Therapeutic Potential of Mesenchymal Stem Cells for the Treatment of Airway Remodeling in Pulmonary Diseases

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ABSTRACT

According to significant improvements in the tissue engineering field over the past several years, lung tissue cells have recently attracted more attention due to the high prevalence and diversity in related diseases.

However, selection of an appropriate cell type, screening of suitable conditions for growth and proliferation, as well as subsequent implantation into the body to repair and regenerate damaged tissues are considered as important issues in this context.

It should also be noted that most studies have been described in animal models, but not in humans. Because of the high regenerative capacity, predominant immunomodulatory feature, and inhibition of T-lymphocyte proliferation, mesenchymal stem cells (MSCs) may play an important role in the reconstruction of damaged tissues including bronchioles in pulmonary diseases.

Interestingly, clinical trial studies demonstrated that MSCs have the significant potential to treat a wide variety of diseases including acute myocardial infarction (AMI), liver cirrhosis, crohn's disease, and graft-versus-host disease (GVHD).

Keywords: Adipose-derived mesenchymal stem cell; Airway remodeling; Chronic bronchitis; Chronic obstructive pulmonary disease; Inflammation; Lung diseases; Mesenchymal stem cell

INTRODUCTION

The respiratory system is one of the most complex organs in the body. Because of its anatomical position and composition after exposed to various damaging factors such as micro particles, carbon granules, toxic gases (including sulfur dioxide, nitrogen oxides, ozone etc), the respiratory system is prone to a wide variety of irreversible damages and alterations.[1-3] Both acute and chronic lung injuries include a variety of symptoms, ranging from mild and reversible such as allergies to severe and irreversible problems such as fibrosis, fatal asthma and chronic obstructive diseases. In most diseases involving the airways, the epithelial linings of

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the airway are found to be at risk of irreversible damages that cannot naturally be repaired. However, the standard treatment approaches involve surgery and lung transplantation. The prolonged use of bronchodilators can be helpful, if the terminal airways or bronchioles are damaged. However, long-term bronchodilator therapy can lead to major problems and safety concerns in the patients.[4] Therefore there is a need for a new therapeutic strategy to overcome these problems.

In recent years, significant advances have been made in the ability of stem cells to treat various diseases. Similar to most tissues, the pulmonary system contains stem cells such as ductal cells, basal cells (BCs), clara cells and alveolar type II cells in the primary bronchi, terminal bronchioles, bronchioles and alveolar regions, respectively.[5,6] Although lung stem cells have the ability to repair and reconstruct lung tissue, this decreases with age and severity of damage.[6-8] However, a number of recent studies indicated that embryonic and mesenchymal stem cells (MSCs) derived from bone marrow are able to convert into airway and epithelial cells in the lung.[9,10]

Based on these studies, it is proposed that the use of stem cells from other sources such as mesenchymal stem cells may provide a logical solution. Although embryonic stem cells have a high proliferation and differentiation capacity, the risks of tumorigenesis and transplantation rejection as well as ethical issues make the clinical application of these cells challenging.[11] Due to the high regenerative potential, along with the immunomodulatory features and inhibition of T-lymphocyte proliferation, the MSCs may play important roles in the reconstruction of damaged tissues.[5] Further experiments demonstrated that the intravenous injection of MSCs increases their localization in the alveolar spaces and airways.[12,13] Nonetheless, one of the most important challenges for the use of MSCs in lung lesions is the low level of cell engraftment in damaged lung tissue.[13]

As progenitor cells of the connective-tissue or multipotent mesenchymal stromal cells, MSCs have been shown to have the therapeutic potential for lung injury.[14] MSCs can be isolated from different adult tissues and differentiated into various cell types. Several studies showed that MSCs mediate beneficial effects in the repair of damaged tissues. After in vitro culture, growth and proliferation, MSC engraftment in damaged tissue may induce moderate inflammatory responses by pro-inflammatory cytokines. In addition,

MSCs lead to a significant immune suppression that can modulate the function and activity of T lymphocyte cells (T cells), Natural killer (NK) cells, and dendritic cells (DCs). Preclinical studies also demonstrated that the use of MSCs in clinical trials can be highly valuable.[15] The clinical properties of MSCs are as follows:[16-20]

1. MSCs can be easily isolated from bone marrow and adipose tissue.
2. Intravenously injected cultured MSCs have the ability of localization without toxicity to the host.
3. MSCs have the potential to differentiate into specific cell types in tissues, with immunosuppressive effects.
4. MSCs are not immunogenic; in other words, engraftment is not associated with rejection, and no immunesuppressive therapy is needed.
5. There are no ethical issues pertaining to the therapeutic use of MSCs.

The data from clinical trials showed that MSCs are the most effective for the treatment of diseases such as acute myocardial infarction (AMI),[21], liver cirrhosis,[22] crohn's disease[23] and graft-versus-host disease (GVHD)[24] in phase III clinical trials, and Type 1 diabetes,[25] arthritis[26] and lung diseases such as COPD[27] in phase II clinical trials.

In this review, we will address both the MSC-associated studies in animal models and MSC-based therapeutic strategies as well as pulmonary diseases.

