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Senescence in Pulmonary Fibrosis: Between Aging and Exposure

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To date, chronic pulmonary pathologies represent the third leading cause of death in the elderly population. Evidence-based projections suggest that >65 (years old) individuals will account for approximately a quarter of the world population before the turn of the century. Genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication, are described as the nine "hallmarks" that govern cellular fitness. Any deviation from the normal pattern initiates a complex cascade of events culminating to a disease state. This blueprint, originally employed to describe aberrant changes in cancer cells, can be also used to describe aging and fibrosis. Pulmonary fibrosis (PF) is the result of a progressive decline in injury resolution processes stemming from endogenous (physiological decline or somatic mutations) or exogenous stress. Environmental, dietary or occupational exposure accelerates the pathogenesis of a senescent phenotype based on (1) window of exposure; (2) dose, duration, recurrence; and (3) cells type being targeted. As the lung ages, the threshold to generate an irreversibly senescent phenotype is lowered. However, we do not have sufficient knowledge to make accurate predictions. In this review, we provide an assessment of the literature that interrogates lung epithelial, mesenchymal, and immune senescence at the intersection of aging, environmental exposure and pulmonary fibrosis.

Keywords: senescence, lung fibrosis, epithelial cells, inflamm-aging, immune-senescence, aging, mesenchymal senescence

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AN INTRODUCTION TO PULMONARY FIBROSIS

Pulmonary fibrosis (PF) is a disease of senescence, weakened anti-inflammatory activation, and aberrant resolution (1, 2). PF is a rare degenerative pathology (overall incidence of just 13–17/100,000 people/year) characterized by temporally and spatially heterogeneous injury. The excessive production and disorderly deposition of extracellular matrix proteins and collagen that accompanies disease progression is driven by (myo-)fibroblasts and immune cells clustering within aberrant alveolar structures (honeycombs) (3). PF pathogenesis and progression is unpredictable, with genetic mutation (surfactant protein B and C, mucin 5B, telomerases) and environmental factors (cigarette smoke, chronic infections) provoking functional debilitating lesions, lethal within 3–5 years of diagnosis (4, 5). Stratified epidemiological analysis clearly illustrates surge in disease incidence and prevalence in relation to age (93/100,000/year and 494/100,000/year in individuals >65 years old) (6–8). Notably, to date chronic lung pathologies represent the third leading cause of death in the elderly population (1, 9, 10). The magnitude of this healthcare problem is represented by national and global census data showing ∼ =15% of the population currently over 65, and over 2 billion individuals projected to surpass that mark by the year 2050 (11–13).

As the medical field battles the COVID-19 global pandemic, scientists and clinicians have come to terms with the notion that we know very little of the mechanisms mediating lung injury and resolution in the aged and susceptible respiratory system (14–16). To overcome these limitations, this manuscript summarizes the evidence linking lung injury and remodeling in the context of biological aging and chemical exposure.

SENESCENCE AT THE BASIS OF CELL DYSFUNCTION AND DISEASE

A series of landmark reports identified nine "hallmarks" of aberrant cellular fitness shared by chronic degenerating conditions such as cancer, fibrosis, and aging: (1) genomic instability; (2) telomere attrition; (3) stem cell exhaustion; (4) epigenetic alterations; (5) loss of proteostasis; (6) deregulated nutrient sensing; (7) mitochondrial dysfunction; (8) senescence; and (9) altered intercellular communication (2, 17–23). During early life/adulthood, a checkpoint system (DNA damage response, apoptosis, unfolded protein response) ensures maximal cellular fitness (24). Aging, environmental exposure, or genetic perturbations in key functional proteins induce seemingly moderate downstream adjustments to these safeguards, but progressively edges the cell closer to developing an irreversible phenotype (**Figure 1**).

Besides the conventional notion that aging is accompanied by replication-dependent telomere erosion and defective recognition of toxic mutations, a number of convergent systemic failures contribute to the development of a dysfunctional phenotype. For instance, chemicals such as lead, nitrosamines, air/traffic pollution, carbon black are linked to telomere shortening (25, 26). Tobacco smoke and chronic ozone exposure generate reactive oxygen species and trigger mitogenesis (also linked with intracellular ROS production), thereby damaging the DNA and shortening telomeres (27). Acetaminophen, acrolein, chlorpyrofos, chloroquine and heavy metals, are all known to disrupt cellular proteostasis (i.e., unfolded protein response, UPR) (**Figure 2**) (28). This response is associated with a shift in nutrient utilization to favor glycolysis (and away from mitochondrial oxidative phosphorylation) (29). Due to the inefficient and slow ATP production of the glycolytic cycle, the cell progressively accumulates excess ADP and AMP and halts its capacity to proliferate, a process that requires substantial and rapidly available energy. AMP is the preferred substrate for AMPK, leading to activation of p53/p21 and pRB/p16 pathways (30). The substantial amount of pyruvate produced through glycolysis is then shuttled to the mitochondria, leading to: mitochondrial swelling; ROS generation, NADH and NADPH depletion; excess AcCoA (31–33). Mitochondrial and cellular swelling is used as a morphological biomarker to identify senescent cells. The excess ROS production promote DNA damage, which further favor the development of a dysfunctional cell and is linked to the development of a SASP (senescent associated secretory phenotype). The enzymatic conversion of pyruvate in the mitochondria generates a surplus Acetyl-CoA which is shunted to the Krebs Cycle, or in the nucleus where it is utilized as an acetyl-donors to remodel histone structure and thus, regulate cell transcription (34, 35). In support of this notion, pre-senescent and senescent cells display widespread loss of histones H3 and H4 and senescence-associated heterochromatin foci (SAHF) at the hands of the histone chaperones Asf1 and HIRA (36, 37). The role of these proteins is particular important as they represent a cell cycle independent mechanism to modify histones, a function fundamental for replication-restricted senescent cells. This epigenetic reprogram further support SASP by promoting expression and release of highly inflammatory

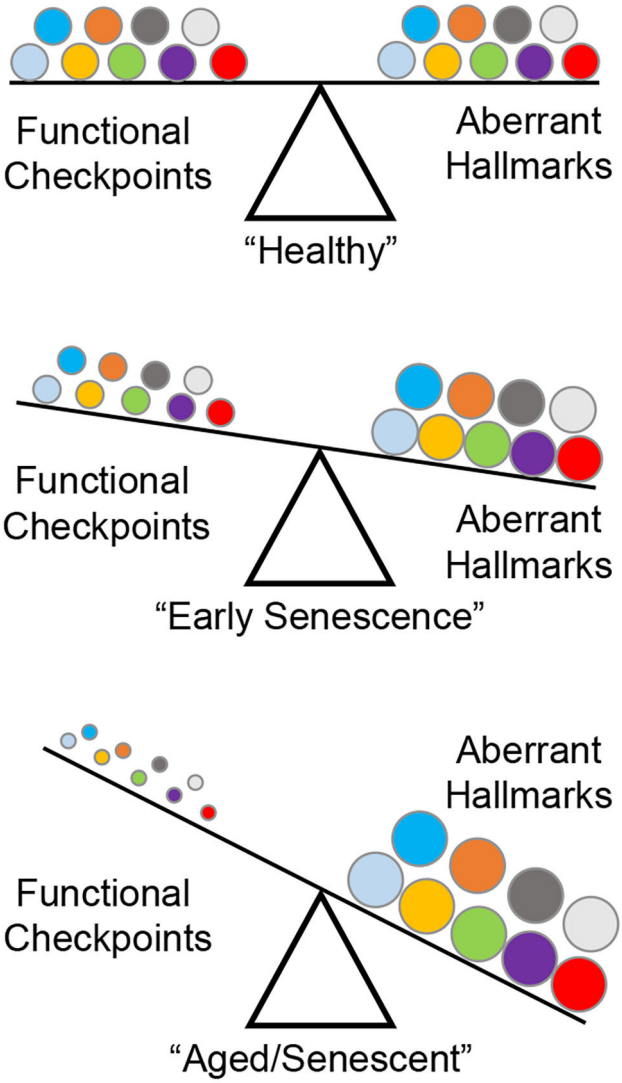

FIGURE 1 | The nine "hallmarks" of cell fitness. Healthy cells rely on a balance between survival checkpoints and aberrant hallmarks. A total of nine factors have been defined: genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication (represented by circles in matching colors). Skewing of this equilibrium generates a progressively senescent phenotype as a result of age-related checkpoint dysfunction driving cellular toxicity.

