Official Journal of the Asian Pacific Society of Respirology
Respirology

INVITED REVIEW SERIES:
STEM CELLS AND THE LUNG
SERIES EDITORS: YUBEN MOODLEY AND PHILIP J. THOMPSON

Adult stem cells for chronic lung diseases

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ABSTRACT

Idiopathic pulmonary fibrosis (IPF) and chronic obstructive pulmonary disease (COPD) are chronic, progressive and lethal lung diseases. The incidence of IPF and COPD increases with age, independent of exposure to common environmental risk factors. At present, there is limited understanding of the relationship between ageing and the development of chronic lung diseases. One hypothesis is that chronic injury drives to exhaustion the local and systemic repair responses in the lung. These changes are accentuated during ageing where there is a progressive accumulation of senescent cells. Recently, stem cells have emerged as a critical reparative mechanism for lung injury. In this review, we discuss the repair response of bone marrow-derived mesenchymal stem cells (B-MSC) after lung injury and how their function is affected by ageing. Our own work has demonstrated a protective role of B-MSC in several animal models of acute and chronic lung injury. We recently demonstrated the association, using animal models, between age and an increase in the susceptibility to develop severe injury and fibrosis. At the same time, we have identified functional differences between B-MSC isolated from young and old animals. Further studies are required to understand the functional impairment of ageing B-MSC, ultimately leading to a rapid stem cell depletion or fatigue, interfering with their ability to play a protective role in lung injury. The elucidation of these events will help in the development of rational and new therapeutic strategies for COPD and IPF.

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Received 8 February 2013; accepted 5 April 2013.

Key words: ageing, chronic obstructive pulmonary disease, idiopathic pulmonary fibrosis, lung, mesenchymal stem cell.

INTRODUCTION

A chronic disease can be defined as one with long duration and slow progression. Symptoms may be continual or intermittent, but the patient usually has the condition for life. In the case of the lung, a chronic disease is characterized by a limitation of airflow due to any pulmonary disease occurring as a result of increased airway resistance or decreased elastic recoil. Clinical entities most often associated with chronic lung disease are chronic obstructive pulmonary disease (COPD) and many of the interstitial lung diseases (ILD). The most common ILD is idiopathic pulmonary fibrosis (IPF) which represents 45% of the ILD patients. The pathogenesis of this group of diseases is diverse, but many them have an acute injury stage as well as a more chronic stage. During the chronic stage, diseases such as IPF have recurrent acute exacerbations that worsen the prognosis and increase the risk of mortality. Acute stages are more frequently associated with inflammatory responses but usually interstitial, intra-alveolar or peribronchial fibrosis occurs during the chronic stages. Chronic diseases can also be associated with persistent stem cell activation which can result in stem cell exhaustion. As a consequence, there is an irreversible loss of biological activity and immunomodulatory properties of the mesenchymal stem cells pools. Furthermore, the incidence and severity of fibrotic and emphysema lung diseases increases with age, but very little is known about how age-related changes affect the mechanisms that underlie disease emergence and progression.[1,2] Normal ageing includes accumulation of deoxyribonucleic acid (DNA) mutations, oxidative stresses, increased susceptibility to apoptosis and telomere length dysfunction. In addition, these inevitable ageing-related phenomena may also cause dysfunction and impaired repair capacity

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doi: 10.1111/resp.12112

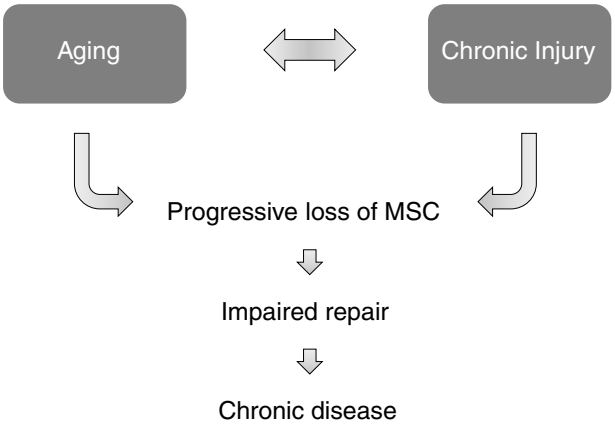

Figure 1 We hypothesize that aged bone marrow derived mesenchymal stem cells (B-MSC) increases the susceptibility to the development of chronic injury and abnormal lung repair triggered after injury. Recently, chronic diseases have been associated with progressive and persistent stem cell activation resulting on stem cell loss or exhaustion. As a consequence there is an irreversible damage on their biological activity promoting the perpetuation of the lung disease.