Stem Cell Dynamics in the Lung Epithelial Cell Layers

The lung tissue, both the proximal and distal airways, contains a wide variety of structurally and functionally distinct epithelial cell types that can also be found in other locations. The bronchial region (large airways) often includes ciliated, mucus-secreting cells, and BCs. Bronchioles (small airways) contain Clara cells as well as alveolar type I and type II cells (Figure 1).[28] Several potential sources of stem cells were so far identified for repairing the airway epithelial layer. They can be classified into endogenous progenitor cells in the respiratory tract, and exogenous stem cells derived from other tissues of the body. Studies showed that secretory and basal cell types are parts of the airway epithelial stem cells, accounting for up to 6-30% of the airway epithelia involved in normal homeostasis and regeneration of injured tissue.[28-30] Several studies demonstrated that dynamic airway epithelial repair includes de-differentiation, cytoskeleton
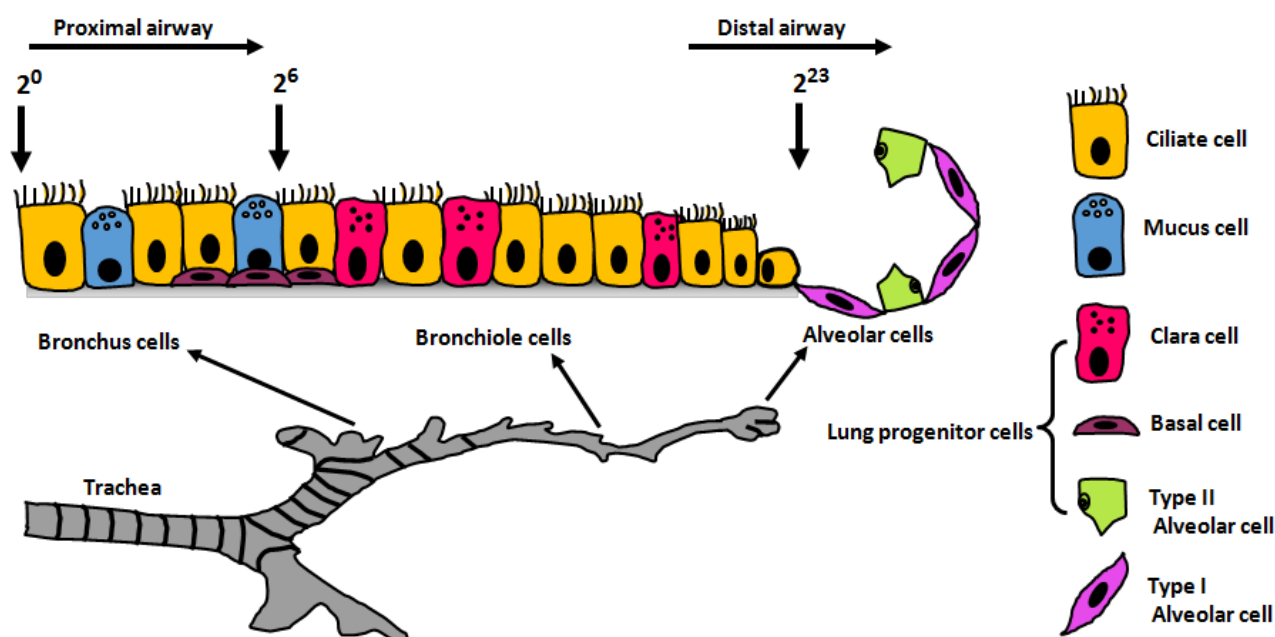
Figure 1. A schematic diagram depicting various parts of progenitor stem cells in the airway. As the progenitor epithelial cells, Clara cells and BCs reside in airways and alveolar type II cells do in the alveolar region.

rearrangement, migration and re-differentiation, respectively.[31-33]

Airway epithelial damage can result from a number of unknown airway diseases, toxins, bacterial or viral infections, allergic reactions, exposures to tobacco smoke and chemical agents (such as chemical compositions of fine particles suspended in the air, or active compounds from industrial and military agents), physical damages and cancers.[34,36] Although the cells of airway walls are able to regenerate and restore their normal structure under normal conditions, if the damage is severe enough, the repair process will be further impaired, leading to more extensive damage that is more difficult to repair, called airway remodeling.[37-39] Airway remodeling includes changes in the epithelial structures such as subepithelial fibrosis, increased mass of smooth muscles, enlargement of mucous glands and angiogenesis (Figure 2).[40,41] TGF-β that is a factor produced by epithelial cells and fibroblasts, plays an important role in rearrangement of airways in chronic lung disease.[42-44] In addition, TGF-β was found to increase the expression of extracellular matrix components such as collagen. On the other hand, TGF-β decreases the expression of matrix metalloproteinases (MMPs), and increases the expression of tissue inhibitor of matrix metalloproteinases (TIMPs), resulting in the collagen accumulation and fibrosis formation.[45-47] In response to the chronic injuries, remodeling includes decreased airflow, airway obstruction and high sensitivity.[48]

In many airway diseases including asthma, chronic obstructive pulmonary disease (COPD) and cystic fibrosis (CF), small airways, especially epithelium, are damaged, exhibiting a marked delay in repair.[49-52] Because of the low half-life of ciliated cells in the trachea and lungs, the ability to renew and repair the epithelial layer is considered to be slow.[6]