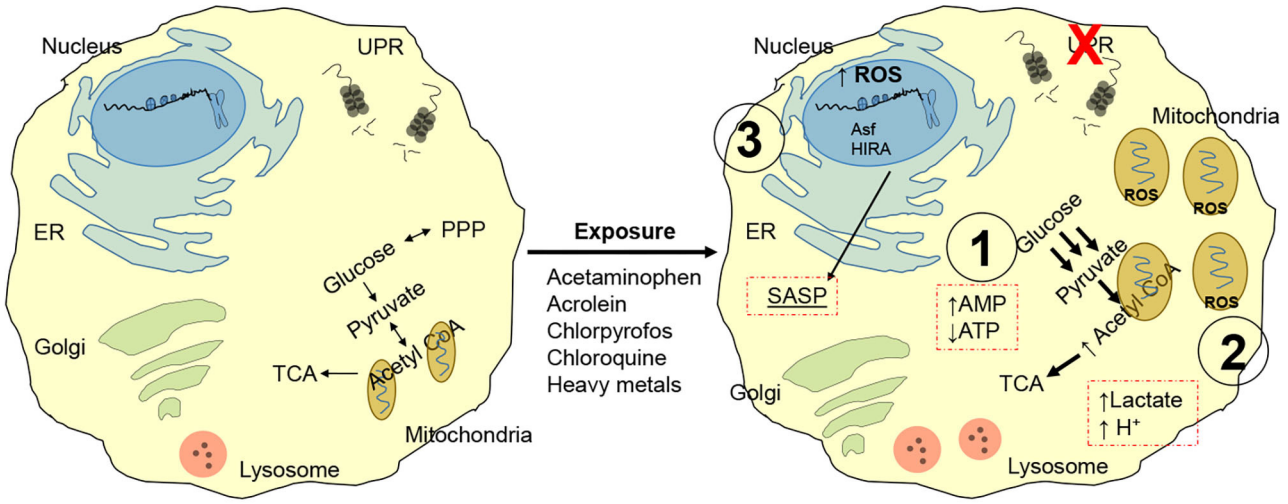

FIGURE 2 | Chemical induced senescence. Acetaminophen, acrolein, chlorpyrofos, chloroquine, and heavy metals exposure disrupts unfolded protein response, UPR. Nutrient utilization is then shifted toward glycolysis, leading to: **(1)** Enhanced AMPK signaling driven by excess AMP, resulting in proliferative latency and pRB/p16 pathway activation. **(2)** Excess pyruvate enters mitochondria for conversion into AcCoA; this leads to mitochondria! overload and swelling, ROS generation and toxic cell acidification. **(3)** Oxidative stress from mitochondria damages cytoplasmic proteins and DNA; AcCoA functions as a acetyl donor for localized histone hyperacetylation, while histone chaperones Asf1 and HIRA greatly remodels chromatin independently of replicative state of the cell. Gene expression resulting from chromatin reprogram results in senescent associated secretory phenotype (SASP), enriched in tumor suppressor proteins, transcription factors, microRNAs, growth factors, proteases, and inflammatory cytokines (e.g., p-galactosidase, p16INK4a, IL-6, CXCR2, IL-1 receptor, C/EBPp, and NF-KB).

factors including tumor suppressor proteins, transcription factors, microRNAs, growth factors, proteases and inflammatory cytokines (e.g., β-galactosidase, *p16INK4a*, IL-6, CXCR2, IL-1 receptor, C/EBPβ, and NF-KB) (38–40). SASP elicits an immense power to reshape the behavior of the surrounding tissue, to the point that just over 20% of senescent cells are sufficient to trigger systemic effects (41).

In light of this evidence, any therapeutic that effectively reduces numbers or activity of senescent cells has significant healthcare potential. While technological advancements produced a broad arsenal of pharmacological moieties, their efficacy against age- and fibrotic-related senescence has been hindered by the paucity of models that mimic the clinical course of disease (42–44).

CHEMICAL EXPOSURE ACCELERATES LUNG SENESCENCE

The current PF paradigm proposes that repeated episodes of alveolar epithelial cell dysfunction triggered by endogenous (genetic predisposition) or exogenous stress (environmental), is necessary to trigger lung fibrogenesis. In particular, the latter remains mechanistically obscure and is often categorized as idiopathic in origin. Mutations of pivotal rheostats such as genes involved in proteostasis, telomere and mitochondrial maintenance have been abundantly mapped in the past two decades (45–51). This has led to the development of robust genetic models, including the naturally occurring senescence accelerated mouse series (SAM-1 to−8) (52), the telomerase reverse transcriptase (TERT) deficient mice (42, 43), the surfactant protein C mutant and null mice (44, 49), or through disruption of cell-cell communication via genetic modulation of key fibrotic signaling pathways (TGF-β or IL-13) (53, 54). These models all display accelerated aging, senescence and pulmonary fibrosis initiated by the lung epithelium and perpetuated by mesenchymal and immune cells (46, 55–57). By comparison, clinically relevant models of chemical induced fibrosis are limited by intrinsic differences among the thousands of environmental, dietary, or occupational stressors the lung comes into contact daily.

Reactive moieties (i.e., ozone), minerals and metals (silica, asbestos, cadmium, beryllium), wildfire and cigarette smoke, particulate matter of sizes 10 μm and below (PM2.5 and PM10), and nanoparticles/nanoplastics are widely used, yet imperfect, surrogates of chemical senescence and fibrosis (58–65). The keen observes may suggest that these models are not dependable or ineffective in predicting senescence and fibrogenesis. This is, in part, true. One clear element may be responsible for this results: age. While epidemiological evidence overwhelmingly show that age disproportionally impacts the outcome of chemical exposure and/or fibrosis (i.e., clinical PF is, on average, diagnosed at age 65), experimental modeling predominantly examines young and healthy animal cohorts. As a result, we have carefully modeled mechanistic datasets that poorly translate to aged murine cohorts, or the human condition. In support of this notion, sterile and infectious challenge (infection, radiation, and cigarette smoke) elicits heightened toxicity in aged mice compared to young ones, a response linked to the development of irreversible senescence (66–69). To overcome these limitations and provide adequate prediction of how do environmental exposures that

lead to cellular senescence lead to different lung pathologies, it is absolutely necessary to test chemical exposure in aged, as well as susceptible cohorts starting, perhaps, from the aforementioned TERT and SP-C mutant mice.

CONDITIONS FOR CHEMICAL INDUCED SENESCENCE/FIBROSIS

In-depth analysis of the fibrogenic effects of environmental exposure on the senescent lung is critical to advance the field and better address the needs of susceptible populations. At least three aspects need to be considered: window of exposure; dose, duration and recurrence of exposure/injury; cell type-specific responses.

Window of Exposure

Exposure to an inhaled toxicant during early life has been suggested to increase susceptibility to disease by reshaping the parenchymal and inflammatory cell milieu (70–72). For instance, *in utero* and early life exposure to tobacco smoke, respiratory viral infections and gestational diabetes impairs lung development and function by reshaping the cellular metabolic and inflammatory machinery at the chromatin level (epigenetic), thus greatly increasing the incidence of chronic pathologies including asthma, COPD and fibrosis (73–76). Widely used as the standard model of acute lung injury, ozone represents the perfect example of an environmental toxicant that produces variable responses across the lifespan, through widespread epithelial and bronchial oxidative damage and inflammation (77, 78). The pattern recognition receptor TLR4 is partially responsible for ozone responses (63, 79). Therefore, the known age-related alteration in expression (low at birth) impacts the cellular and structural responses elicited upon exposure (80, 81).

