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Molecular Pathways Underlying the Therapeutic Effect of Stem Cells during Asthmatic Changes

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ABSTRACT

Allergic asthma is a chronic inflammatory disease characterized by airway remodeling, hyperresponsiveness, and exacerbated inflammation. While most patients respond well to current treatments, a small subset remains resistant necessitating new therapeutic strategies. Due to their immunomodulatory properties, stem cells have been proposed as a promising treatment option for asthma. Stem cells can reduce airway inflammation and restore immune balance, demonstrating positive outcomes, particularly in cases of steroid-resistant asthma. However, the mechanisms underlying lung tissue repair are not clearly defined. On the other hand, there are limitations in using these cells and for clinical use of mesenchymal stem cells, which must be produced in accordance with Good Manufacturing Practice. This review article discusses the mechanisms by which stem cells may aid in asthma treatment and addresses and explores the challenges associated with their use. By addressing these areas, we can better understand the potential and limitations of stem cell therapy in asthma and develop more effective strategies to harness their therapeutic benefits for patients with uncontrolled asthma.

Keywords: Airway inflammation; Asthma; c-Kit cells; Mesenchymal stem cells; Therapeutics

INTRODUCTION

Asthma is a chronic and common disease related to the respiratory system. It affects approximately 5% to 10% of the population and is associated with considerable mortality, morbidity, and economic burdens. Asthma prevalence is high in economically developed countries; its rate is increasing in middle- and low-income countries. It is one of the most prevalent chronic diseases in childhood, affecting nearly 1in 10 children.[1-3]

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The asthma pathophysiology is complex and involves airway inflammation, bronchial hyper-reactivity (AHR), hypersecretion of mucus, reversible airflow obstruction, and airway remodeling, all of which lead to a decreased ability to expel air, hyperinflation, and air trapping. The progressive pathological features associated with the clinical respiratory asthma symptoms include wheezing, dyspnea, breathlessness, chest tightness, coughing, and limitation of expiratory airflow, which differ between individuals and over time. Asthma ranges from an intermittent or mild disease to a persistent and severe disease, which is difficult to treat.[2,4]

AHR is a cardinal feature of asthma, defined as an exaggerated response to several endogenous and

exogenous stimuli. The degree of AHR relates to the magnitude of clinical symptoms of asthma. The exact mechanism underlying AHR in asthma is unknown. A variety of physical and pharmacological agents, including dust, cold air, several irritants and allergens, viral infections, exercise, histamine, prostaglandins, leukotrienes, and methacholine, can trigger bronchoconstriction and asthma attacks. Moreover, genetic factors can contribute to asthma presentation and severity.[5,6]

Several lines of studies have proposed airway inflammation as a potential mechanism involved in the maintenance and development of bronchial hyper-reactivity in asthma. Inflammation includes infiltration of inflammatory cells, predominantly T lymphocytes, eosinophils, and abnormal deposition of the extracellular matrix (ECM).[7,8] Moreover, in asthmatic airways, inflammation not only involves the bronchi and trachea but also spreads to the terminal bronchioles. The uses of bronchoscopy and biopsy have helped us better understand the immunopathological changes of the airways in asthma. Bronchoscopic biopsy shows that the airways are often erythematous and swollen. Moreover, lavage and biopsy of the airways have provided evidence for increased numbers of mast cells, eosinophils, and T lymphocytes, as well as macrophage activation, which all confirm inflammatory responses.[9,10] Besides, studies have demonstrated that pathological airway remodeling, as a result of prolonged inflammation in asthma, comprises epithelial cell shedding, an influx of myofibroblasts, subepithelial fibrosis, collagen deposition, proliferation of blood vessels, hypertrophy of airway smooth muscle, and edema leading to progressive loss of lung function.[11]