of B-MSC.[3] This review summarizes what is currently known about age-related molecular changes in the lung and B-MSC that are implicated in the pathobiology of chronic lung disease. (Fig. 1)

LUNG AGEING

Epidemiological studies indicate that ageing is associated with an increased incidence of two common chronic respiratory diseases: COPD and IPF. Although COPD and IPF are different clinical entities, they are both characterized by an increased prevalence in ageing individuals and increased collagen deposition either at the small airways or the interstitium. Notably, both conditions can occur in the same individual suggesting that there are common molecular mechanisms that might contribute to the pathobiology of both COPD and IPF.[4,5]

AGED MESENCHYMAL STEM CELLS AND REPAIR

Bone marrow-derived stem cells can be divided into hematopoietic (B-HSC) and mesenchymal (B-MSC) stem cells. Accurate characterization of B-MSC has been a complicated issue since there are no specific cell surface markers. Enrichment of B-MSC from crude bone marrow suspensions is achieved by selection for a plastic-adherent population that expresses neither hematopoietic nor endothelial cell surface markers but is positive for the expression of adhesion and stromal markers. Due to a lack of a defined panel of unambiguous markers distinguishing B-MSC, a criterion for establishing B-MSC phenotype is to use adherent cells isolated by cell sorting that (i) express CD44, CD73, CD90 and CD105, (ii) lack the expression of hematopoietic markers like CD45, CD34 and CD31 and (iii) in a trilineage differentiation assay, confirm their plasticity by the ability of the cells to differentiate into multiple cell types like adipocytes, osteocytes and chondrocytes.[6] An interesting aspect of B-MSC biology is that they can migrate to the lung in response to a lung injury[7–9] and participate in lung repair either by differentiating into cells destined to repopulate denuded damaged sites[10,11] or by modulating inflammation.[12] It is known that B-MSC can traffic into the lung after bleomycin treatment.[13] Although the majority of studies have been focused on B-MSC's role in wound healing and fibrosis, probably due to their therapeutic potential, there are a small group of adherent B-HSCs (Fibrocytes) that have been recently implicated in the pathobiology of pulmonary fibrosis. Fibrocytes are bone marrow adherent cells that express leukocyte cell markers such as CD45 and CD34, can traffic to the lungs in response to CXCL12 in a bleomycin injury murine model and can produce type I collagen.[14–16] High levels of circulating fibrocytes have been associated with poor prognosis in IPF.[17]

Compared to younger individuals, B-MSC from elderly people have different morphology, increased production of reactive oxygen species and oxidative damage,[18] DNA methylation changes affecting cell differentiation[19] and, slower proliferation rate in culture,[20,21] shorter telomeres[21] and a larger proportion stain positive for senescence-associated beta-galactosidase.[22] Studies in mice with accelerated ageing show an increase in fibrocyte mobilization as well as higher levels of CXCL12. In parallel, a decrease in B-MSC is observed in ageing mice. In general, B-MSC are in a quiescent state with low metabolic activity in the G0 phase of the cell cycle which preserves the long-term proliferative potential of B-MSC. However, the quiescent G0 state also has disadvantages since B-MSC do not undergo cell cycle-dependent DNA damage checkpoints and repair pathways. The accumulated DNA damage of B-MSC leads to stem cell apoptosis and consequently to stem cell depletion or exhaustion in older individuals. The critical effect of accumulation of DNA damage in ageing B-MSC has been recently demonstrated using a progeroid mouse model with defects in DNA repair associated with deficiency in the excision repair cross-complementation group (Ercc1) protein. In an unpublished study, using the murine model Ercc1, we instilled bleomycin into 12-week-old Ercc1-/Δ mice and littermate controls and demonstrated that Ercc1-/Δ mice have increased susceptibility to lung fibrosis. At day 21, Ercc1-/Δ mice developed more severe pneumonitis and collagen deposition than controls. In addition, there was an increase in the number of cells in the bronchoalveolar lavage (BAL) with elevated expression of interleukin (IL)-6 and tumour necrosis factor-alpha (TNF-α) in plasma. These data demonstrate the increased susceptibility to lung injury and fibrosis in Ercc1-/Δ mice.