Dose, Duration, and Recurrence of Exposure

There is extensive evidence that, although often well-tolerated, repeated toxic exposure promotes progressive genetic instability, epigenetic remodeling (i.e., cadmium); proteostatic and mitochondrial dysfunction (i.e., ozone); genesis of SASP (i.e., multi-walled nanotubes, asbestos); or all of the above (i.e., cigarette smoke and radiation) (82–87). The (often) cyclical nature of environmental/occupational exposure is also linked to exhaustion of the stem cell reservoir, their depletion, and SASP (88–90). These effects progressively diminish the ability of the lung to respond to subsequent challenges even of modest intensity. **Figure 3** portrays possible outcomes resulting from to acute and chronic exposure of susceptible individuals. For instance, aging of an individual presenting somatic predisposition (i.e., SP-C mutation) may lead to fibrogenesis, compared to a health individual. Similar differences can be observed following sublethal chronic exposure, with susceptible population developing chronic pathologies. Ozone and particulate matter/dusts, once again, provide the perfect examples of moderate/sublethal and potentially recurrent stressors linked to fibrotic disease. Indeed, modeling short term

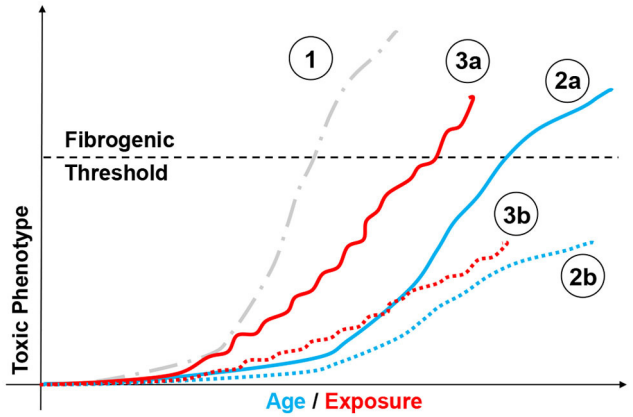

FIGURE 3 | Modeling chemical exposure on the fibrotic phenotype. Depiction of possible outcomes resulting from aging and chemical exposure and their relationship to fibrogenesis. Highly fibrogenic chemical exposure (**1**, gray dotted line,) may drive rapid and possibly lethal fibrosis after a single exposure. By comparison, aging may lead to different disease profiles based on factor such as genetic instability (i.e., SP-C mutation). In such case, an individual presenting somatic mutations may be predisposed to develop a fibrotic phenotype without toxic challenge (**2a,** blue line,), compared to a healthy individual (**2b**, blue dotted line,). Similar responses can be observed following mild/moderate repeated exposure, with susceptible population (**3a,** red line,) passing the "fibrogenic threshold", whereas healthy cohorts will not. (**3b,** red dotted line,) or never reach that threshold depending on factor such as genetic susceptibility. Similarly, aging may be associated with fibrotic and non-fibrotic outcomes depending on individual biological clocks.

acute ozone exposure produces neutrophilic, monocytic, or eosinophilic responses at doses ranging from 0.8 to 3 ppm (91–93). Repeated low-dose ozone exposure (0.8 ppm, 4 h/day, 9 days) generates subchronic multicellular inflammation with extensive airway and goblet cell involvement (94), progressing to fibrosis following 6 weeks of exposure (95). Genetic manipulation of the inflammatory collectin surfactant protein-D further supports the notion that a lifetime of sub-toxic inflammation and oxidative stress reshapes parenchymal function (senescence) and could possibly impact lung responses to exposure later in life (80, 96).

Cell Type-Specific Responses

The lung contains more than 40 types of cells representing epithelium, interstitial connective tissue, vasculature, hematopoietic and lymphoid tissue, and the pleura (97). Each of these cell types participate to the correct function of the lung and it is therefore central to consider how chemical exposure differentially affects each cell type to better comprehend the pathogenesis of disease. For instance, mutations of the surfactant protein (SP)-A and -C, or ATP binding cassette subfamily A member 3 (ABCA3) represent well-described examples of alveolar epithelial type 2 distress resulting in chronic lung disease and fibrosis (98, 99). A number of genetic constructs successfully leveraged these mutations to produce lung fibrosis and accelerated senescence triggered by a stressed epithelium (49, 100). While to date is still unclear what effects environmental exposure elicits on such susceptible parenchyma, work from our group and others intends to fill this knowledge gap.

By comparison, chemical exposure does not promote such a cell specific response, often leading to varying degrees of stress, ranging from susceptibility to disease later in life to irreversible senescence and pathogenesis of disease (101, 102). Particulate matter exposure has been widely studied for its importance in lung health (**Figure 4**). This environmental and occupational mixture is known to induce ROS production, activating the inflammasome pathways and triggers unfolded protein response in lung epithelial cells (103). Depending on size (2.5 or 10 μm), dose, and duration of exposure PM elicits epithelial cell death (acute), as well as exacerbation of chronic pulmonary conditions (asthma and COPD) and epithelial to mesenchymal transition (104). Based in these responses it is unsurprising that PM promote fibroblast and myofibroblast proliferation, a response fundamental for fibrogenesis (105–107). At the levels of mucus producing cells (goblet cells), PM results in MUC5B hypersecretion (103). Furthermore, PM engages and functionally impacts innate like cells type 1 (ILC1), thereby blunting their interferon gamma (IFN-γ) production and cytotoxic function; induces antigen-presenting cell-mediated inflammatory responses, while impairing their migration; enhanced neutrophil and eosinophil responses; and shifts lymphocyte differentiation toward an effector phenotype (Th1-like) (108–111). Lastly, the effects on endothelial function (disruption of tight junctions) have significant repercussion on the susceptibility to cardiovascular disease and infarction (112–115). Notably, modeling the toxicity of PM is further complicated by the fact that intrinsic composition (levels of metals, polycyclic aromatic hydrocarbons, carbon black content) and secondary chemicals being carried (LPS and allergens) ranges across time of the year and location where it is collected (116–118). In what seems like a prohibitive task (investigating the effects of hundreds of chemically diverse moieties on dozens of cell types), technological advancements in single cell sequencing analysis and multi-omics approaches significantly eased these challenges. The next section will summarize epithelial, mesenchymal and immune cell senescence induced by aging or chemical exposure.

CELLULAR RESPONSES IN SENESCENCE AND FIBROSIS

Lung Epithelium

While it is well-established that initial respiratory functions are achieved in the nasal epithelium and upper airways, the largest share of research is devoted to the study of alveolar type-1 pneumocytes (AT1) due to their central role in gas exchange. A second alveolar epithelial population, termed AT2, has gained broad recognition as a multipurpose unit in charge of pulmonary surfactant production; regulation of fibroblast proliferation; communication with resident immune cells during homeostasis and injury; control of vascular endothelium permeability to peripheral leukocytes; and replenishment of damaged AT1 cell and mesenchymal (epithelial-mesenchymal transition) pool in stressful conditions (119–121). It is therefore unsurprising that epithelial cell dysfunction produces such a multifaceted phenotype, ultimately linked to senescence and lung remodeling. Supported by clinical evidence, TERT and surfactant protein-C mutant lines today represent robust platforms for modeling epithelium-driven fibrosis (44, 49, 50, 122–124). Mutations in the cystic fibrosis-linked ubiquitin ligase NEDD4-2 produces aberrant epithelial Na+ channel (ENaC) and pro-SP-C localization, processing and degradation, leading to airway surface liquid depletion, impaired clearance of inhaled irritants and progressive architectural and functional alterations consistent with cystic fibrosis-like disease (44, 49, 50, 122–125). The tremendous influence that these proteins elicit on epithelial cell survival and proliferation prioritizes their evaluation in the context of aging. While not directly examining the role of SP-C mutations, experimental evidence linked age-related senescence of the surfactant protein machinery to poor survival following sublethal bacterial challenge (LPS) (126). Similarly, there is clinical evidence that TERT mutations, both familial or exposure-induced (i.e., cigarette smoking), is accompanied by premature deaths (127). The paucity of data that comprehensively examines the responses of a dysfunctional lung epithelium to environmental challenge blurs our ability to determine the mechanistic overlap preceding fibrogenesis. To the same point, chemical challenge has been predominantly examined in juvenile/healthy lungs. While these studies helped defining senescent-like phenotype following exposure, a conspicuous scientific gap remains.

At its essence, the most complex element associated with the study of aging, chemical exposure, or fibrosis, is the asynchronous nature of cellular senescence that accompanies pathogenesis of the phenotype. Application of diffusion pseudotime, a computational single-cell method that can trace the dynamics of biological processes and predict cell fate, may elegantly address this problem (128). Recent use of this methodology to study the evolution of the epithelial cell milieu during bleomycin induced lung fibrosis identified a unique transitional stem cell state, defined by *Krt8* expression, involved in regeneration and healing (129). Lineage tracing techniques and human-derived organoid cultures recently identified a similar population of AT2 cells on their way to terminally mature AT1 cells (130, 131), while single-cell transcriptomics of human IPF and COPD lungs have morphologically (termed basaloid cells for their distinct non-AT1/squamous non-AT2/cuboidal structure) and transcriptionally linked this regenerative subset to fibrotic remodeling (132). While investigation of these transitional epithelial cells is still in its infancy, it is tempting to propose their involvement in resolution of chemical-induced injury; their progressive dysfunction in the context of aging; and to ask whether repeated stress can "exhaust" their replicative potential (133). Addressing each of these questions may advance out understanding of senescence.