Clinically, the disease is categorized into allergic and non-allergic asthma based on the absence or presence of elevated immunoglobulin E (IgE). Allergic asthma is the most common case, in which the immune system is hypersensitive and responds adversely to harmless environmental triggers.[12,13] However, in both forms, helper T (Th) cells infiltrate the airway and secrete cytokines, mainly interleukin (IL)-13, IL-4, and IL-5. These cytokines, also called Th2 cytokines, interact with different resident lung cells like fibroblasts, smooth muscle, and epithelial cells, activate mast cells, and enhance the production of eosinophils, leukocytes, and B lymphocyte (B)-cell IgE. Regulatory T cells (Tregs) downregulate the inflammation-causing T cells by secreting anti-inflammatory cytokines, such as IL-10.[14-16]

Managing asthma is difficult, expensive, and frustrating. Current therapies are often useful in decreasing inflammation and treating acute airflow obstruction; however, their effect in preventing chronic structural changes has not been observed and does not address long-term symptoms.[17] The current treatment options for asthma patients include inhaled corticosteroids, β-agonists, antagonists of leukotriene receptor, omalizumab, theophylline, or tiotropium, which are either expensive or have potential side effects.[18] The majority of patients suffering from asthma respond to these drugs. However, asthma symptoms remain uncontrolled in 5% to 10% of patients after receiving common treatments. The costs of treating refractory asthma account for around 50% of all asthma instances and have a notable financial impact on individuals, families, and society.[19] Moreover, these drugs only relieve the symptoms of asthma but do not change the immune system. Biological immune therapies are considered a pharmacological method to treat severe asthma. However, the diversity in asthma pathogenesis poses challenges in developing sustainable treatments for patients. Furthermore, prolonged use of these medications raises concerns about potential side effects that could restrict their utilization.[20] Therefore, there is an urgent need to discover safe, new, and effective alternative treatments.

Based on research findings, it can be understood that the treatment of asthma with stem cells has beneficial results. Mesenchymal stem cells (MSCs) can differentiate into multiple cell lineages as they are multipotent progenitor cells[21] and provide remarkable immunoregulatory and anti-inflammatory properties, which can repair damaged tissues.[19] MSCs exert their therapeutic effects on asthma by inhibiting the function and proliferation of B and T cells, dendritic cells, and natural killer, suppressing $CD8^+$ T lymphocytes, reversing Th17/Tregs imbalance, restoring Th1/Th2 balance and promoting the switch of macrophages (M)1 to M2.[22-24] Moreover decrease eosinophilia in the bronchoalveolar lavage fluid (BALF), serum concentrations of IL-4 and IgE, mucus production, and goblet cell hyperplasia, inhibit Th2 polarization,[25] increase transforming growth factor beta (TGF-β) and raise Tregs in the lungs,[26] thereby suppressing allergic response.

These cells avoid airway remodeling by diminishing the deposition of collagen and the thickness of the basement membranes in the airways.[24] MSCs decrease

oxidative stress by reducing excessive reactive oxygen and nitrogen species generation, thereby reducing airway inflammation, AHR, and mitochondrial dysfunction in the lung tissue in allergic asthma.[27] MSCs decrease asthma symptoms and pathological changes through downregulation of microRNA (miR)-133 and miR-155[28] and by reducing apoptosis in epithelial lung cells.[29] Besides, MSCs suppress airway inflammation in steroid-resistant neutrophilic asthma.[30,31] Thus, MSCs may be an effective and promising treatment for asthma. The MSCs' low immunogenicity is one of their important advantages for treating asthma. MSCs exhibit minimal levels of major histocompatibility complex (MHC)class 1 molecules. They also lack MHC class 2 molecules; thus, the body's immune system does not recognize them and they do not cause an immune reaction.[32,33] In addition, MSCs secrete a series of immunosuppressive molecules such as IL-10, which play a role in MSC-induced immunosuppression.[34] Some preclinical tests have acclaimed that using allogeneic MSCs for asthma has not caused any complications in terms of immunogenicity and also, significant therapeutic effects have been obtained.[35-37] However, reports state that MSCs can stimulate an immune response.[38]