We have previously demonstrated the importance of the integrity of the genome of B-MSC in individual's lifespan. Using parabiosis, pairing young WT with an Ercc1-/Δ mouse, resulted in a more than doubling

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the lifespan of the Ercc1-/Δ mice in direct correlation with maintenance of body weight. Recently, our observation was confirmed by Lavasani *et al.*,[23] who, using Ercc1 KO mice, demonstrated that their life span could be increased solely by the infusion of young WT B-MSC. Life span did not changed by infusion of natural old B-MSC, or murine embryonic fibroblasts as control. These results suggest that B-MSC can have a direct effect in the lifespan of mice.

In a rat model for cardiomyopathy, human B-MSC from aged donors did not perform as well as the ones from young donors.[24] B-MSC from old donors failed to differentiate *in vitro* into neuroectodermal cells,[25] and early passage B-MSC were more efficient in promoting the proliferation and maintenance of hematopoietic progenitor cells.[26] In one study, the administration of stem cells from young mice restored cardiac angiogenesis in senescent mice when stems cell from old mice failed to do so.[27] The superiority of very young B-MSC can be explained by several aspects of their biology.[28] Briefly, B-MSC of foetal or amniotic origin maintain the expression of markers of pluripotency (Oct-4, Nanog, Rex-1, SSEA-3, SSEA-4, Tra-1–60 and Tra-1–81), have longer telomeres and greater telomerase activity. Foetal MSC are also more readily expandable and senesced later in culture than their adult counterparts. In conclusion, ageing and extensive *in vitro* culture of B-MSC defines telomeric length, pluripotency, proliferating potential and overall their ability to execute their regenerative role[28,29]

B-MSC-lung interaction and ageing

The extracellular matrix (ECM) provides structural support by serving as a scaffold for cells, and as such the ECM maintains normal tissue homeostasis and mediates the repair response following injury. The composition of the ECM varies according to the tissue localization and physiological conditions. Tension applied through collagen fibrils at the ECM-cell interface might lead to protein synthesis, cell mitotic activity and changes in gene expression via activation of mitogen-activated protein kinase phospho-relay systems.[30,31] The major ECM proteins of the distal lung are collagen, elastin and proteoglycans. They are critical to determine the mechanical properties of the ECM and the mechanical forces that can influence the physiological function of the lung parenchyma. Collagen represents 15–20% of the total lung dry weight and represents the most important component of the ECM to modulate cellular responses to injury. Fibronectin, a fibrillar protein is also a major component of ECM that is deposited earlier than collagen during tissue injury. The dynamic composition of the matrix can affect the adhesion, proliferation and migration of the cells including B-MSC. Age-related changes in the ECM can contribute to the decline of lung function with ageing. In general, in the ageing connective tissue, the collagen type I content of ECM is increased, whereas the collagen type III content is decreased along with elastin fibres. Proteoglycan content also appears to decrease with age. The exact mechanism of how the ECM of the ageing lung influences the cellular responses to injury is still unclear. Studies in animal models indicate that fibronectin can be an important player of this response. Cellular fibronectin is a glycoprotein produced by mesenchymal, epithelial and inflammatory cells that contains variable proportions of the extra type II domains A and B (fibronectin extra domain-A (EDA) and extra domain-B (EDB)). EDA fibronectin has been implicated in fibroblast activation and wound healing.[32–34] Ageing mice show an augmentation of the EDA fibronectin, and this isoform is also increased in lungs from mice treated with bleomycin, suggesting a connection between fibronectin EDA, age and fibrosis.

The pro-fibrotic factor TGF-β1 promotes the EDA inclusion into fibronectin through the PI3K/Akt/mTOR and Smad3 signalling pathways. EDA fibronectin is necessary for the TGF-β1 induced myofibroblast transformation of stromal cells. Recently, it was documented that an age-dependent increase of TGF-β1 and TGF-βR1 expression in old lungs coincides with increased Smad3 messenger ribonucleic acid (mRNA) and protein expression. It has also been observed that fibronectin EDA is elevated in patients with IPF, whereas EDA deficiency protects mice from fibrosis.[33,34] Taken together, these findings suggest that the elevation of TGF-β with age induces changes in the ECM composition, and it is the higher proportion of EDA fibronectin that drives fibrosis and repair responses.