Following injury, the repair process initiated in the airway epithelia includes acute inflammatory responses, recruitment of immune cells (such as neutrophils and macrophages), migration and proliferation of epithelial progenitor cells. Additionally, factors such as epithelial and fibroblast growth factors, chemokines and interleukins [Interleukin-1β (IL-1β), -2, -4 and -13)] as well as prostaglandins (such as PGE2) are released during the lung injury.[34-36] It should be noted that although injury can occur in the distal and proximal airways, the initiation of injury in epithelial cells is similar to that of different sources. Progenitor cells migrate and the epithelial-free surfaces are lined by a layer of epithelial cells.[35]

Clara cells, known as progenitor cells of the bronchial epithelium,[47,53] seem to play an important functional role of stem cells in airways.[6]
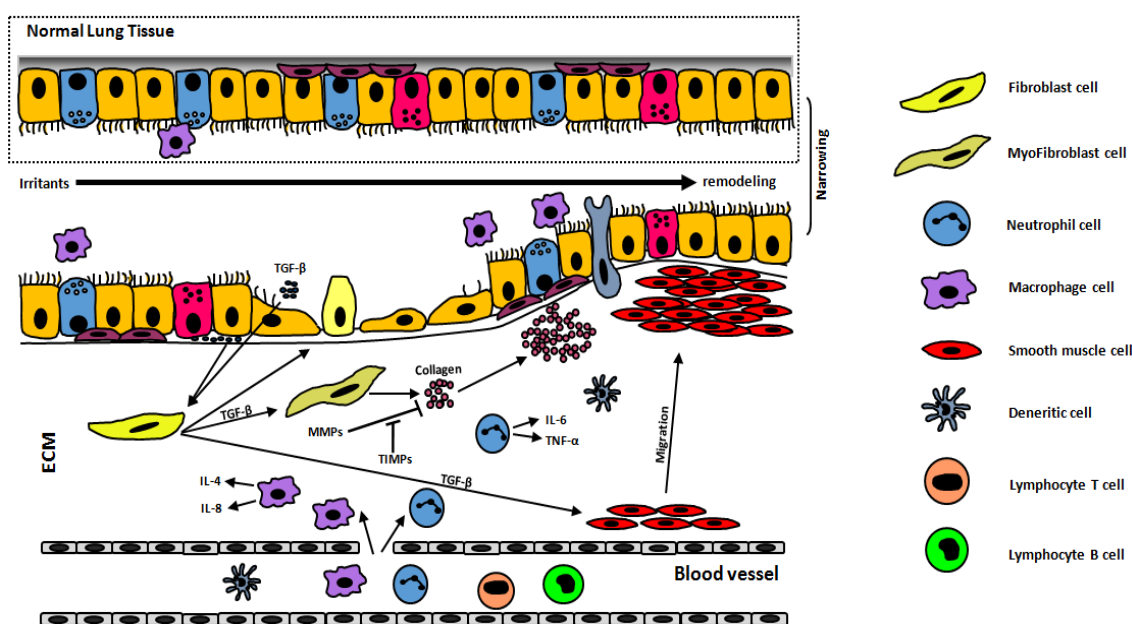
Figure 2. Damage stages of the airway epithelial layer and incomplete repair processes after injury.

During lung injury, ciliated cells damaged by chemical gas may be replaced by Clara cells starting to proliferate.[54] When the intact mouse is exposed to chemical agents such as bleomycin, more Clara cells die due to toxic metabolic activities. Ciliated cells are shed, leaving the bronchial basal membrane naked. Subsequently, only a few Clara cells survive and begin to proliferate. Bleomycin-resistant progenitor cells, a set of Clara cells residing within neuroepithelial bodies, are able to generate a mass of stem cells.[55] The Second epithelial stem cell population found in an area of distal airways is called alveolar type II cells which include 16% of the total alveolar cells. Seven percent of the alveolar surface was covered with alveolar type II cells, expressing high levels of the surfactant protein C.[56]

Molecular mechanisms governing the generation and separation of such cell types in the airways are not well understood. However, it has been found that airway progenitor cells fail to generate epithelial cells under severe injury conditions. In addition, mucus-producing cells are most active after injury in the lungs; this is more common in patients with severe asthma. The presence of inflammatory cytokines, such as IL-9, IL-10 and IL-13, is involved in difficulty breathing and chest tightness.[57-59]

Characteristics and Homing of MSCs

There are complex sets of non-hematopoietic cells in bone marrow called mesenchymal progenitor cells (MPCs). MSCs are well-known as multipotent cells that have the ability to self-renew and differentiate into a great variety of cells.[60,61] MSCs can be isolated from bone marrow, umbilical cord, peripheral blood and adipose tissue, and cultured in specific media. MSC colony formation, which is known as marrow-like stromal cells and MPCs, is similar to fibroblast colony forming unit (CFU-F) in in vitro condition.[62,63] According to the International Society for Cellular Therapy (ISCT), MSCs can be easily detected or identified from other cells using flow cytometric analysis to detect specific surface markers (Table 1).[14,64,65]

Table 1. List of MSCs surface markers in cell culture

Surface markers	Expression (+) or No expression (-)
CD29	+
CD31	-
CD34	-
CD44	+
CD45	-
CD73	+
CD90	+
CD105	+
HLA-DR	-
HLA-ABC	+