A number of recent reports have provided a considerable foundation on the biology of the aging lung epithelium. By combining single-cell RNA-sequencing analysis and proteomics, Angelidis and colleagues generated a comprehensive cell-type specific atlas of the 3 and 24 month old murine lungs (unchallenged), and convincingly presented both ultrastructural and functional changes associated with senescence, including extracellular matrix deposition and epithelial inflamm-aging

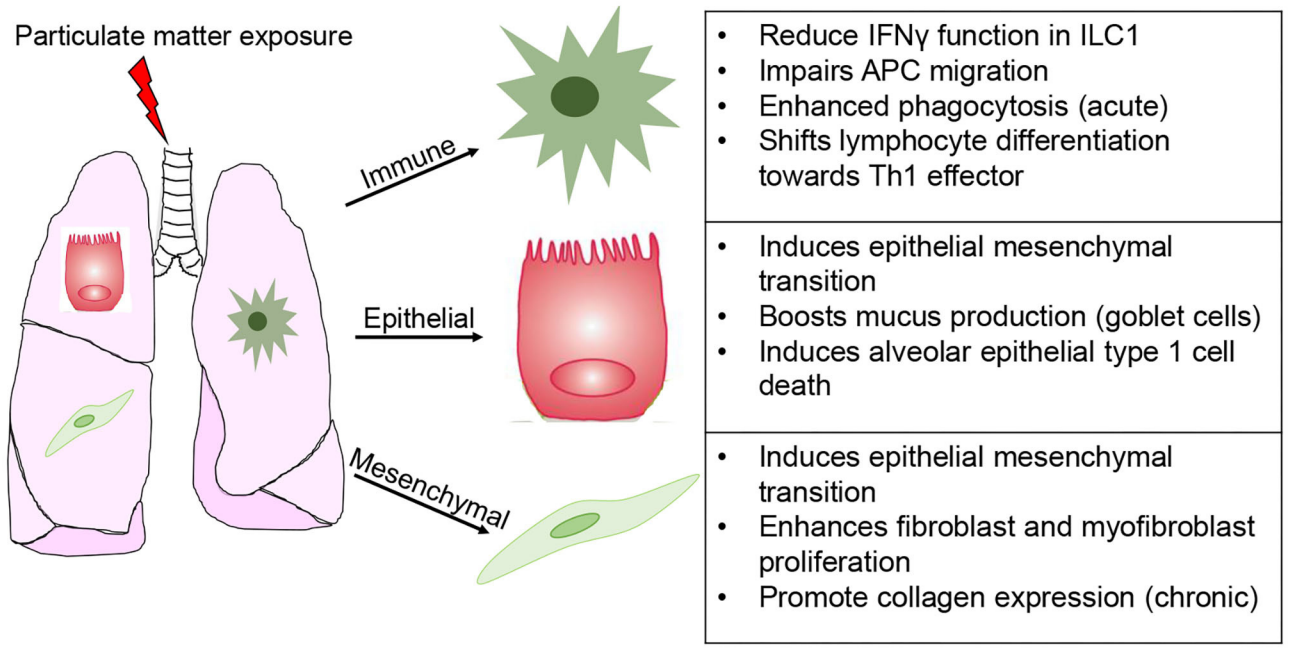

FIGURE 4 | Modeling cell specific responses to chemical exposure. Simplified depiction of the divergent effects of particular matter exposure in immune, epithelial (inclusive of alveolar and mucus producing goblet cells), and mesenchymal (fibroblasts and myofibroblasts).

(134). These epithelial changes reflect, at least in part, organ level dysfunction of the aging lung, characterized by reduced mechanical tissue remodeling, aberrant alveolar derecruitment and impaired oxygen saturation (44, 135). The fibrotic lung presents comparable epithelial and organ wide (functional) alterations, oftentimes in an accelerated and more widespread fashion. The heterogeneity of the injury also complicates assessment of epithelial senescence from a whole organ perspective. As discussed above, instability generated by TERT, ABCA3, SP-A, and SP-C mutations or fibrogenic exposure is accompanied by an epithelial phenotype that aligns with age-induced senescence (cell cycle checkpoint disruption and SASP) (136–138). To date, only correlative evidence (epidemiological) links ambient pollution exposure to acute inflammatory exacerbations that rapidly accelerate lung function decline in IPF patients (102, 139, 140). By combining this observational evidence with the currently available pulmonary disease models, the next decade of pulmonary research has important clinical implications to our pursuit of mechanistic answers of sublethal toxicity in the susceptible lung.

Mesenchymal Cells: Fibroblasts and Stromal Cells

Fibroblasts are highly proliferative cells crucial in maintaining alveolar structural integrity and architecture during homeostasis and throughout the injury resolution process. Understanding the biology of fibroblast senescence is central to link aging and exposure to chronic lung pathologies (141), as their uncontrolled proliferation and/or senescence causes aberrant alveolar remodeling (i.e., impaired gas exchange function). Fibroblast senescence can be triggered directly (somatic mutations and environmental stress), or indirectly (TSLP, IL-25, and IL-33 rich milieu produced by neighboring senescent cells) (142–144).

Aging significantly impacts the extent of fibroblast senescence. For instance, toxic challenge of young primary fibroblasts triggers a "reversible" senescent state, still capable of eliciting programmed cell death mechanisms or resolution pathways. By comparison, cells from aged mice undergo myofibroblast trans-differentiation and develop a profibrotic phenotype (145–147). Analysis of accelerated senescence models (TERT and SAM1-8 murine lines) or human IPF fibroblasts demonstrate aberrant replicative responses (increased expression of cyclins, renin-angiotensin peptides, insulin-like growth factor–binding proteins 3 and 5, Wnt signaling pathway), and altered survival signaling (i.e., apoptosis and autophagic flux) (148–151). A number of environmental stressors, such as ozone and particulate matter, are known to trigger similar oxidative stress and survival pathways. While environmental/occupational exposure to these chemicals is often time subtoxic, chronicity and window of exposure progressively burdens healthy fibroblasts to develop a senescent phenotype (151, 152). This notion is corroborated by experimental modeling using "high-impact" fibrogenic stressors such as bleomycin and gamma radiation, which produces a senescent phenotype comparable to that described in aging (aberrant proliferation and survival, as well as MCP-1, PAI1, TNF-α, MMP10, MMP12, Col1a1, TGFβ, p16, and p53 overexpression) (153).

Mesenchymal Stromal/Stem Cells (MSC): For the past 20 years the acronym MSC has been used to define several subsets belonging to the same mesenchymal family (mesenchymal stem

cell, mesenchymal stromal cell, and multipotent stromal cell). Today, it defines multipotent mesenchymal stromal cells, a nomenclature that separates them from mesenchymal stem cells on the basis of self-renewal and the capacity to differentiate down multiple lineages. While there is no truly unique MSC marker, a combination of hematopoietic progenitor markers CD73, CD90, and CD105 appears to be well accepted (154). This population is canonically associated with a bone marrow origin, but it has been reported that lung resident MSCs are involved in local sustenance of the mesenchymal compartment (155). Although still debated, some evidence also suggest that lung MSCs might be susceptible to differentiate into myofibroblasts and promote airway fibrosis (156).

Dampened stromal signaling in aging immune organs such bone marrow, thymus, lymph node, and spleen is thought to be responsible for the progressive contraction in lymphoid cell numbers and loss in adaptive immune system function in the elderly population (157). The inflammatory microenvironment described with age (inflamm-aging) also triggers stromal cells to produce activation factors (TNFα IL-1β, IL-6, MCP1, MMP-12, MMP-13) that contribute to the phenotypic reprogramming of peripheral myeloid cells prior to their egression to the lung (1, 158, 159). Chemical exposure to pesticides, doxorubicin, bleomycin and radiation demonstrate that the added pressure provided by acute or chronic exposure accelerates stromal cell senescence, thereby contributing to the pathogenesis and progression of the fibrotic response (153, 160, 161). Indeed, primary bone marrow MSCs from IPF demonstrate a highly senescent phenotype, characterized by mitochondrial dysfunction, debilitating DNA damage, and the secretory capacity to induce senescence in normal fibroblasts (162).