Another type of stem or progenitor cell is c-Kit$^+$ cells, which possess the typical features of stem cells. Intratracheal delivery of c-Kit$^+$ cells decreases airway remodeling and reduces the number of goblet cells, IL-13, IL-4, and IL-5 by increasing IL-10 in the BALF of asthmatic animals.[39] Suppressed inflammation and nitrosative stress,[40] reduce asthma-related pathologies by controlling the expression of miR-133 and miR-126.[41] These cells also reduce inflammation in asthmatic mice by reducing the level of CD8$^+$ cells, inhibiting the extracellular signal-regulated kinases/nuclear factor kappa B (ERK/NF-κB) signaling pathway, and differentiating into lung epithelial cells.[30] These effects confirm the c-Kit$^+$ cells' immunosuppressive effect, which resembles the well-known action attributed to MSCs. c-Kit$^+$ cells have the advantage over MSCs in that they can be used immediately after extraction and do not need to be cultured. Culture conditions might change the proliferation, regeneration, and differentiation capability of cells.

In addition to MSCs, active molecules secreted from these cells, called secretome or MSC-CM, are of great importance in creating regenerative effects.[42,43]

In fact, compared to stem cells, MSC-CM has more advantages. Since they have no cells, no donor-recipient matching is needed to prevent rejection issues and they are easier to pack and transport.[44] Currently, there are different protocols in many articles with regard to the source of their preparation and production methods.[45]

However, several challenges limit the effectiveness of MSC and MSC-CM-based therapies. These include the limited number of cells that can be extracted from tissues, nutrient and oxygen deficiencies in the culture medium, cell senescence, MSC apoptosis, alterations in protein secretion profiles, and a reduction in the quality of MSC-CM. Collectively, these factors reduce the efficiency of these therapies, ultimately limiting their therapeutic potential for treating asthma.[46]

Thus, the production of these factors for clinical use requires observing the standards of good manufacturing practices (GMP). This is necessary to ensure product quality and safety; however, it faces many challenges and obstacles.

This review discusses the potential therapeutic effects of administering MSCs, their secreted bioactive molecules, and c-Kit$^+$ populations in treating an experimental asthma model with a specific concentration on the cellular and molecular mechanisms of action and the challenges associated with their utilization.

MATERIALS AND METHODS

This review article focuses on the clinical application of stem cell-based treatments, highlighting the fundamental therapeutic mechanisms. To understand the prominence of MSCs, we summarized the immunomodulatory effects and evaluated their therapeutic potential in clinical trials and animal asthma models. This is the result of reviewing several articles indexed in PubMed and Google Scholar. This search was conducted using the keywords of asthma, c-kit cells, mesenchymal stem cells, and treatment. Guidelines, clinical experience, observational studies, and other reviews were included. Also, while checking the quality of studies, data were extracted and summarized. Qualified studies published up to 2024 were used to extract articles, and 3416 articles were explored. During the screening of articles, some articles were eliminated based on titles or abstracts. All the duplicate papers were discarded; finally, 200 research papers were selected for review.

Stem Cell Therapy

In the recent decade, the suitability of cell therapies and bioengineering methods for lung tissue regeneration has been thoroughly studied. There is a growing number of publications applying a variety of progenitor cells to promote functional and structural repair of lung tissue. These studies have generally focused on their paracrine and anti-inflammatory properties rather than their differentiation abilities.[24]

The therapeutic efficacy of bone marrow (BM)-derived stem cells has been observed in several models of lung injury. BM-derived stem cell populations consist of endothelial progenitor cells (EPCs), hematopoietic stem cells (HSCs), and MSCs. Recent studies have provided evidence of the efficacy of adult stem/progenitor cells in animal models of lung injuries for instance chronic obstructive pulmonary disease (COPD), bronchopulmonary dysplasia, and asthma.[24]

