side of alveolar endothelial cells that permits intraluminal transport of water into pulmonary vessels, thereby alleviating hydrostatic- and osmotic-driven water transport into interstitial space. A recent study demonstrated that senescent pulmonary endothelial cells showed decreased levels of aquaporin isoforms, aquaporin-1 and aquaporin-5, when compared with younger controls (14). This relative deficiency in aquaporin expression was accordingly associated with significant decreases in intraluminal hydrostatic and osmotic water transport when compared with younger hosts in a murine model of pulmonary edema. Deficient expression of aquaporin water channels in senescent pulmonary endothelial cells could further be restored with administration of dexamethasone, providing a putative mechanism for the efficacy of steroids in the treatment of acute respiratory distress syndrome (15).

Impaired NO Signaling During Senescence

The pulmonary vascular bed regulates regional vascular resistance through elaboration of NO, a vasorelaxant gaseous signaling molecule that increases intraluminal diameter (16). NO is produced in endothelial and smooth muscle cells lining pulmonary vessels by synthases of which there are three isoforms, endothelial, neuronal, and inducible. Endothelial NO synthase is the predominant pulmonary endothelial synthase isoform, whose catalytic activity is governed by the intracellular calcium/calmodulin complexes as well as the formation of stable protein complexes with subcellular protein chaperones such as heat shock protein 90 and endoglin (17).

Endoglin (CD105) is an ancillary receptor for transforming growth factor-β superfamily ligand including bone morphogenetic protein (18,19). It is primarily expressed in pulmonary and systemic endothelial cells. Homozygous deficiency in endoglin expression is lethal in utero, resulting in angiogenic defects and severe cardiac morphologic defects during midgestation (20). Heterozygous endoglin deficiency, however, results in age-dependent formation of extensive intrapulmonary arteriovenous malformations in low arteriole resistance territories including the lung, liver, and brain—a syndrome characteristic of hereditary hemorrhagic telangiectasia (20,21).

A recent study showed hemodynamic, histological, and micro-computed tomographic imaging data to support the notion that endoglin null heterozygotes develop pulmonary arterial hypertension in an age-dependent fashion (22). Relative to wild-type hosts, endoglin+− heterozygotes showed significantly increased right ventricular systolic pressures compared with age- and strain-matched controls, starting around 8 weeks of age. These hemodynamic alterations were accompanied by significant increase in right ventricular mass, positive pulmonary arteriolar remodeling with increased mural diameter, and rarefaction of abundant small distal pulmonary vessels in lieu of fewer but larger caliber muscularized vessels. These changes occurred exclusively in senescent hosts but not in younger strain-matched hosts.

Due to the age-dependent pulmonary phenotype, the effects of pulmonary endothelial senescence in heterozygote endoglin-deficient hosts were recently examined by two groups of investigators (22,23). Aged endoglin-deficient heterozygote hosts (8–12 weeks old) showed destabilization of heat shock protein 90 and endothelial NO synthase protein complexes relative to heterozygote newborn hosts (5–8 days old) and heterozygote prepubertal hosts (3 weeks old) in coimmunoprecipitation experiments following pulmonary endothelial cell induction with ionomycin, an ionophore causing intracellular calcium influx. Although this destabilization neither altered the overall vasorelaxant properties of pulmonary endothelium nor changed the local NO levels, endoglin−+ hosts showed significant age-dependent accumulation of measured ROS when compared against endoglin heterozygote newborns and prepubertal hosts. This increased production of ROS was abolished with the NO synthase inhibitor, L-NG-Nitroarginine methyl ester. Together these data collectively demonstrate that endoglin deficiency leads to the uncoupling of the electron transfer reactions necessary to produce NO, resulting instead in the production of free radical species. Age-related decrement in endothelial NO synthase activity has been well-documented and is reviewed in Hayashi and colleagues (24).