Immune Cells

Myeloid and lymphoid cell senescence can be described as a combination of heightened inflammatory tone in homeostatic conditions (inflamm-aging), and abnormal immune reaction following challenge (immune-senescence) (163, 164). These responses result in reduced pathogen clearance and inflammatory resolution at the innate immune level. By comparison, senescence of the adaptive system is associated with blunted humoral response and failure to recognize "self" and increases the susceptibility to develop autoimmune disorders. As we dive into cell specific mechanisms of senescence, it is important to define its divergence from "exhaustion," the altered differentiation state observed in chronic infection and cancer. Granted, these two states share a number of features inherent to the function of key transcription factors, metabolic derangement, and a failure to transition to quiescence. However, proliferative dysfunction (irreversible in senescence) and activation state (incompetent in exhausted T cells) is profoundly different (165, 166). Justification of these differences may provide important insights to our understanding of both cellular conditions.

As introduced in the previous sections, senescence is triggered by biological aging and accelerated by fibrotic remodeling, whether induced by somatic mutations or secondary to external challenges (167, 168). Aberrant activation of the immune system has profound effects on disease outcome. To counter excess immune activation in pulmonary injury and fibrosis we adopted broad spectrum immunomodulation (corticosteroids, anti-cytokine antibodies) (169, 170). However, its efficacy has been sporadic and a number of dilemmas remain, as to whether steroid immunomodulation is at all effective in toning down the activation of a senescent immune cell; or if the doses used to treat elderly individuals are adequate to address the myeloid lineage expansion typical of aging (171, 172).

The ever-increasing toolbox of immunological models available to investigate immune cells in pulmonary fibrosis have rarely been used to examine aging and senescence. These include, germ line and inducible knock outs targeting M-CSF, GM-CSF, CD68, and CCR2, as well as Cre/Lox and diphtheria toxin depletion lines targeted against $CD25^+$, $LysM^+$, $CD11b^+$, and $CD11c^+$ cells (93, 173–177). Adoptive transfer, bone marrow chimerism, parabiosis, and lineage tracing have also provided substantial data on inflammatory cell dynamics in lung injury (178, 179). The advent of CRISPR/Cas9 technology has expanded even further the range of possibilities at hand to investigate these questions (180, 181).

It is fundamental to recognize that the behavior of immune cell subtypes is unique and often dependent on the surrounding environment. The failure of broad-spectrum immunomodulatory therapy in PF is a reminder of this. The next section summarizes the current knowledge of age- and chemical-induced senescence on a cell by cell basis.

Macrophages (an *in vitro* Preamble)

Macrophages are considered the archetypal resident guardians of any tissue, acting in unison with their neighboring cells to mount the adequate response to challenge. Today's deep understanding of macrophage phenotype and function results from extensive *in vitro* testing of immortalized cell-lines and bone marrow-derived macrophages to generate two phenotypic states: M1/pro-inflammatory (classically activated; elicited using IFNγ or LPS) and M2/anti-inflammatory (alternatively activated; induced by IL-4/13 or IL-10) (**Table 1**) (182). This research provided the necessary insights to define macrophage extreme plasticity and a bench work for comparison against *in vivo* responses. While *in vitro* assessment of aging is prohibitive, extrapolation of the effects of age-related senescence on macrophage function demonstrated positive results. Utilizing analogous stimulatory conditions (M1: LPS or IFNγ; M2: IL-4) evidence show that macrophage isolated from old mice and conditioned with M1 or M2 prototypical activators exhibit blunted activation compared to macrophages isolated from young mice (iNOS, IL-6, TNFα, and IL-1β, as well as YM-1 and Arginase), a response consistent with the notion of immune-senescence (198). Similarly, bone marrow derived macrophages isolated from p16 knock out mice demonstrate a phenotype resembling that of IL-4 treated M2 cells and inability to elicit pro-inflammatory functions upon IFNγ challenge (199). This wealth of information provided the foundation of *in vivo* research on fibrosis, senescence and aging, and emphasized the perception that macrophage phenotype and activation is organ-specific and non-dichotomous (200, 201).

TABLE 1 | Phenotypic characterization of nine prototypical macrophage populations including M1, M2a/b/c/d, Mox, Mhb, and M4.

Phenotype	Trigger	Transcription Factor	Function	Activation signature
M1	IFN-γ, TNF-α, and LPS	STAT1/5, IRFs, NF-κB and AP-1	Antibacterial, Destructive, Th1 immunity; Type-IV hypersensitivity, tumor resistance	TLR-2/4, CD80, CD86, iNOS, and MHC-II on the surface. Produce TNF-α, IL-1α, IL-1β, IL-6, IL-12, IL-23, CXCL9, CXCL10, CXCL11
M2a	IL-4/-13	STAT3/6, glucocorticoid receptor, (PPAR)-γ and -δ, STAT6, IRF4, JMJD3	Repair and remodeling (pro-fibrotic); Th2 immunity; endocytic activity; cell growth	YM1, FIZZ1, Arg-1, CD206, IL1R surface expression. Produce IL-10, TGF-β, CCL17, CCL18, and CCL22
M2b	TLR ligands + IL-1β		Th2 immunity; Immunoregulatory (breadth and depth of inflammatory responses)	CCL1, TNF-α, IL-1β, IL-6, and IL-10
M2c	Glucocorticoids, IL-10 and TGF-β		Immunoregulation, tissue repair, matrix remodeling; Clearance of apoptotic tissue	TLR-1/8, Arg-1, CD163, CD206 surface expression. Produce IL-10, TGF-β, CXCL13, CCL16, and CCL18
M2d	Adenosine + TLR2/4/7 antagonists		Pro-angiogenic; clearance of apoptotic tissue	IL-10R, IL-12R surface expression; no Dectin-1 expression. Produce VEGF, IL-10 and iNOS; low levels of TNF-α and IL-12; intermediate Arg-1
Mox	Oxidized phospholipids	Nuclear factor erythroid 2–related factor 2 (NRF2), Nurr1	Pro-atherogenic. Reduced phagocytic and chemotactic function	TLR-2 surface expression. Produce NRF2 response genes, reactive oxygen species, IL-1β and IL-10
Mhb	Haptoglobin	–	Phagocytic (erythrocyte clearance)	HO-1 and CD163 surface expression
M4	CXCL4	KLF2	Pro-atherogenic; no phagocytic capacity	No CD163, MHC-II, and HO-1 expression. Produce TNF-α, IL-6, CCL2, CXCL8, MMP-12, and S100A8

References: (182–197)

Table describes the chemical mixture necessary to trigger phenotype in vitro; transcription factors involved in macrophage activation; biological function; and activation signature. Appropriate references are listed at the bottom of the table.

Circulating Monocytes

Peripheral monocytes (Mo) can be simplistically defined as immature myeloid intermediates recruited to sites of injury through chemical gradient. Three major populations of circulating monocytes have been recognized both in mice ($CD11b^+CD11c^-Ly6C$) and humans ($CD11b^+HLA^-DR^+CD169^-$): classical monocytes ($CCR2^+Gr1^-Ly6C^{hi}$ in mice; $CD206^-CD14^{++}CD16^-$ in humans) are short lived (mean survival ≈1 day), acting as a reservoir to replenish tissue resident cells following injury. Notably, restoking the resident macrophage compartment was recently shown to produce a highly fibrogenic monocyte-derived subtype in experimental models of primary allograft rejection, rheumatoid arthritis, and fibrosis (100, 179, 202–204). Intermediate monocytes (mean survival ≈4 days) patrol the vasculature and are known to transition to non-classical monocytes. Non-classical/intravascular ($CX_3CR1^+Gr1^-Ly6C^{lo}$ in mice/$CD206^+$ $CD14^+CD16^+$ in humans) represent subset known to patrol the endovascular space by tightly adhering to the microvasculature (205–208). These cells survive up to 7 days in the circulation and replenishing the interstitial and alveolar compartment in conditions of stress, and playing important role in inflammatory termination and tissue remodeling (179, 209).