Stem or progenitor cells can be administered systematically intraperitoneally, via intravenous infusion, or directly into the lungs via intratracheal and intranasal administration (Table 1).[47-49] They have not yet been able to determine the most suitable way to deliver stem cells in lung injuries. However, among these routes, intratracheal cell delivery has specific advantages, such as minimizing the risk of colonization of other organs, decreasing the number of required cells, and depositing them in the place of need with no need for passing through the vessel wall, all of which enhance the therapeutic benefit of cell therapy.[49]

Table 1. In vitro and in vivo studies on the therapeutic effects of stem cells or extracellular vesicles in asthma models.

MSC Type/ c-Kit cells	Study Type	Mechanism	Routes	Ref.
Rat BM-c-Kit cells	Ova-induced acute asthma in rats	↓Number of leukocytes in BALF, ↓IL-4, ↑IL-10, ↑IFN-γ, ↓deposition of collagen in lung	Intratracheal	50
EV-hBMSCs	Ova-induced acute asthma in mice	↓Numbers of inflammatory cells in lung, ↓Eotaxin-2 in lung and BALF	Intranasal and Intraperitoneal	51
Rat BM-c-Kit cells	Ova-induced acute asthma in rats	Differentiated into lung epithelial cells, ↑$CD4^+$ and ↓$CD8^+$ cells in blood, ↓p-ERK/ERK ratio, and NF-ƙB in lung	Intratracheal	14
Rat BM-c-Kit cells	Ova-induced chronic asthma in rats	↓IL-1β and TNF-α in BALF, ↑INF-γ, ↓IL-4, VCAM-1, and ICAM-1 in lung,	Tail vein	40
hADMSCs, hBMSCs	Ova-induced chronic asthma in mice	hADMSCs: ↓AHR, ↓Th2-cytokines, Both MSCs: ↓airway remodeling and inflammation, ↑RELM-β, hBMSCs were more effective than hADMSCs	Tail vein	52
EV-hUCMSC	Ova-induced chronic asthma in mice	↓Eosinophils and Total inflammatory cells, ↓IL-13, and IL-4, ↓Airway remodeling, ↓Collagen-1, ↓α-SMA, ↓TGF-β1/Smad signaling pathway,	Tail vein	53
Rat BMMSCs,	Ova-induced acute asthma in rats	↓Total number of BALF immune cells, ↓collagen deposition, ↓goblet cells Proliferation, ↓airway remodeling, ↓Wnt/β-catenin signaling pathway	Tail vein	54
hAD-MSCs, hB-MSCs	Ova-induced acute asthma in mice	hADMSCs or hBMSCs: ↓AHR, ↓BALF cell counts and inflammation, double treatment with hMSCs: ↓inflammatory cell infiltration and Th2 cytokine levels.	Tail vein	55

Table 1. Continued...