In addition, they have the ability to generate differentiated tissues such as bone, cartilage, adipose, astrocyte, tenocyte and myocyte in in-vitro conditions (Figure 3).[66-68] MSCs can also differentiate into ectodermal and endodermal cells.[69] It has been shown that adipose-derived MSCs are able to differentiate not only into the primary muscle cells, but also into the skeletal and smooth muscle cells, following as an appropriate stimulation.[70-72] There is a variety of potential advantages supporting the application of MSCs in cell therapy, such as plasticity properties, ease cultural, easy isolation from bone marrow or other tissues, immunomodulatory function and lack of ethical considerations.[73-75]

The process of MSC homing is initiated by cell migration into vascular endothelium, followed by MSC localization within the tissue. However, the data revealed that localization and homing of MSCs are very infrequent after the infusion. There are several factors that play critical roles in the MSC homing.[76,77] It is supposed that the migration of MSCs is directed by a concentration gradient of the chemokines. Elevated levels of inflammatory chemokines are the key mediator for trafficking of MSCs to the site of injury. Chemokines are released after tissue damage, and induce MSCs to express several receptors for the chemokines.[78,79] The interaction of MSCs with chemokine gradients is an important step in the activation of MSC mobilization towards the site of injury (Figure 4).[80]

During multiple passages in vitro, MSCs exhibit a series of surface receptors with increased or decreased expression profiles, such as CXCR3, CXCR4, CXCR6, CCR9 and CCR10, which might involve homing ability. CXCR4 is an SDF-1 receptor displaying increased expression in bone marrow stem cells, damaged tissue and tumor cells, while not usually expressed on the MSC surfaces in continuous cultures.[81] The interaction of SDF-1α, also termed CXCL12, with the CXCR4 receptor enhances the MSC activation, survival, proliferation and migration to the site of injury. The SDF-1α chemotactic factor produced at the site of injury leads to the recruitment and activation of MSCs for repairing the damaged tissue. However, when damage is severe enough, healing is not completed.[82-84] In response to SDF-1, MSCs express several adhesion molecules as well as chemokines such as CXCL16, CCL21 and CCL19.[85-87] SDF-1/CXCR4 is responsible for both cell cycle arrest and inhibition of homing the CXCR4-expressing cells to the site of injury.[88]

During the homing process, different mechanisms are involved in MSC trafficking, which is performed very slowly within the bloodstream. Large size of MSCs or thin capillaries can reduce their speed due to the physical interactions. In small veins, MSCs display the leukocyte-like processes (Tethering and Rolling) using p-selectin and VLA-4/VCAM-1.

MSCs anchor vascular epithelial cells, diffuse across vascular membranes and reach the site of injury in the epithelial layer through ECM[89-91] (Figure 4).
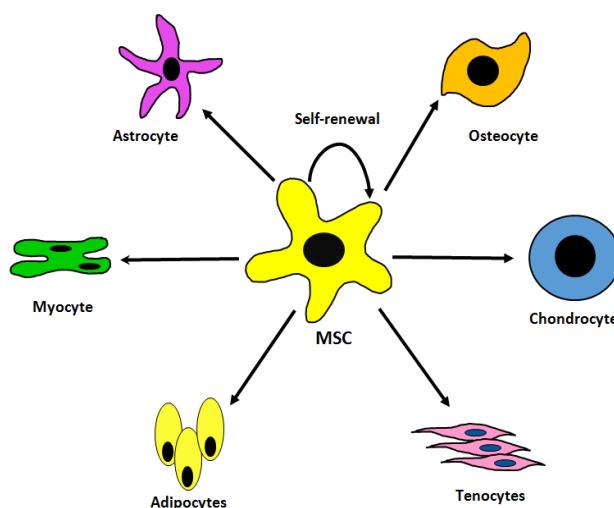

Figure 3. The ability of MSCs to self-renew and differentiate into various tissues such as bone, cartilage, adipose etc.
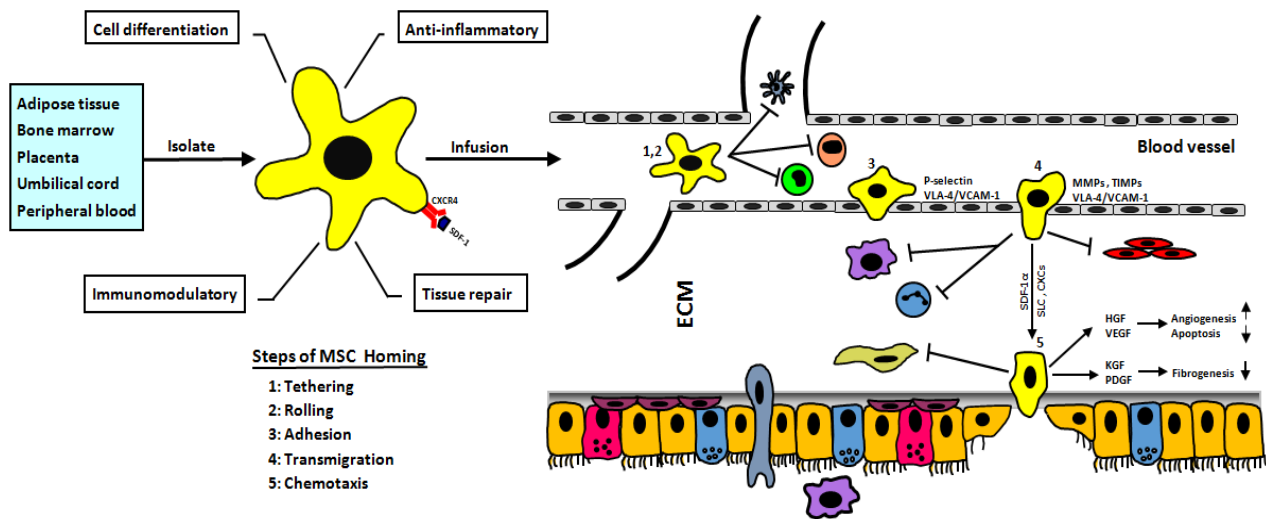
Figure 4. Steps of localization and homing of MSC in lung tissue and recruitment with chemokine gradient concentration.