The limited invasiveness of human blood monocyte collection and the simplicity of phenotypical characterization on the basis of surface expression has provided a useful tool to interrogate their role in aging and pulmonary disease. Although aging does not affect total monocyte counts, non-classical $CD14^+CD16^+$ monocytes significantly increased with age, but display reduced HLA-DR (aka MHC-II) and CX_3CR1 surface expression in the elderly; by comparison, classical $CD14^{++}CD16^-$ monocyte numbers are not affected by age (210, 211). In the context of pulmonary disease (fibrosis and COPD), number of CX_3CR1^+ anti-inflammatory non-classical monocytes are inversely proportional to disease severity; this is juxtaposed to the increases in classical $CD14^+$ and $CCR2^+$ monocytes in patients with poor prognosis (212–214). Evidence of monocyte functional senescence is demonstrated by analysis of blood monocytes collected from aged cohorts present blunted responses to bacterial infection (LPS) as a result of decrease in TLR1 and TLR4 surface expression (215), as well as substantial age-associated defect in CD80 expression and functional engagement (89, 216). With blood derived monocytes are clinically (easily) accessible, it is surprising to see such limited number of datasets screening for transcriptional changes across an individual lifespan in conjunction with the progression of fibrosis. It would be a tremendous achievement to direct research efforts onto this question and combine it with the current advancements in therapeutics aimed at control myeloid cell phenotype (217–219).

Alveolar and Interstitial Macrophages

In terms of pulmonary immunobiology, alveolar macrophages (AMs) represent the most studied population. These long-lived resident sentinels constantly communicate with the surrounding parenchyma to elicit innate and adaptive immune

activities, promote immune-tolerance, and participate in lung surfactant reuptake (209, 220, 221). One notable immunological discrepancy between the murine and human lung is represented by the frequency of AMs. Westphalen et al., estimated one macrophage per three alveoli (222). By comparison, the human lung is composed by ∼480 million alveoli and 2.1 billion alveolar macrophages, thus suggesting a ratio of over four macrophages/alveolus (223, 224). The evaluation of human alveolar macrophage ontogeny can only be inferred from murine evidence (225). The advent of fate mapping approaches defined fetal liver origin for murine AMs, while attributing their homeostatic sustenance throughout life to local proliferation (209, 226–228). These cells can be characterized by a unique signature of transmembrane integrins and sugar binding lectins associated with maturity ($MerTK^{+}F4/80^{+}SigF^{+}Ly6C^{lo}CD64^{hi}$). Consistent with this notion, human AMs can be defined by their expression of $CD11b^{+}HLA\text{-}DR^{++}CD206^{++}CD169^{+}$ (225, 229, 230).

A second resident macrophage population is represented by interstitial macrophages (IMs). These cells originate from sequentially recruited progenitors in the yolk sac and bone marrow, an observation that suggests functional diversity across resident lung macrophages on the basis of ontogeny (227). CX_3CR1 reporter mice has helped visualization of IMs within the bronchial parenchyma, in the proximity of the lymphatic vessels. Flow cytometric characterization of IMs reveals strong similarities to blood monocyte's in mice and humans (mouse IMs: $MerTK^{+}CX_3CR1^{+}F4/80^{+}SigF^{-}Ly6C^{lo}CD64^{hi}$ and monocytes: $MerTK^{-}F4/80^{+}SigF^{-}Ly6C^{lo}CD64^{int}$; human IMs: $CD11b^{+}HLA\text{-}DR^{++}CD206^{+}CD169^{-}$; and monocytes: $CD11b^{+}HLA^{-}DR^{+}CD206^{-/+}CD169^{-}$), thus indicating blood monocytes replenish the interstitial compartment across the lifespan (227, 231–234). Unbiased (single-cell) and biased (cell sorting) RNA-sequencing was utilized to functionally define unique IM subpopulations. The resulting datasets independently found Lyve-1 and MHC-II as discriminants. While providing nuanced differences, these studies demonstrated: (1) heterogeneity within the interstitial compartment; (2) transcriptome divergence from alveolar macrophages; (3) existence of parallel populations in heart, fat, and dermis; (4) monocytic origins ($Ly6C^{hi}$); (5) mobilization/residency to specific tissue sites, wither adjacent to nerve bundles ($Lyve1^{lo}MHCII^{hi}$) or blood vessels ($Lyve1^{hi}MHCII^{lo}$); (6) immunomodulatory functions (their depletion exacerbates fibrosis following challenge) (231, 235, 236).

A third population of resident macrophages is represented by monocyte-derived alveolar macrophages (MoAMs). This population develops in response to significant challenge of the lung, sufficient to recruit peripheral monocytes. These immature myeloid cells have been shown to mature into macrophages, replenish the alveolar compartment, and persist in the tissue for extended periods after bleomycin induced injury, where they elicit a fibrotic phenotype (100, 179, 237).

Senescence of the resident macrophage compartment has been investigated across the spectrum of lung health (genetic susceptibility, acute and chronic chemical challenge, and biological aging), but seldom in combination. Exposure to the warfare agent, nitrogen mustard is accompanied by early pro-inflammatory macrophage activation, followed by transition to a pro-resolution/pro-fibrotic phenotype (238). While identification of a senescent phenotype was beyond the scope of these studies, RNA-sequencing analysis of lung macrophages at a time coordinated with fibrosis found features consistent with senescence, including apoptosis, p53, and cell cycle signaling, paired with morphologically aberrant appearance (foamy) (239, 240). In the context of biological aging, tissue-resident macrophages persist in the lung without input from bone marrow-derived monocytes. Aged alveolar macrophages demonstrate increased signs of inflamm-aging (interferon signaling) and down-regulated cell cycle signaling, phagocytotic and antigen recognition function (241, 242). Preliminary evidence by McQuattie-Pimentel et al., elegantly shows that resident macrophages from aged cohorts adoptively transferred to young lungs acquires a transcriptome prolife reflecting the age of the recipient, thus suggesting that lung microenvironment governs macrophage behavior (243). While the work is still in preprint form, the authors bring up significant points related to the differential responses of lung resident AMs and MoAMs to a second challenge. This (and other) work could reshape how we view macrophage biology. A more complete understanding of monocyte biology in the context of aging and fibrosis could help identify unique signatures to define monocyte-derived alveolar macrophages, and even achieve selective targeting during the fibrogenic process.

Eosinophils

Eosinophils are extensively studied in the context of eosinophilic esophagitis, hyper-eosinophilic syndrome, asthma, allergy, and parasitic infection. There is observational evidence linking eosinophils to fibrosis and COPD; however, not much has been done to show their functional role (244–249). Eosinophils perform a number of functions important in tissue remodeling, including modulating lymphocyte recruitment and homeostasis and coordinating Th2 polarization (250). As seen with macrophages, ontogeny impacts cell behavior and physiological function. Indeed, resident eosinophils ($SigF^{int}CD62L^{+}CD101^{lo}$) have been reported to reside within the lung airspace where they display a regulatory phenotype, while bone marrow cells exhibit a highly destructive phenotype (IL-5 dependent, $SigF^{hi}CD62L^{-}CD101^{hi}$) (251, 252).

Eosinophilia is infrequent and not well-understood in pulmonary fibrosis. Nevertheless, blood eosinophil counts have been shown to be a valuable biomarker predicting development of acute inflammatory exacerbations and prognosis in chronic pulmonary disease (253–255). These intermittent events are currently treated with corticosteroids, although a number of large clinical trials demonstrate that prednisone has minimal positive impact (and could even be detrimental) on patient survival during or after acute inflammatory exacerbations (256–258). Asthma therapy provides important data on the pro-apoptotic effects of broad spectrum corticosteroid therapy (prednisone, dexamethasone) (259). In the absence of stratified analysis to determine whether eosinophilic IPF patients represent the group most responsive to steroid therapy, it is tempting to argue that

eosinophil blood count could provide a valid aid to improve therapeutic regimen and, perhaps, disease outcome. One caveat could be related to age-related diminution in peripheral blood eosinophil counts and function (reduced IL-5 stimulation) across a lifespan, since the median age of PF diagnosis is ≥65 and by this time eosinophil development from bone marrow progenitors is significantly reduced (260–262). This could, in turn, reduce steroids responsiveness in the elderly to a level comparable to that seen in asthma and COPD individuals resistant to glucocorticoid therapy (262–266). In this context, it is interesting that parabiosis or adoptive transfer attempting to replenish old mice with juvenile/younger eosinophils successfully prevented age-related declines in physical and immunological functions (267).

Dendritic Cells

Dendritic cell (DC) represent a cellular link between innate and adaptive immunity. Their ever-expanding taxonomy reflects their myeloid/lymphoid lineage differentiation as conventional DCs ($CD103^+CD11b^+CD11c^+$MHC-II^+), plasmacytoid DCs ($CD11c^+PDCA1^+B220^+$), monocyte-derived DCs ($CD11b^+CD141^+$) (268). DCs are primarily tasked to perform antigen recognition functions. Their age-related senescence has been associated with increased immune response against self-antigens (269). This effect, combined with diminished secretion of innate cytokines such as type I and III interferons by plasmacytoid DCs, as well as reduced expression of the anti-inflammatory cytokine, IL-10, significantly impacts the principal functions of these cells with respect to impaired vaccine responses in the elderly (270). It is currently unclear whether DC's functional senescence results from permanent remodeling of the epigenome leading to aberrant response to challenge, and identification of inaccessible DNA regions during the inflammatory response is an active area of DC research (via ATAC-sequencing analysis alone and/or in combination with single-cell RNA-sequencing) (271).