As we mentioned above, the matrix composition can affect the function of B-MSC. This could be of critical importance for the rational design of cell-based therapies with B-MSC. While B-MSC have been shown consistently to modulate the immune response and prevent lung damage in numerous preclinical studies of acute and chronic lung injury, the use of B-MSC during the fibrotic phases of the repair response can be deleterious. Using a murine model of radiation-induced lung fibrosis, it has been shown that the use of B-MSC at early time points (4 h) after injury conferred protection and presented low levels of engraftment. In high contrast, B-MSC infused at later time points after injury (60 and 120 days) were engrafted in the lung interstitium and expressed markers of myofibroblasts. This study clearly demonstrated that the function and differentiation of B-MSC occurred in the injury site as a response to local mediators. Thus caution is warranted when considering the use of B-MSC during ongoing fibrotic responses as this cell-based therapy could cause worsening of the disease process.

B-MSC AND IDIOPATHIC PULMONARY FIBROSIS

The ILD comprise a group of diffuse pulmonary parenchymal diseases that are classified together because of similar clinical, radiological, physiological and/or pathological manifestations. IPF is the most common of the interstitial pneumonia. It is usually a progressive condition resulting in respiratory failure and death within 2–5 years of diagnosis. IPF is characterized by an inability to repair the injured epithelial cells, activation and proliferation of fibroblasts/

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myofibroblasts, abundant collagen deposition and loss of normal lung architecture. Classically, IPF lungs show infiltrates on chest radiographical imaging, and when biopsy is performed, the hallmark of the pathological findings are the presence of fibroblastic foci and the presence of microscopic honeycombing.

The occurrence of IPF increases in both prevalence and incidence in the sixth decade of life.[35,36] Symptoms typically occur at age 50 to 70 years, and most patients are >60 years of age at the time of clinical presentation.[37] Risk factors for IPF include a history of cigarette smoking, male gender and age. The only effective therapy for IPF is lung transplantation. Although the majority of the cases of pulmonary fibrosis are sporadic, a small percentage of patients suffer from familial pulmonary fibrosis associated with mutations of the surfactant proteins, and telomerase genes hTERT and hTR. The most accepted theory in the pathobiology of IPF is the increased susceptibility of alveolar epithelium to lung injury by a combination of endogenous and exogenous factors, including genetic disorders, age-associated changes in the repair responses and environmental exposures, such as viral infection and microaspiration.

Ageing and IPF have been associated with cellular senescence, oxidative stress, abnormal shortening of telomeres and increased apoptosis.[2] Animal studies also support the link between ageing and susceptibility to fibrosis by demonstrating an increased vulnerability of the aged lung to injury. Our group has shown that a single episode of lung injury by bleomycin[38] or lung infection with a murine gamma herpes virus[39] will cause severe progressive pulmonary fibrosis only in naturally aged wild type mice compared to young mice. These observations, in some way, challenge the traditional concept that progressive fibrosis is the result of chronic injury. Chronic injury leads to an accelerated ageing by shortening telomere, increasing DNA damage, diminishing mitochondria function of the stem cells with a consequent depletion and inability to repair. It is notable that a single injury in the ageing lung can result in progressive fibrosis.

B-MSC and animal models of fibrotic lung disorders

We and others have shown that infusion of B-MSC can protect the lung from severe inflammation and fibrotic scarring induced by bleomycin.[8,9,40] Intratracheal instillation of bleomycin leads to lung fibrosis which occurs in three stages. Bleomycin-induced cytotoxicity results in apoptosis of the alveolar epithelial cells and necrosis of the alveolar epithelium, followed by recruitment of neutrophils and macrophages into the lung with inflammation peaking at day 7 post-bleomycin. At days 14–21 post-bleomycin, there is increased fibrosis characterized by collagen deposition, aberrant repair and tissue remodelling.[41] The protective effect of B-MSC on bleomycin-induced was first reported by Ortiz and colleagues.[8] They injected B-MSC from male mice into female mice immediately after a bleomycin challenge. The results showed a decreased in the lung matrix metalloproteinase mRNA and lung collagen content. In contrast, the protective effects of B-MSC were negligible when the infusion was performed 7 days after the bleomycin challenge.