MSCs Type/ c-kit cells	Study Type	mechanism	Routes	Ref
EV-ratADMSCs	In vitro	↑IL-10, ↓IL-6, and TGF-β in dendritic cells suppress maturation of bone marrow-derived dendritic cells	-	56
Placenta-MSCs	In vitro	↓IL-5 level ↓Proliferation CD8+, and T CD4+ cells,	-	57
EV-hBMSCs	In vitro	↑immune-suppression capacity of Tregs and Proliferation, ↑TGF-β1 and IL-10	-	58
Dental follicle MSCs	In vitro	↓Proliferative of CD4+ T cells, ↓IL-4, and GATA3, ↑frequency of Treg cells, ↑IFN-γ, and IL-10 expression, down-regulate inflammatory responses via TGF-β and IDO pathways, suppress monocytes-stimulatory molecules	-	59
hiPSC-MSCs BM -MSCs	Ova-induced chronic asthma in mice	↓Airway remodeling ↓AHR, modulates airway inflammation via TGF-β1/Smad pathway.	Tail vein	60
hiPSC-MSCs	Ova-induced acute asthma in mice	↓Th2 cytokines, ↓mitochondrial dysfunction of epithelial cells, mitochodrial transfer from iPSC-MSCs to epithelial cells, ↓inflammation	Intratracheal	61
hiPSC-MSCs	Ova-induced acute asthma in rat	↓Neutrophilic airway inflammation, ↓p-STAT3, Th17 cells and IL-17A, ↓mRNA levels of RORγt andGATA3	Tail vein	30
Mice BMMSCs	House dust mite-induced acute asthma in mice	↓Airway hyper-Responsiveness and Bronchoconstriction, ↑switch of M1 to M2,	Tail vein	62
Rat BMMSCs	Ova-induced acute asthma in rat	↓Total WBC and $CD3^+CD4^+$, ↑$CD3^+CD8^+$, ↓tracheal responsiveness	Femoral vein	63

AHR: airway hyperreactivity; BALF: bronchoalveolar fluid; BMMSCs: bone marrow mesenchymal stromal cells; BM: bone marrow; DOI: indoleamine 2,3-dioxygenase; EV: extracellular vesicles; ERK: extracellular signal-regulated kinases; GATA3: a transcription factor; hADMSCs: human adipose MSCs; hBMSCs: human bone marrow MSC; hUC-MSC: human umbilical cord MSC; IFN-γ: interferon-gamma; ICAM-1:intercellular adhesion molecule-1; iPSC-MSCs: induced pluripotent stem cells MSCs; IL-4: Interleukin-4; MSCs: mesenchymal stem cells; NF-ƙB: nuclear factor kappa B; OVA: ovalbumin; p-ERK: phosphate ERK; p-STAT3: phosphor Signal transducer and activator of transcription 3; RELM-β: Resistin-like molecule beta; RORγt: nuclear hormone receptor; α-SMA: α-smooth muscle actin; TGF-β1/SMAD: transforming growth factor-β1/ standardized mean difference; Th2: T helper 2; TNF-α: tumor necrosis factor-alpha; TGF-β: Transforming growth factor-β; VCAM-1: Vascular cell adhesion molecule-1.

The lung also contains stem cell/progenitor cells, which are typically quiescent in normal conditions and can self-renew and proliferate during injury to restore the lung epithelium. There are niches in distinct anatomical regions of the lung containing lung stem/progenitor cells, including Clara cells, basal cells, alveolar type II cells, bronchoalveolar stem cells, and submucosal gland stem cells, which contribute to

homeostasis and repair following damage to the epithelium.[64,65] Moreover, the human lung possesses a pool of undifferentiated c-Kit$^+$ cells that are multipotent and self-renewing.[66] Evidence shows that transplantation of human lung c-Kit$^+$ cells, into an injured mouse lung regenerates epithelial and vascular endothelial lineages.[67] In contrast, Liu et al, showed that c-Kit$^+$ cells have a vascular endothelial cell fate but do not contribute to epithelium during lung repair and homeostasis.[68] Although the lung tissue contains endogenous stem/progenitor cells, due to their complexity, stem cells from extrapulmonary sources receive more attention in cell therapy. Studies show that regardless of which tissue the stem cells are extracted from progenitor cells lodge in the lung or release a range of soluble mediators.[47,69,70]

The results of a systematic review have confirmed the potential profits of stem cell therapy in mice models of asthma, showing that stem cell therapy reduces inflammation, AHR, eosinophil accumulation, and Th2 cytokines production, and improves histopathological changes, such as epithelial thickness, goblet cell hyperplasia, and tissue remodeling.[71]