Molecules Secreted by MSCs

MSCs secrete large molecules such as growth factors, chemokines, cytokines and hormones that not only play different roles in cellular functions, but also have paracrine effects on immune cells and their migration to target sites of injury.[92]

MSCs secrete various biological factors either with proliferating or with anti-inflammatory effects, including Interleukin-37 (IL-37), transforming growth factor-β1 (TGF-β1), vascular endothelial growth factor (VEGF), prostaglandin-E2 (PGE-2), hepatocyte growth factor (HGF), nitric oxide (NO) and hemeoxygenase-1 (HO-1). These factors secreted by MSCs can exert an immunosuppressive effect through the inhibition of T-cell proliferation and induction of Treg cell proliferation.[66,93]

MSCs inhibit DC, T helper cell and NK cell function through the inhibition of pro-inflammatory cytokine secretion such as interferon-gamma (IFN-γ), IL-1β and tumor necrosis factor (TNF-α), whereas inducing increased expression of anti-inflammatory cytokines such as IL-10.[94] IL-10 prevents the apoptosis, and decreases the reproduction and activation of lymphocytes.[12,95]

The Immunomodulatory Properties of MSCs

MSCs are able to modify immune responses including cell proliferation and cytokine production, as well as inhibit cytotoxicity of T cells and NK cells. In chronic inflammatory diseases, studies have shown that MSCs can reduce innate immune responses and may surprisingly have the ability to change adaptive immune responses through their impact on T-cells. In this regard, there are a number of cell surface and intracellular molecules that elicit a strong immune response or induced immune suppression. Adhesion molecules and major histocompatibility complex (MHC) antigens play a key role in the interaction with immune cells, co-stimulatory molecules and ligand-Fas receptors, which are essential for the activation of T cells.[106,107] MSCs induce the immune tolerance not only through a reduction in some surface antigens like MHC class I and II, and co-stimulatory molecules such as B7-2 and B7-1, but also through an increase in the expression of some co-inhibitory molecules like B7-H1 and B7-H4.[108,109] In contrast to the other adhesion molecules, ICAM1 is only expressed under induction conditions. MSCs also express Toll like receptors (TLRs), indicating their immunomodulatory properties.[110,111] Studies have demonstrated that MSCs are able to alter cell proliferation, migration, differentiation and immunosuppressive potentials of other immune system cells by activation of TLR ligands on their surface.[112,113] In addition to differentiation potentials, MSCs display immune regulatory activities like inhibition of various innate and adaptive immune cells, including antigen-presenting cells (APCs), B cells, macrophages, DCs, NK cells, CD8+ cytotoxic T lymphocytes (CTL) and regulatory T cells (Tregs). Some of these complex
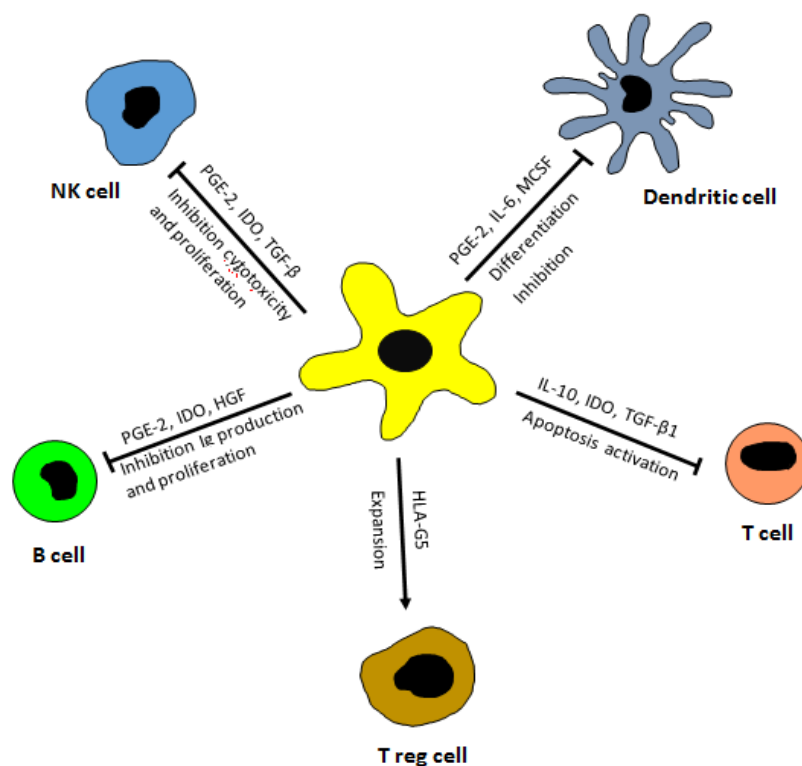
Figure 5. The interaction between MSCs and different cell types involved in both innate and cellular immunity. The release of secreted factors from MSCs leads to a decrease in the proliferation of T and B lymphocytes, NK cells and DCs, as well as an increase in the induction of Treg cells.

