**Context:**

*Focus of the study:* Each day, the lung is exposed to ~10,000L of air containing environmental particulates and potentially infectious materials1,2. And so, the lung must perform dichotomous functions of maintaining a permeable layer for air exchange while acting as a strict barrier for external stimuli1–4. In addition, inflammation in the lung can lead to impaired gas exchange, manifested clinically by shortness of breath, cough, and may culminate in respiratory failure and death1,5. So, to allow adequate permeability for proper homeostasis and functioning, the lung environment must be maintained as a minimally inflammatory environment. However, during aging there is an increase in increase in basal inflammation called inflamm-aging which modifies the normal homeostasis within the lung. This increased basal inflammation which results from aging is associated with an increase in a susceptibility to many diseases including interstitial lung diseases such as Idopathic pulmonary fibrosis (IPF)6,7.

IPF is a rare, orphan interstitial lung disease (ILD) which affects around 14, 000 Canadians (0.1% prevalence) mostly in the elderly population (>60) but with an incidence that increases with age8,9. The aetiology of IPF is by definition unknown, and, thus, the diagnosis of IPF requires the exclusion of other forms of interstitial lung diseases, such as those associated with connective tissue disease, autoimmune dysfunction and environmental/occupational exposures10. Overall, patients diagnosed with IPF have a poor prognosis with a typical survival of 3 to 5 years after diagnosis. In addition, IPF is unpredictable, with sudden, rapid and often unexplainable acute exacerbations8–12. Due to a rapid disease progression and lack of efficacious treatments, IPF accounts for a higher than expected share of Canadian mortality (1.4% of population mortality)8,9,11,12.

The current paradigm of IPF pathogenesis is that recurrent damage to the alveolar epithelium of the aging lung drives an abnormal wound-healing response. This results in fibrosis and a loss of lung elasticity rather than repair 2,5,11–13. IPF is characterized by increases in fibrogenic mediators produced by immune and epithelial cells. These mediators lead to the activation, migration and proliferation of the normally sparse fibroblasts in the healthy lung1,14,15 and their differentiation into activated myofibroblasts8,9,11,12. Currently, there are two drugs available for the treatment of IPF: nintedanib and pirfenidone16,17. These drugs have been shown to slow the decline of lung function but do not substantially improve survival or quality of life 10–12,18,19.

*Research Concept:* Macrophages are tissue-resident immune cells that are central to tissue homeostasis and host defense. As such, they are responsible for controlling the inflammatory tone of the lung but have also been implicated in the development of IPF5,20,21. In the healthy lung, alveolar macrophages (AMs) reside at the interface between the airways and parenchyma where they intercept environmental contaminants and dampen the inflammatory response of the lung epithelium. Unlike the macrophages of the interstitial lung region (IMs) or those derived from peripheral blood mononuclear cells (PBMCs), AMs promote an anti-inflammatory environment through the production of anti-inflammatory signals such as immunosuppressive prostaglandins and transforming growth factor-β2,22. However, macrophages are susceptible to inflamm-aging, resulting in modified responses6,23. Inflamm-aging, combined with other risk factors, modifies the lung environment to increase the inflammatory potential of AMs or replaces AMs with more inflammatory IMs of PBMCs13. This is associated with an accumulation of scar tissue and aberrant levels of surfactant which result in a lower capacity for air exchange in IPF and, ultimately, respiratory failure20,21,24. For this study, I will use a combination of large genomics datasets and state-of-the-art machine learning models to describe the mechanism by which macrophages drive inflamm-aging in the development of fibrosis.

*Hypothesis*: Age related increases in basal inflammatory responses by macrophages, called inflamm-aging, predispose to the development of IPF.

*Aims:* The overall goal of my research program is by using both pre-clinical and clinical approaches to:

1. Determine the function and genomic changes of lung macrophage and PBMC populations associated with inflamm-aging
2. Describe the changes of lung macrophage and PBMC populations in IPF which are associated with inflamm-aging

**Methodology of the aims.**

*Aim 1. Determine the function and genomic changes of lung macrophage and PBMC populations associated with inflamm-aging.* Inflamm-aging results in changes in gene expression and epigenetics which skew the response of inflammatory cells from maintenance seen in the young to more inflammatory and inflammation prone activation in the elderly6,7. In circulating monocytes and lung macrophages, TLR receptors and inflammasome members are upregulated while phagocytic capacity and the ability to resolve inflammation and repair damage is impaired6,7,25. Along with changes in gene expression, there is a change in the circulating monocyte population with an increase in monocyte populations which are typically associated with inflammation (CD16+ in human, Ly6C+ in mice)26,27. Changes in circulating monocyte population are likely a result in changes in hematopoiesis and may suggest a change in the hematapoietic environment that is driving differentiation, a process called trained immunity26. In the lung, AMs are reduced in aged mice, resulting in longer neutrophil retention and increased tissue damage28,29. Overall, these data suggest that monocytes/macrophages may participate in inflamm-aging through both a shift in cell populations and macrophage/monocyte trained immunity.