Dendritic cell presence in the lung is dependent on bone marrow recruitment of pre-DCs, that mature locally into DC subsets (272). Fate-mapping analysis shows that $CD103^+$ cells arise almost exclusively from common DC progenitors (maturing from macrophage-DC progenitors), while that number drops drastically for $CD11b^+$ DCs (273). Plasmacytoid DCs are critically dependent on IRF8 and STAT3 signaling, while GM-CSF and signaling via STAT5 inhibit their maturation (274). It is therefore unsurprising that STAT3 signaling declines with aging, a response that may explain the systemic contraction of this DC subset (275, 276). The role of lung resident dendritic cells in pulmonary fibrosis remains largely unstudied. Single cell sequencing analysis of IPF lungs shows only minimal increases in dendritic cell population (132), while experimental modeling (adenoviral-TGFβ overexpression) indicates that fibrogenesis is accompanied by increases in $CD11b^+$ and $CD103^+$ DCs, but that their ablation does not affect lung remodeling (277).

B Cells

The lung adaptive/humoral immune response is carried by highly specialized lymphoid populations, including B cells and T cells. There are some important differences between humans and mice. Much of the information on B cell cellular and molecular pathways described here was derived in murine models. Canonical B cells are mostly known for their adaptive (immunoglobulin-dependent) function against infections and cancer (278). $CD19^+$ B cells populate ∼5% of the lung immune compartment, and their numbers almost quadruples in non-small cell lung cancer (279). Two self-renewing B cell subtypes, B1A and B1B, have been described to develop in the fetal liver from a distinct progenitor and perform innate-like functions in the lung (280). These subsets can be distinguished by their differential expression of CD5 and CD43. They mobilize from the bone marrow to the respiratory tract in a CXCL13 dependent fashion (281), and trigger an IgM and IgA response following IL-5, IL-10, and TLR-agonist signaling (282). Their role in aging and fibrotic senescence is not well-established, but mounting evidence suggests that fetal exposure may produce a battery of specialized subtypes that favor pathogenesis and progression of lupus, diabetes and asthma (283–285).

Evaluation of telomere length as a proxy for senescence is abnormal in B cells since naïve and germinal center B cells exhibit long telomeres, while circulating and memory B cells show extremely short ones (286). By comparison, B cells functional senescence is more linear, as demonstrated by increased incidence of infection in the elderly (287, 288). Decline in bone marrow stromal cell IL-7 production significantly impacts B cell numbers and their maturation. As a result, a highly autoreactive and pro-inflammatory (inflamm-aging) senescent B cell is noted (287–290). Mechanistically, this response appears to be driven by progressive reliance on inflammatory TLR7/TLR9 engagement, rather than the B cell receptor, to trigger a humoral response (287, 291–293). In turn, these autoantibodies lead to chronic lung lesions that can be only resolved via scarring/fibrosis. Perhaps as a compensatory mechanism, aberrant expansion of ectopic inducible bronchus-associated tissues (iBALT) independently supports the maturation and selection of B cells ($CD5^+$ and $CD20^+$) in aged, COPD, and IPF clinical cohorts, in particular those with cigarette smoking history (294–296). Single cell sequencing and proteomic analysis of lungs and peripheral blood cells from IPF individuals shows strong evidence of B1 and $CD38^+CD138^+$ plasma cells accumulation, while experimental modeling of fibrosis generated strong correlation between the degree of pulmonary fibrosis and B-cell numbers in the germinal center (297–300). The involvement of B cells in sterile (environmental) injury is not as well-established, aside from allergen induced IgE production in aging asthma cohorts (301). Nevertheless, the potential role of memory and B1 B cells in shaping humoral immunity against senescent cells should not be overlooked (302, 303).

T Cells

There are several non-lung resident T cell subsets defined by their surface expression and cytokine production (**Table 2**). These cells reside in primary and secondary lymphoid organs (bone marrow, spleen, thymus, lymph nodes), and can be promptly mobilized to the lung through the lymphatic and vascular system. By comparison, only a handful of lymphocyte subsets are recognized as "lung resident," with their appearance

conditional to pathogen exposure across the lifespan (i.e., flu) (311–314). These include tissue-resident memory T cells (TRM), innate (innate lymphoid cells, ILCs), and unconventional T cells (invariant natural killer T cells, iNKT; CD8αα$^+$ cells; mucosal-associated invariant T cells, MAIT; γδ T cells; and intestinal intraepithelial lymphocytes, IELs) (304, 305). Our understanding of tissue resident lymphocyte senescence is limited by subset novelty or their abundance (TRMs expressing a specific antigen are infrequent). Nevertheless, there are numerous reports describing senescence of the machinery in charge of T cell differentiation and selection as well dysfunction of peripheral lymphocyte populations due to toxic microenvironment. Due to the abundance of T cell subtypes to be discussed, a single section would not be sufficient. There are comprehensive reviews that highlight T cell senescence and human health (315–317).

Lymphocyte senescence, and the health associated effects it produces, is the product of direct (cellular dysfunction) and indirect (declining T cell selection machinery; exposure) mechanisms. Clinical and experimental evidence indicate that CD57 and KLRG-1 expression defines senescent conventional T cells (316, 318). Epigenetic analysis of aged senescent lymphocytes demonstrate progressive expansion of reactive effector cells (i.e., Th17) through DNA hypermethylation of transcriptional regulators essential to T cell responses (LGALS1, IFNG, CCL5, GZMH, CCR7, CD27, and CD248) and differentiation (SATB1, TCF7, BCL11B, and RUNX3) (319). These responses are complemented by locus specific chromatin remodeling favoring terminal differentiation of naïve T cells into effector cells (CD8 and Th17) (320). As a result, evaluation of Th17-to-Treg ratio in aging cohorts indicates progressive skewing toward effector Th17$^+$ (high affinity for self-antigens) in lieu of suppressive Tregs (312, 321–323). Similarly, aging is associated with progressive loss in mucosal-associated invariant T cell and iNKT numbers and variant diversity due to functional decline in CD8 negative/double negative ratio in the thymus of aged cohorts (324–326). Further analysis also identifies time related shift in CD4$^+$ MAIT, and within this subtype a decrease in interferon IFNγ/IL-4 ratio indicating Th1 to Th2 transition (327). Their importance should not be understated, as they represent up to 10% of circulating T cells and accumulate in large numbers in the bowel and airways and perform central role in epithelium protection from pathogens (328). Notably, clinical and experimental evidence suggested they play juxtaposing role in homeostasis (protective) and chronic kidney, bowel and non-alcoholic liver disease (profibrotic) (329–331). In the context of asthma, MAITs communicate with B cells and eosinophils to promote allergen-induced airway inflammation (332). The latter function is shared with iNKT cells, a population for which we have more detailed information. These effector cells have been shown to promote T cell dysfunction in aging murine cohorts through proliferative inhibition of splenic T cells (333). Their intrinsic (functional) senescence is still not well-understood. However, clinical and experimental evidence shows that cigarette smoke promotes their accumulation and activation in the lung, a response linked to the development of COPD (334, 335). Nevertheless, iNKT cell's role in recognizing and clearing senescent cells may unlock their potential in attenuating progression of already established age-related disorders (336).

Experimental modeling of environmental and occupational hazard exposure corroborates the notion that external stress accelerates T cell biological senescence. For instance, inhalation of vanadium fumes, used as an additive in the production of steel, and exposure to titanium oxide nanoparticles impacts "normal" T cell selection and differentiation by depleting thymic antigen presenting cells and extensive inflammation (337, 338). Chronic inflammation induced by particulate matter exposure triggers T cell senescence, with external stimuli or neighboring senescent cells producing an inflammatory microenvironment (SASP) that reprograms the metabolic machinery of CD8$^+$ T cells by boosting mitochondrial biogenesis, and thus ROS production, distinctively defined as inflamm-aging (339). Cigarette smoke from human studies show an increase in inflammatory CD4+ Th17 lymphocytes at blood- and pulmonary level in smokers (340, 341).