mechanisms are not yet clearly understood (Figure 5).[114-118]

The Crosstalk Between MSCs and Immune cells

It is clearly demonstrated that MSCs inhibit T cell proliferation in cell culture conditions.[119] In fact, the expression of some surface markers in lymphocytes decreases in the presence of MSCs. As a result, MSCs are usually used to inhibit CD8^{+} and CD4^{+} T cells.[120] In the presence of MSCs, T-cell proliferation is suppressed at G0 phase of cell cycle.[93]

MSCs modulate the immune responses and lymphocyte proliferation through the induction of Tregs by two ways including cell-to-cell contact between MSCs and T CD4^{+} cells and also secretion of PGE-2 and TGF-β1.[115]

MSCs are able to generate Treg cells through differentiation induction of CD4^{+} T cells into Th1 and Th17 pro-inflammatory cells.[121] Several studies indicated that there are beneficial effects of MSC injection along with Th17 suspension into a mouse model of autoimmune diseases. The increased levels of

lymphocytes such as CD4^{+} CD25^{+} Foxp3^{+} cells are reported in the early stages of the disease.[121] Inhibition of co-stimulatory molecules such as HLA-G by specific blocking antibodies leads to increased T lymphocyte proliferation, suggesting that these molecules are involved in the immune suppression function.[122,123]

Due to PGE-2, IDO and HGF secretion, MSCs inhibit both B-cell proliferation and antibody production.[114,124] MSCs inhibit IL-2-induced proliferation of NK cells via immunosuppressive factors such as TGF-β and PGE-2.[115,125] MSCs also inhibit effective functions of NK cells, such as cytotoxicity against virus-infected cells with a reduction of IFN-γ secretion.[21]

DCs play an important role in survival and regulation of immune responses through the activation of antigen-specific T cells and stimulation of innate immune system cells.[126,127] Recent studies represented impacts of MSCs on the function of DCs. In contrast to their effect on effector immune cells, MSCs have the ability to mediate a low expression of stimulatory molecules on DCs, thus preventing differentiation of

monocytes into mature DCs.[128]

Consistent with this interpretation, a lot of reports have shown that MSCs establish an immunosuppressive microenvironment through the induction of pro-inflammatory T cells that have a regulatory phenotype. These results may provide a chance for MSC utilization in the treatment of inflammatory and autoimmune diseases.[121]

Functional Evaluation of MSCs in Animal Airway Treatment

Several methods are used to evaluate MSC function in animal models. Stem cells are able to localize at the site of injury, proliferate and reproduce airway epithelial cells. To achieve this features, many researchers have developed mouse models with airway diseases to investigate the repair process using different approaches of MSCs administration .

In a mouse model of E.coli endotoxin-induced acute lung injury, the concentration of the KGF molecule on alveolar fluid was evaluated during two stages (before and after the application of human MSCs). The high levels of a KGF factor are found during the administration of MSCs, demonstrating MSC migration at the site of lung injury. Since secretion of considerable amounts of KGF by MSCs is required for MSC renewal. KGF can be also used as a marker for assessing MSC function in the site of injury.[105]

Protein and cytokine profiles in Bronchoalveolar lavage (BAL) fluid, such as macrophage inflammatory protein 2 (MIP-2), which is a murine neutrophil chemokine, can be measured by ELISA. However, protein concentration in BAL fluid can be consider as a marker for lung epithelial and endothelial permeability.[129]

Using lung protein sample, SDS-page and western blotting analyses, it is possible to monitor the presence of vascular cell adhesion molecule 1 (VCAM-1), cytochrom C (Cyt C), intercellular adhesion molecule 1 (ICAM-1) and TNF-α. In addition to the above-mentioned approach, analysis of inflammatory gene expression (mRNA), apoptosis and oxidative stress in the damaged lung parenchyma can also provide suitable diagnostic tests for Adipose-Derived MSCs (ADMSC) functions of the lung. According to this interpretation, mRNA expression of three inflammatory markers, IL-1b, TNF-a and MMP9, should be increased, in comparison to anti-inflammatory markers, endothelial nitric oxide synthase (eNOS), IL-10 and adiponectin. Furthermore, mRNA expression of three antioxidant indicators, hemeoxygenase (HO) -1, glutathione reductase (GR) and glutathione peroxidase (GPx), might be decreased in patient groups. These findings indicated that administration of ADMSC can induce increased antioxidant effects .[130]