Molecular Regulation of Endothelial Cell Lifespan

Cellular life expectancy is governed by the length of telomeric DNA sequences, which become shortened with progressive cell divisions. Due to incomplete replication of the lagging strand of DNA during cellular replication, some telomeric DNA are lost as cells divide enabling a predetermined number of divisions and facilitating replicative senescence once this limit has been reached (25). Aside from physiologic telomere loss due to cell division, pathological erosion of telomere length may also occur secondary to genotoxic damage by ROS (26,27), oncogene-induced replicative stress (28,29), and deficiency in binding of telomere-stabilizing proteins
Gestated in a prior study whereby the CD34 surface stem cell marker was localized to pulmonary endothelial cells in human lung tissue sections (38). A side population of multipotent self-renewing stem cells was recently isolated and characterized in surgical lung tissues from healthy donors (39). Human donor lung samples were immunostained with c-kit (CD117), a proto-oncogene that encodes a transmembrane tyrosine kinase receptor that functions as a cytokine receptor for stem cell factor (steel factor). C-kit is implicated in morphogenesis of multiple tissues and as such has been implicated in primitive human solid organ tumors like small cell lung cancer, gastrointestinal stromal tumors, and testicular seminomas (40) as well as liquid malignancies including acute myelogenous leukemia and mast cell tumors (41).

The c-kit positive cells from human pulmonary tissue sections showed a primitive surface marker phenotype (negative for clara cell 10-kd secretory protein and cystic fibrosis transmembrane conductance regulator) that was furthermore negative for surface antigens expressed in intermediate stages of differentiation in mesenchymal stromal cells (CD44, CD90, CD105), and mast cells (CD45, CD29, CD49d, CD49e). Further, although in culture, these cells demonstrated proliferative potential with a self-renewing phenotype. Importantly, this population of stem cells, when adoptively transferred into cryoinjured pulmonary tissues in vivo, gave rise to functional pulmonary structures including bronchioles, alveoli, and pulmonary vessels that were structurally and functionally integrated with native pulmonic tissues. These regenerative changes occurred rapidly, within 2 weeks of iatrogenic cryoinjury.

Although the frequency of pulmonary stem cell precursors is low, dynamic expansion of stem cells in vivo in response to acquired lung disease has been observed. In a model of canine pulmonary hypertension, CD133+ cells representing a putative pulmonary endothelial progenitor population were serially sorted by flow cytometry 48 hours and then 6 weeks following dehydromonocrotaline-induced pulmonary hypertension (42). The investigators found that the number of circulating CD133+ cells significantly increased at 48 hours but then became significantly decreased after development of pulmonary hypertension, characterized by increased pulmonary artery pressure and right ventricular systolic pressure as well as persistent hypoxia. Relative to CD133+ cells taken at 48 hours after dehydromonocrotaline treatment, the CD133+ cells isolated at 6 weeks after development of pulmonary hypertension further demonstrated decreased ability for vasculogenesis as shown in tubule-forming assays and displayed increased frequencies of β-galactosidase expression, a marker for senescence whereby cells display viability but inability to proliferate.

These studies suggest an important role for resident pulmonary stem cells to repair and potentially regenerate functional pulmonary structures, including the pulmonary endothelium. Key questions that remain are (a) which stem cell population has the greatest reparative and pluripotent potential, (b) how aging process affects their pluripotency, (c) the role of TERT, TERT overexpressing hosts were crossed with hosts expressing increased median life span (32).

A study in systemic endothelium implicated senescence in arterial endothelial dysfunction in atherosclerosis (33). Coronary arteries obtained from participants with ischemic heart disease showed higher beta-galactosidase activity, a marker for cellular senescence. To recapitulate senescence in vitro, TERT was pharmacologically inhibited in arterial endothelial cells, leading to increased adhesion molecule expression and decreased NO synthesis. These senescent-related changes could be reversed with TERT overexpression, implicating a role for senescence-mediated endothelial cell dysfunction during coronary artery disease. The local milieu of advanced atherosclerotic plaques, rich in ROS, also contributes to endothelial senescence. TERT enzymatic activity in atherosclerotic lesions is mechanistically linked to local formation of ROS, ROS chelation and blockade of ROS formation with pharmacologic agents such as aspirin (34), statins (35), estrogen (36), and thiazolidinediones (37) block ROS-mediated export of TERT and delay replicative senescence in arterial endothelial cells (35). Though accumulated evidence suggests that TERT-mediated delay in senescence occurs in systemic endothelium, it remains to be seen whether this is the case in pulmonary vasculature.