At the crossroads between innate and adaptive immunity, innate lymphoid cells represent a rare and powerful population that can shape the behavior of surrounding cells during stressful conditions. Of the three subtypes described to date, ILC2 and ILC3 have been clearly implicated in lung injury, wound healing, and fibrogenesis. Their role is primarily linked to the production of mediators that promote extracellular matrix destruction and remodeling (IL-17, TGFβ, IL-5, IL-13) [extensively reviewed by (342, 343)]. Recent reports describe transcriptional and functional ILC2 senescence/exhaustion in the aging lung and brain, resulting in inability to restock the ILC2 pool in aged mice (344). Interestingly, these effects were, at least in part, alleviated by adoptive transfer of activated ILC2s (345). While identification of these tissue resident lymphocytes is progressing fairly rapidly thanks to single-cell techniques, analysis of their roles in specific disease states still at its infancy. Thus, further functional examination of these cells in the context of a senescent state (aging and fibrosis) represents a promising strategy to advance the field and perhaps therapy.

BIOLOGICAL SIGNATURES AND THERAPY

A few non-specific serum biomarker link disease progression and patient's survival to senescence (IL-6 and TNFα) or pulmonary fibrosis (SP-A, SP-D, KL-6, MMP-7), while epigenetic remodeling has gained considerable traction in early detection both conditions (346–348). Changes in global and site-specific DNA hypermethylation (gene silencing) patterns are well-described in the literature and are been considered therapeutically (349–351). Blood screening of elderly populations and individuals with interstitial lung abnormalities identified complex microRNA signatures linked to senescence, proliferation, cell survival, transcript processing, translation, and immune function (348, 352). While the overlap between PF and aging miRNA signatures is not currently available, a three-arm analysis examining young, aged, aged fibrotic lung could be very informative. At the transcriptional level, the Least Absolute Shrinkage and Selection Operator (LASSO) regression method was recently used to

TABLE 2 | Phenotypic characterization of peripheral and tissue resident lymphocyte populations including CD4, CD8, Th17, Treg, innate (innate lymphoid cells, ILCs), and unconventional T cells (invariant natural killer T cells, iNKT); CD8αα$^+$ cells; mucosal-associated invariant T cells, MAIT; γδ T cells; and intestinal intraepithelial lymphocytes, IELs).

Phenotype	Trigger	Transcription Factor	Function	Activation signature
CD4$^+$ (Cellular Response)	Antigen presenting cell and epithelial signals (CXCL10 and CXCR3)	STAT4, Tbet	Th 1 Immunity (Enhance macrophage killing activity, proliferation of cytotoxic CD8$^+$ T cells)	IL-2, IL-12, IFN-γ, and TNFα
CD4$^+$ (Humoral Response)	Antigen presenting cell and epithelial signaling (IL-33, IL-25, and TSLP)	STAT6, GATA3	Th 2 Immunity (Recruit/activation of eosinophils, basophils, mast cells, and B cells	IL-4, IL-5, IL-9, IL-10, IL-13, and IL-25
CD8+ (Cytotoxic T)	Antigen exposure	Tbet, EOMES, RUNX3	Intracellular pathogen defense, tumor surveillance	(a) TNF-α, IFN-γ secretion; (b) cytotoxic granule release; (c) direct cytotoxicity (Fas/FasL)
Th9	IL-4 and TGF-β	STAT6, PU.1	Anti-parasitic	IL-9
Th17	Antigen dependent and independent activation, IL-6 and IL-23	RORγt, STAT3		(IL-17A, IL-17F, IL-21, and IL-22)
Th25/Treg	TGF-β	FOXP3	Immunosuppressive; prevent autoimmunity	IL-10, TGFβ, IL-35
Tissue resident memory T (TRM)	Pathogen exposure, epithelial signaling (IL-25)	BCL6, Blimp-1	Immunological memory	IL-5, IL-17, and CCR7
Unconventional T (Innate-like, NKTs, IELs, MAITs, γδTs)	IL-33, IL-25, TSLP	PLZF, RORγt, Tbet		IFNγ (all); IL-4, IL-13 (MAIT); IL-17 (MAIT, γδT);
References: (304–310)				

Table describes the milieu necessary to trigger phenotype; transcription factors involved in subset activation; biological function; and activation signature. Appropriate references are listed at the bottom of the table.

identify a signature spanning across age- and chemical-induced senescence. This machine learning approach using training datasets from chronic exposure to cigarette smoke and radiation, allowed to build a transcriptomic age model that accurately predicts chronological age in untreated mice and the deviations associated with certain exposures based on a 57-gene signature including Cyp1a1, Lcn2, MMPs, and immunoglobulins (87).

There is no panacea or elixir to counter the effects of age- or chemical-induced senescence. Nevertheless, recent evidence highlighted a number of bioactive nutrients, supplements and therapeutics (termed senolytics), that blunt oxidative damage and reprogram the cells' inflammatory, metabolic and death machinery (353). Two such chemicals are the macrolides azithromycin and rapamycin, shown to clear senescent fibroblasts and thus reduce SASP-related factors through autophagic modulation (354). The anti-diabetic drug metformin and the immune-suppressor rapamycin have shown significant affinity to modulate AMP-activated protein kinase (AMPK), thereby reducing cellular apoptosis, and extending cell longevity (355–357). Lastly, a long line of natural compounds (i.e., resveratrol, fisetin, piperlongumine, and quercetin) that activate Nrf2 (nuclear factor erythroid-derived 2-related factor 2) are shown to be cytoprotective while also inducing senescence or apoptosis in damaged and potentially precancerous cells (358). There is increasing evidence that these chemicals are effective beyond aging, with ample research focusing on their anti-cancer benefits. Examination in the context of fibrosis is still in its infancy, with clinical and in experimental data (bleomycin) supporting the feasibility of quercetin, as well as metformin, against fibrosis. This appears to be achieved via AMPK activation in myofibroblasts, enhanced mitochondrial biogenesis and regulation of apoptotic sensitivity, which aids reversal of SASP and collagen deposition (359–362).

A second potentially groundbreaking approach to reverse pulmonary fibrosis and aging induced senescence, is represented by allogeneic injections of mesenchymal stem cell/multipotent stromal cell/marrow stromal cell (MSCs). A number of experimental and clinical evidence reveals promising results in chronic pulmonary disease, fibrosis and age-related frailty (363–365). These cells can be obtained *in vitro* through expansion of adherent bone marrow mononuclear cells and may function as a trophic source to support immune-senescent inflammatory cells (366).

CONCLUSIONS AND FUTURE PERSPECTIVES

This review exposes the wealth of evidence that pertains to aging, environmental exposure, and fibrosis. While demonstrating some degree of mechanistic redundancy across the spectrum of senescence, it also highlights a number of knowledge gaps that need to be addressed to impact human health (i.e., therapeutics). Based on this foundation, the next cycle of

research questions should test: (1) whether senescence of the remodeled/fibrotic lung occurs faster and through the same mechanisms as an architecturally pristine one? (2) Since it is understood that age-related dysfunction lowers the threshold necessary to trigger an irreversible senescent phenotype, can we model and accurately predict such levels? (3a) Can we identify mutual factors involved in senescence across the spectrum of chemical exposure? (3b) Can we identify shared and exclusive factors that drive senescence across the lung disease spectrum? For instance, how does PF senescence compare to that observed in COPD, emphysema, asthma? (4) Can we modulate/reprogram the behavior and communication of specific cell types, and thus amplify an anti-senescent signal? (5) Can senolytics modulate seemingly irreversible changes in the fibrotic (and aging) lung?

As we look beyond the next decade, it is absolutely necessary that we boost our effort to define sex and hormonal differences as they relate to lung function, senescence, response to environmental toxicants and fibrosis. In the context of immune cell function, it is shown that females elicit an estrogen driven humoral response (Th2 like), while testosterone supports Th1 immunity (367). Peripheral blood analysis shows that aging males exhibit marked epigenomic alteration linked to naïve T and B cell decline and increased monocyte cytotoxicity (368). How these hormones, or their imbalance during menopause and andropause, support or protects the lung from exogenous stressors and disease is not well-understood. This is likely the most important puzzle piece to understand clinical datasets. Yet, there is a canyon-sized knowledge gap in front of us.

Although we are a long way away from getting all the answers, it is comforting to see an increasingly collaborative scientific community and frequent technological advancements that help us comprehensively study cell biology. The successes of the next decade of research lies in good hands.

AUTHOR CONTRIBUTIONS

AV prepared and edited the manuscript.

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