In order to determine further mechanisms mediating B-MSC protection following injury, we administered bleomycin to busulfan-induced myelosuppressed mice and studied the local and systemic response to B-MSC infusion. The infusion of GFP^+ B-MSC was performed 6 h after bleomycin treatment. We demonstrated that the immunosuppressed mice that received bleomycin had a mortality rate close to 80%, in contrast those who had received the B-MSC infusion showed a reduced mortality rate of less than 10%. Morphometric analysis revealed increased engraftment of B-MSC in the lung at day 14 in myelosuppressed animals compared to mice with intact bone marrow, demonstrating the protective effect of B-MSC infusion against bleomysin-induced lung injury. We found that mRNA levels of T_H1 cytokines (IL-2, IL-1β, IFN-γ) were significantly decreased in the lung 14 days after bleomycin while there was an increase in IL-4, G-CSF and GM-CSF. These results indicate B-MSC alter the cytokine milieu to favour repair.

B-MSC AND CHRONIC OBSTRUCTIVE PULMONARY DISEASE

COPD of the lung are progressive syndromes resulting in the destruction of alveolar septa leading to airspace enlargement and resulting in decreased alveolar surface area.[42] Emphysema, defined as airspace enlargement of the adult lung, frequently develops as a component of COPD. In 1990, COPD was the sixth leading cause of death globally, but it is projected to become the third by 2020.[43] In the United States, COPD is currently the fourth cause of death.

The main risk factors associated with emphysema in adults are cigarette smoking and air pollution. The pathogenesis of emphysema remains to be understood, yet the disease is well characterized and consists of increased inflammatory processes leading to apoptosis of epithelial cells, proteolysis of the terminal air spaces and lung extracellular matrix components. B-MSC may have a therapeutic action in COPD since they promote type I and type II cell regeneration as well as their immunomodulatory effects.

To date, one study has investigated the effects of B-MSC administration in a rat model of emphysema.[44] Rats were exposed to Co60 irradiation and intratracheal papain treatment after which bone marrow-derived MSC were infused intravenously. After 28 days, lungs were harvested, and histological changes were compared between all treatment groups. As anticipated, emphysematous changes in the lung, as quantified by mean linear intercept, increased in irradiated, papain treated rats. B-MSC treatment by infusion significantly protected against air space enlargement and reduced epithelial cell apoptosis. Lung sections analysed by immuno-histochemical assays revealed co-staining of engrafted B-MSC with the type II-epithelial cell marker, surfactant protein-C, suggesting that B-MSC may have differentiated into

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lung-cell types. These data suggest that B-MSC can protect against progression of emphysema by increasing epithelial cell regeneration and reducing alveolar apoptosis.

Currently, a Phase II clinical trial is underway to establish the safety and efficacy of multiple administrations of allogeneic B-MSC in patients with moderate to severe COPD (clinical trials identifier—NCT00683722). To date, 62 patients have been enrolled, receiving, in a randomized double-blinded protocol, intravenous infusions of either allogeneic B-MSC or vehicle control. Patients have received four monthly infusions (100×10^6 cells/infusion) without any sign of acute or chronic toxicity, cells have been well tolerated.[45] Findings have yet to be released.

CONCLUSION

As a self-repair mechanism, living organisms have stem cells that are attracted to sites of injury. Chronic injury as well as ageing could exhaust and impair stem cell reparative capacity as well as diminish number of available stem cells. The mechanism(s) by which alterations in the homeostasis of stem cells pools are involved in the pathogenesis of chronic lung diseases is unknown. If stem cell exhaustion and ageing is the cause of morbid states, stem cell-based therapies will be able to prevent and treat them. Restoration of stem cells has shown promising therapeutic benefits for certain lung pathologies. Particularly, the immunomodulatory capacity of B-MSC has been shown to be beneficial for lung diseases with exacerbated inflammatory responses. However, a generalized use of B-MSC in chronic lung diseases must be considered with caution, and careful studies are still required to establish safety and efficacy of such use.

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