To define inflamm-aging, we will sort macrophage populations of the lung and monocyte populations in the blood from C57Bl/6 (fibrosis susceptible) and Balb/c (fibrosis resistant) mice at four age groups: adolescent (8 weeks), adult (6 months) and two populations of elderly (1 and 1.5 years and humans at three age groups: healthy young (24-36 y), healthy middle age (44-55 y) and non-frail old (67-83 y). We will then define and contrast the genomic landscapes of these age groups. This will include defining the active and poised transcriptome via RNA-seq and genome run-on (GRO)-seq. We will also define the epigenetic profiles of these human and mice samples through the active and poised promoters and enhancers via overlap of H3K4me2 (a mark of enhancers and promoters) and ATAC-seq (a mark of open chromatin) signals and relating these to GRO-seq signal for eRNA or mRNA. With this, we will define the signatures in alveolar macrophage and peripheral monocyte populations which contribute to inflamm-aging. Further, we will perform scRNA-seq on the hematopoietic compartment and lung of mice and BAL of 2 patient samples per group to identify the cell populations which change during aging. In both mouse and humans, we will also perform ELISA on BAL for a panel of cytokines to define changes in inflammation. We will, then, perform machine learning using an attention neural network based on PyTorch to correlate changes in RNA and epigenetic signatures, which are a result of increased basal cytokine activation, with aging.

The immediate term goals of this work are to define signatures of inflamm-aging, to define the macrophage populations which contribute to inflamm-aging and to epigenetically define changes in the macrophage population of the lung in mice and humans. We will also describe similarities and differences between and within populations which may define species specific differences for interpreting animal models and genetic variation which drives responses. The longer term goals are to define the signaling environment which modifies the lung macrophages population to drive an altered homeostasis in the aging lung.

*Aim 2. Describe the changes of lung macrophage and PBMC populations in IPF which are associated with inflamm-aging.* Macrophages are important adapters of immune modulation to inflammation and damage throughout the body. In the lung, macrophages maintain surfactant homeostasis, control environmental particulates and are important drivers of wound healing1,5,13,14,30. As such, macrophage involvement in the lung is paramount in the resolution of disease.

Within the lung, there are two populations of macrophages which can be differentiated by RNA expression patterns in single cell RNA sequencing and Siglec-Fhigh/CDC11bhigh staining by flow cytometry20,21,24. Previous work in mice suggest that a shift in the macrophage population from predominantly AMs to IMs exacerbates the signals which lead to damage and fibrosis20,21,31,32. These two macrophages populations receive the same signals but interpret them in drastically different ways. To study fibrosis development in mice, we will interrogate two well-established models: a sublethal dose of intranasal bleomycin and a virally delivered TGF-β in adolescent C57Bl/6 and use this standard dose for all of our studies 33,34. We will define the sensitivity (EC) to these pro-fibrotic agents in sensitive (C57Bl/6) and resistant mice (Balb/c) in the previously defined age cohorts (8 weeks, 6 months, 1 year, 1.5 years). In humans, we will collect BAL and whole blood from patients with stable IPF and patients experiencing acute exacerbations. We will similarly collect RNA, ATAC, and GRO as well as scRNA sequencing to define the core gene signature involved in the macrophage response to disease. Initially, we will define the lung and PBMC cell populations in an unbiased way using scRNA sequencing. We will then use RNA velocity on the IPF and aging scRNA seq samples to the predict the changes of cell populations and origins of disease causing macrophages through disease development. Using the scRNA-seq as a guide, we will use our machine learning models to predict genomic signatures associated with disease progression from the bulk RNA sequencing. Similar analysis will be performed on the mouse samples, examining the effects of aging on the development and severity of fibrosis. In particular, we will define a list of genes/genomic markers which are differentially expressed in our resistant and susceptible mouse strains. From this data, we will determine shared signatures of disease progression between human and mouse including those which are differentially expressed in our two mouse strains. We will then study these targets in disease progression and survival in mouse knockout models of our mouse fibrosis models to confirm our predictive modelling.

The immediate term goal of this work is to examine the differences between macrophage populations during fibrosis progressions and predict the genomic determinants which drive these changes. The longer-term goal of this project is to establish a core set of pathways involved in fibrosis development shared between mice and humans and to experimentally verify these targets in fibrosis development in our mouse models.

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