Mesenchymal Stem Cell Characteristics

MSCs are multipotent adult progenitor cells that can differentiate into multiple cell lineages, including bone, tenocytes, chondrocytes, cardiomyocytes, and adipocytes, thus serving as a cell repository for regenerative medicine.[21] Furthermore, MSCs exhibit immunosuppressive properties through paracrine mechanisms.[72] Given that MSCs can adopt the phenotype and morphology of parenchymal cells, in the lung tissue, MSC engraftment can serve as a progenitor and precursor for lung alveolar epithelium type I and II, pneumocytes, and bronchial epithelial cells.[73,74] Additionally, they release numerous growth factors and express surface molecules enabling them to influence the function of host cells at the injury site.[75,76] In contrast to the adverse effects of hematopoietic stem cell transplantation, MSC delivery does not have any serious adverse effects.[24]

MSCs have been confirmed to suppress lung injury and inflammation and reverse airway remodeling in several mouse models of inflammatory lung diseases, namely asthma.[53,54,71,77] MSCs modulate immune responses by decreasing inflammation and suppressing macrophage and T-cell activation, thereby promoting wound healing. They exert anti-inflammatory and immunomodulatory effects independently of histocompatibility and have been suggested as a possible treatment modality, which can reverse or inhibit inflammation in chronic asthma.[24]

Upon systemic or intratracheal administrations of MSCs, they accumulate in the lung, home to the damaged tissue, and release a range of mediators, which affect damaged lung tissue through their paracrine immunomodulatory activities.[49,54]

Then, within 1 to 3 days, most of the administered MSCs are cleared from the lungs, possibly through the pathways of apoptosis and alveolar macrophage phagocytosis, raising the question of how they produce such long-term immunosuppressive effects.[78]

The BM is a common source of MSCs due to its relatively high concentration of MSCs. Although BMSCs are considered the best cell source in tissue repair, the number of MSCs obtained from BM is limited. Moreover, some studies have shown that MSCs derived from the BM are incapable of secreting the vital levels of critical factors required for regeneration and remodeling.[79]

On the other hand, BMSCs possess significant immunomodulatory, inhibit the function and proliferation of T and B cells, natural killer cells, and dendritic cells, and suppress CD8$^+$ T lymphocyte responses.[13,22,80] Notably, BMSCs show context-dependent immunomodulatory plasticity. In the presence of high levels of pro-inflammatory cytokines, for example, interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α), They adopt a suppressive T cell proliferation and immune-suppressive phenotype by secreting elevated levels of various factors, such as prostaglandin E2 and indoleamine 2,3-dioxygenase. Nevertheless, in the absence of an inflammatory environment, they switch to a proinflammatory phenotype and promote the chemokines secretion that attracts lymphocytes to the inflammation site enhancing T-cell responses.[81]

Alternative sources for isolation of MSCs include adipose tissue, skeletal muscle, periosteum, tendons, bone, lung tissue, synovial membranes, peripheral blood, umbilical cord blood, placenta, skin, and the nervous system.[82] Interestingly, Melief et al have reported that BMSCs have a lower immune-regulatory potential compared to adipose tissue-derived MSCs.[83] Moreover, adipose tissue MSCs are abundant and capable of reducing inflammation in experimental allergic asthma. In addition, compared to BMSCs, lung MSCs have long-

lasting persistence in damaged lung tissue following systemic administration.[84]

The placenta can produce more MSCs than BM.[23] There are some similarities in morphology and function between placenta-generated MSCs and BMSCs; however, placental-MSCs exert robust immune inhibitory effects on T-cell proliferation and activation.[71,85] Additionally, human placenta are more easily available, proliferated, and differentiated; hence, they are more suitable for culture on a large scale than BMSCs. In addition, they retain their immunotolerance properties and present fewer ethical concerns due to the placenta being a medical waste.[25,85] Li et al, reported that administrating human placental MSCs in ovalbumin (OVA)-sensitized and challenged rats decreased IL-17 but increased IL-10 levels in lymph and blood and corrected Th17/Treg balance concomitant with reduced AHR and airway inflammatory cell infiltration.[23] Another study also found that human placenta-deviated MSC engraftment decreased eosinophilia in the BALF, serum concentrations of IgE and IL-4, hyperplasia of goblet cells, and mucus production, and inhibited Th2 polarization in OVA-sensitized rats.[25] Recently, an in vitro study reported that dental follicle-derived MSCs suppressed the lymphocyte apoptosis and proliferation of $CD4^+$ T lymphocytes in cultures of peripheral blood mononuclear cells from asthmatic patients sensitive to house dust mites.[86]