Jianguo et al. studied C57BL/6-Tg mice with bleomycin-induced lung injury that expressed high levels of GFP in all their cells. Following injection of MSCs, the lungs of mice were processed and stained with hematoxylin and eosin (H&E) for histopathological studies. The histopathological findings were analyzed to detect inflammatory mediators such as INF-γ, IL-1b, MIP-1a, IL-8 and IL-12 and to evaluate the morphological and functional characteristics of the inflammatory cell.[131]

BCs, normally residing in the lung and tracheal airway epithelium of both mice and human beings, are believed to be stem cells. The activation of BCs and Nichs is regulated through a series of signals (mp7, jagged2,Wnt3a etc), receptors (Ngfr, Ptch2, Egfr etc) and antagonists of intracellular signaling pathways (Socs3, sprouty1, Sfrp1etc), all of which can be used as diagnostic markers to determine the BC function.[132]

Clara cells express specific factors such as Clara cell secretory protein (CCSP), which can be readily analyzed through examining RNA levels before and after cell therapies.[32] In a study performed by Loi et al. (2007), bone marrow cells of male mice were used in a female mouse model of cystic fibrosis. The cells concentrated in the lung and differentiated into epithelial cells were evaluated via the FISH technique for Y chromosome.[133] The results demonstrated that a small number of bone marrow cells can home in the target tissue. During the homing process, the increased expression of surface markers CD34 and SCA-1 was found on the bone marrow cells.[134]

In another experiment, cells from a female mouse with a lacZ/neomycin resistance gene cassette were used as donors. LacZ expression was detected by X-gal staining in the recipient mouse.[135] After administration of MSCs, a mouse model of airway injury exhibited the decreased and increased expression levels of TGF-β and IL-10, respectively.[132] However, one of the most important points to be noted here is differences between the human and mouse models. Detection of cell therapy problems after injection in proximal (bronchus) and distal (alveolar) airways is the main concern in this type of research.

The Role of MSCs in Treatment of Toxic- and Chemical-induced Lung Injuries

Over the past decade, a number of studies have shown that MSCs display great plasticity and are able to differentiate into bronchial epithelium, alveolar, vascular epithelium and different interstitial cells.[136] In addition, several studies indicated that the conversion of the cells in the normal lungs is relatively low.[137-139] In the normal lung, adult stem cells reside in specific areas called niches to maintain homeostasis of the lung tissue. Further knowledge on the characteristics and dynamics of cell lineages in the airway, alveolar epithelial and cell sections of lung MSCs are important principles for the treatment of airway remodeling.

Clinical Trials

In 2012, a group of US researchers investigated the potential efficacy of MSC administration for the treatment of airway diseases, which is currently being investigated in a phase II clinical trial. In that study, it was shown that after 2 years of follow-up post MSC administration, no improvement was observed in the patients with moderated symptoms. However, the overall purpose of the project was to determine the safety and efficacy of MSCs.[140] In the studies by Jeong Chan et al., the safety of human Adipose Tissue-Derived Mesenchymal Stem Cells (hATDMSC) for the treatment of toxicity and tumorigenesis have been demonstrated in animals and human subjects.[141]

In another study in 2013, Angelini et al. examined the possibility to use stem cells in the treatment of lung diseases caused by different chemical warfare agents and biological toxins. In their study, it was revealed that damaging elements can lead to the acute lung injury and respiratory distress syndrome. The researchers suggested that the different stem cells, particularly MSCs, can be used for the treatment of patients.[142]

In light of this, a study was performed by Liang et al. in 2012 for the treatment of bronchiolitis obliterans syndrome (BOS). It was found that intravenous injection of bone marrow-derived MSCs facilities more rapid repair in patients, without side effects; pulmonary function tests and Spirometric measurements were analyzed to evaluate their potential role for the treatment of patients.[143]

In 2007, Vladimir et al. showed that bone marrow-derived MSCs are able to engraft in a small and large airway injury from 2 to 6 days after lung damage.[144]

Gomperts et al. in 2007 also demonstrated the effects of keratinocyte growth factor to repair epithelial tissues in bronchial ducts. The study showed that keratinocyte growth factor can stimulate the migration of epithelial progenitor cells from the bone marrow into blood circulation and subsequently into the sites of injury.[145]

In a clinical trial by Tzouvelekis et al., adipose-derived mesenchymal stem cells were used for the treatment of idiopathic pulmonary fibrosis. Preliminary results showed that no allergic reactions, infections, acute exacerbations or ectopic tissue formations have been attributed to the stem cells. In addition, based on 6-months follow-up data there were no significant differences in the 6 min walk test (6MWT) and forced vital capacity (FVC).[146]

In a study by Nichols et al. in 2012, histologically normal lung tissues were used to construct a three-dimensional scaffold for lung tissue engineering. These scaffolds have a high potential to support the cell proliferation in the lung tissue and can have many applications in lung tissue repair.[147]