Age-Dependent Deficits in Pulmonary Cellular Repair and Regeneration

There has been a recent increase in investigative interest in stem cells as a therapeutic modality to autologously rebuild damaged tissues or to synthesize functional organs de novo. Exploiting their multipotent potential, stem cells have the putative capability to repair age-related damage to pulmonary tissues, including the endothelium. The existence of a pulmonary endothelial stem cell population was suggested in a prior study whereby the CD34 surface stem cell marker was localized to pulmonary endothelial cells in human lung tissue sections (38). A side population of multipotent self-renewing stem cells was recently isolated and characterized in surgical lung tissues from healthy donors (39). Human donor lung samples were immunostained with c-kit (CD117), a proto-oncogene that encodes a transmembrane tyrosine kinase receptor that functions as a cytokine receptor for stem cell factor (steel factor). C-kit is implicated in morphogenesis of multiple tissues and as such has been implicated in primitive human solid organ tumors like small cell lung cancer, gastrointestinal stromal tumors, and testicular seminomas (40) as well as liquid malignancies including acute myelogenous leukemia and mast cell tumors (41).

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These studies suggest an important role for resident pulmonary stem cells to repair and potentially regenerate functional pulmonary structures, including the pulmonary endothelium. Key questions that remain are (a) which stem cell population has the greatest reparative and pluripotent potential, (b) how aging process affects their pluripotency,
and (c) whether their frequency in the lungs is affected by aging. A prior study suggested that the frequency of pulmonary endothelial precursor population as identified by CD34 staining was greater in children and decreased with age (38). However, further validation of this finding and characterization of their functionality is necessary to better define the role of these cells in health and disease.

**Conclusions and Future Avenues of Investigation**

Elderly patients have a higher susceptibility to disease processes such as pulmonary sepsis and pulmonary hypertension that at least in part involves the molecular mechanisms presented here (Figure 2). These phenotypic associations are fundamentally driven by an array of pathological molecular processes that, despite recent work, remain in the nascent stages of investigation. The putative role of alternative pathways associated with senescence such as apoptosis (43,44) or G-protein–coupled receptor signaling networks in pulmonary endothelium (45,46) has not been thoroughly explored in the senescent pulmonary vasculature. Ongoing identification and elucidation of the molecular underpinning of these processes are critical for the rational design of therapies to palliate or reverse the worsened pulmonary outcomes in elderly patients.

Native systems of regeneration involving resident stem cell populations in pulmonary tissues may be exploited for end-organ repair during the natural process of senescence or in acquired pulmonary disease in aged individuals. However, analogous to cardiac-resident stem cells, these cells reside at a very low frequency and likely bear limited regenerative capability in mature, adult tissue. Therapeutic reversal of this age-dependent interdiction of regenerative function is a potentially fertile area of future study. Furthermore, although pulmonary stem cells bear promise as a therapeutic modality, research into alternative, noncellular pathways of repair and regeneration is necessary. Ectopic potentiation of cellular repair mediated by activation of endogenous proteins such as TERT (25) and sirtuin deactylases (47) may allow for circumvention of histocompatibility barriers and potential tumorigenic side effects associated with heterologous and autologous stem cell therapies.

In conclusion, the pulmonary endothelium undergoes a number of molecular alterations during the aging process. These molecular alterations drive a number of pathological processes including mishandling of ROS, pathological NO production, and defective mobilization of pulmonary stem cells. Despite the myriad advances in the understanding of pulmonary endothelial senescence, further research is necessary to further elucidate the pathological processes associated with pulmonary endothelial aging in an attempt to therapeutically modulate or reverse the course of pulmonary endothelial senescence.

**Funding**

HL095654 from the National Institutes of Health/National Heart, Lung, and Blood Institute to H.J.C. and Physician Scientist Early Career Grant from the Howard Hughes Medical Institute to H.J.C.

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