Additionally, compared to BMSCs, human-induced pluripotent stem cells (iPSCs) have better cell proliferation capability, minor immunogenicity, less cell senescence, longer lifespan, and greater survival and engraftment following transplantation.[87,88]

Note that the source of the MSCs affects holding in the lung. In this regard, human umbilical cord blood-derived MSCs are shown to be cleared more quickly from the lungs than human BMSCs,[89] possibly because of variations in the size of MSCs from different sources, as well as differences in the expression of specific proteoglycans and integrins. Different sources of MSCs have divergent gene expression profiles and secretome properties even though they share key distinctive characteristics.[90,91]

Effects of MSC Application on Airway Inflammation and Histological Changes in Experimental Asthma Models

MSCs have been administered in some experimental studies related to asthma, which have yielded promising results concerning efficacy.[49,92] Nemeth et al, have reported that intravenous administration of BMSCs during the airway antigen (ragweed) challenge inhibited eosinophil infiltration, decreased Th2 immunoglobulins in serum, reduced IL-13, IL-5, and IL-4 levels in bronchial lavage, and lowered mucus production in the lung of mice. They revealed that BMSCs suppress allergic response by increasing TGF-β production or recruiting Tregs to the lung.[92] Similarly, Kavanagh et al, delivered 100 μL of long bone MSCs through the tail vein on days 7 and 14 following OVA exposure in mice and assessed allergen-driven airway pathology and responses of inflammation in the lung on day 28. Their results confirmed that BMSCs could suppress OVA-driven airway pathological changes and reduce inflammatory cytokines in allergen-specific IgE and BALF but increase IL-10 in BALF and $CD4^+FoxP3^+$ T cells in the lung. On the other hand, the depletion of Tregs enhanced pathology and increased airway eosinophilia confirming that Tregs are required for the protective effect of MSCs.[93] Dong et al, showed that MSC-derived extracellular vesicles (MSC-EVs) remodeled in chronic asthmatic mice and diminished allergic airway inflammation. They ameliorated the eosinophils, total cells of BALF, and proinflammatory mediators IL-13 and IL-4.[53] MSCs remarkably reduced eosinophilia, total cell count, and serum OVA-specific IgE levels in mice asthma models. They reported that MSCs significantly increased the Treg cytokines level and decreased the expression of Th17 and Th2 cytokines.[94]

Intravenous delivery of mouse compact bone-derived MSCs in an experimental chronic asthma model has also been shown to migrate to the lungs of OVA-sensitized mice 2 weeks after injection and suppress histopathological alterations in both proximal and distal airways including goblet cell hyperplasia, mononuclear cell infiltration, and airway remodeling by enhancing Treg expansion in the lungs.[95]

A recent study showed that intravenous administration of adipose tissue-derived MSCs 24 hours before or 2 hours after the OVA challenge inhibited the airway inflammation development and eosinophilia increased IL-10 levels in OVA-sensitized mice but reduced BALF levels of IL-13, IL-5, and IL-4. Still, monocyte depletion by systemic administration of CCR2 antagonist blocked MSC-induced suppression of airway inflammation.[96] Braza et al's studies have shown that an injection of BMSCs inhibits airway inflammation, which