Animal Models and ex vivo Studies

In a study in 2007, Gupta et al. evaluated the ability of MSCs to repair Escherichia coli endotoxin- induced lung injury. They found that the overall survival rate of engrafted MSCs was significantly increased, compared with the control group. At the same time, the amount of edema and epithelial tissue infiltration were greatly reduced, when compared to the control group. Approximately 4 hours after the exposure, the direct distribution of MSCs in the air spaces resulted in a significant reduction in pro-inflammatory cytokines, such as TNF-α and MIP-2, and an increase in the anti-inflammatory cytokine IL-10 in lavage fluid and plasma. Finally, that study demonstrated that intrabronchial infusion of MSCs is able to reduce the effects of endotoxin, and to prolong survival of mice.[12] In addition, in 2009 Leblondet al. showed that intrabronchial injection of stem cells could be a promising way for the treatment of animal models of airway injury.[148]

In another study by Wong et al. in 2007, bone marrow cells were used for the targeted treatment of naphthalene-induced airway injury. The study showed that these cells can survive for 120 days in the damaged tissue and stimulate its repair. Analysis performed on airways and alveoli also revealed the increased expression of lung epithelial proteins, Clara cell

secretory proteins and the surfactant protein C.[149] In a mouse model with damaged airway, decreased and increased expression levels of TGF-β and IL-10 were found following the use of MSCs, respectively.[150]

Recent study on mice model with chronic allergic inflammation has shown that after intravenous injection of 2×10^6 cells, bone marrow-derived mononuclear cells (BMDMC) are able to reduce airway remodeling at the site of damaged tissue. Interestingly, BMDMC therapy reduces eosinophil infiltration, expression of actin-specific smooth muscle cells, subepithelial fibrosis, and myocyte hypertrophy as well as hyperplasia. This results in reduced airway hyper responsiveness and lung mechanical parameters. Although being able to increase the expression of insulin-like growth factor, BMDMC reduces IL-5, TGF-β, platelet-derived growth factor and mRNA expression of vascular endothelial growth factor. The results indicated that cell therapy can be used in a model of chronic allergic airway inflammation as an effective method in the repair of airway epithelial cells, inhibition of inflammatory processes and airway remodeling.[151]

In the mouse model following bleomycin treatment, the administration of MSCs was able to decrease inflammatory cell infiltration significantly, reducing the inflammation.[152] The application of MSCs in the C57BL/6 mouse model of acute lung injury (ALI) resulted in reduced pulmonary edema, lung neutrophil numbers, and inflammatory cytokines in BAL fluid and serum.[131] In studies by Yagi, Olivera, and Sun et al. in a rat model with acute lung injury induced by endotoxin, the administration of MSCs led to an increase in survival time, and a decrease in the symptoms of lung injury, pulmonary edema, numbers of neutrophils in BAL fluid, and pro-inflammation cytokines.[130,153,154]

In another experimental study, the effects of endotoxin in two strains of mice, BALB/c and C57BL/6, were examined. Significant improvements were found following MSC administration, including regeneration of alveolar fluid filtration, increased survival rate and reduced pro-inflammatory cytokines.[95]

A potential use of MSCs to repair bleomycin-induced lung damage in rat model showed improvements associated with reduced pulmonary fibrosis, increased survival and decreased airway necrosis.[155] In another study, the therapeutic effects of human umbilical cord-derived MSCs were examined on a mouse model of plant toxin-induced injury; however, the findings of the study showed a decrease in pro-inflammatory cytokines, lung injury, pulmonary edema and numbers of neutrophils in BAL and an increase in BAL fluid protein levels.[156]

In a study by Andrade et al. in 2007, the Gelfoam sponge and rat lung cells were used for lung tissue repair. The results obtained from cell-based tissue engineering for lung regeneration showed that use of sponges after injection can produce a porous structure similar to that of native lung tissue; this can be effective in furthering the lung tissue engineering.[157] Lee et al. studied oleic acid-induced lung injury in ex vivo, as well as the potential role of MSCs in cell therapy. The results of that study demonstrated a reduction of endothelial dysfunction and pulmonary edema with an increase in endothelial barrier function.[105] Fang et al., studied the effect of human MSCs on human alveolar type II cells in cell culture in the presence of inflammatory cytokines (IL-1β, TNFα, IFNγ). The results indicated a reduction in epithelial permeability to proteins, in turn resulting in inflammation reduction.[158]

CONCLUSION

Since airways are severely damaged in pulmonary problems, drug therapy is not effective enough. Immunomodulatory and growth characteristics of MSCs have the great potential to treat various diseases in preclinical studies and in phase I to III clinical trials. The advantages of cell therapy over other treatments include innocuous side effects and lower costs. Hopefully, bone marrow-derived MSCs can be isolated, cultured and manipulated under ex vivo conditions, then released into the lung to prompt regeneration of damaged lung tissue. Although the use of MSCs has reduced the severity of diseases, the long term data for safety of this therapeutic method is demanded. In addition, there is a need for more information about different functional and proliferative properties of MSCs derived from bone marrow or adipose tissues of patients versus healthy subjects. To prevent transmission of prion and viral agents into the recipient's cell population, the improvement of stringent safety conditions is essential to culture MSCs. Additionally, the use of allogeneic MSCs may increase the risk of acquiring infectious diseases from the donor. Hence, it is necessary to assess all phenotypic characteristics of MSCs to facilitate preclinical studies.

However, there are potential risks in the clinical application of human MSCs. According to immunosuppressive features of the immune system in the treatment of inflammatory and immune-mediated diseases, MSCs can be considered as a great promise for future treatments in humans.

ACKNOWLEDGEMENTS

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