was evidenced by the reduction in the number of BALF eosinophils, neutrophils, lymphocytes, and bronchoconstriction in an acute allergic asthma model induced by house dust mite. Moreover, they showed that transplanted MSCs induce suppressive alveolar macrophage phenotype through phagocytosis, which consequently attenuates ongoing inflammation and stimulates regeneration and repair in the asthmatic lungs.[62] Similarly, it has been reported that intravenous administration of MSCs from an allogeneic donor 1 hour prior to the allergen challenge suppressed Th2-mediated AHR and airway inflammation in house dust mite-sensitized mice, even though the infiltrating type 2 innate lymphoid cells number was not affected.[97] Firinci et al, have reported that a single intravenous injection of BMSCs migrated to the lung tissue and helped to repair the inflammation of the lung tissue by ameliorating serum nitric oxide levels, the thickness of the basement membrane and subepithelial smooth muscle layer, and the quantity of mast and goblet cells in a mice chronic asthma model induced by OVA.[98] Human bone marrow-derived MSC treatment remarkably reduced AHR, inflammation, remodeling, and Th2-cytokine levels in the murine chronic allergic asthma model using OVA sensitization.[52]

Evidence shows the effect of the route of administration on the beneficial effects of MSC therapy in asthma. In this regard, Royce et al investigated the effect of chronic (14 days) intravenous or intranasal delivery of mesenchyme-angioblast (MCA)-derived MSCs on chronic asthma in OVA-sensitized mice. They found that both intravenous and intranasal delivery of MCA-MSCs decreased airway inflammation, TGF-β levels, AHR, and histological changes associated with asthma, including goblet cell metaplasia, epithelial thickening, collagen accumulation, and total lung collagen concentration-induced by OVA challenge. In a comparison of two techniques of MSC delivery into the lungs, direct delivery via the intranasal route provided greater protective effects than the intravenous route.[99] Another study demonstrated that intratracheal administration of murine MSCs from three sources, namely BM, lung tissue, and adipose tissue, differently affects the remodeling and inflammatory processes in OVA-induced allergic asthma, possibly due to releasing different mediators. Moreover, the effects of BMSCs were more pronounced than those of adipose tissue or lung MSCs.[90] However, it was reported that intratracheal delivery of BM-derived mononuclear cells resulted in a higher number of cells trapping within the lung parenchyma when compared to the intravenous route, both routes similarly modulated inflammatory and remodeling processes in OVA-sensitized mice.[47]

Evidence also shows that intraperitoneal delivery of BMSCs in allergic asthma mice model is effective as well. When MSCs were intraperitoneally delivered, they migrated to the lung and nasal tissues, attenuating airway remodeling and inflammation through inhibition of Th2-mediated airway inflammation.[100] Moreover, intraperitoneally administered BMSCs ameliorated histopathological changes of the lung tissue associated with reduced cytokines levels, namely TNF-α, IL-2, IFN-γ, and in the BALF in a mouse model of bronchiolitis obliterans.[101]

Most experimental studies delivered MSCs directly after initial allergen sensitization followed by allergen challenge episodes; then, the immunologic and pathologic parameters were assessed. However, Trizl et al administered 6 intravenous infusions of MSCs bimonthly in an experimental chronic asthma model in cats (sensitized with Bermuda grass) and examined the long-term results longitudinally for 1 full year. They found that MSCs failed to reduce AHR and inflammation (the percentage of eosinophils in BALF). However, MSC treatment could reduce airway remodeling by month 8 (6 months after the last MSC infusion).[102] Another pilot study investigated the long-term effects (after 9 months) of 5 intravenous administrations of allogeneic MSCs within the initial 130 days following asthma induction in the feline. Their results showed that in points, airway eosinophil percentage was not affected by MSCs administration and decreased to the percentage of the normal group by month 9. Nevertheless, MSC treatment diminished AHR, lung attenuation, and bronchial wall thickness on day 130.[103] These studies support the effectiveness of MSC therapy in a large animal model of asthma (Figure 1).

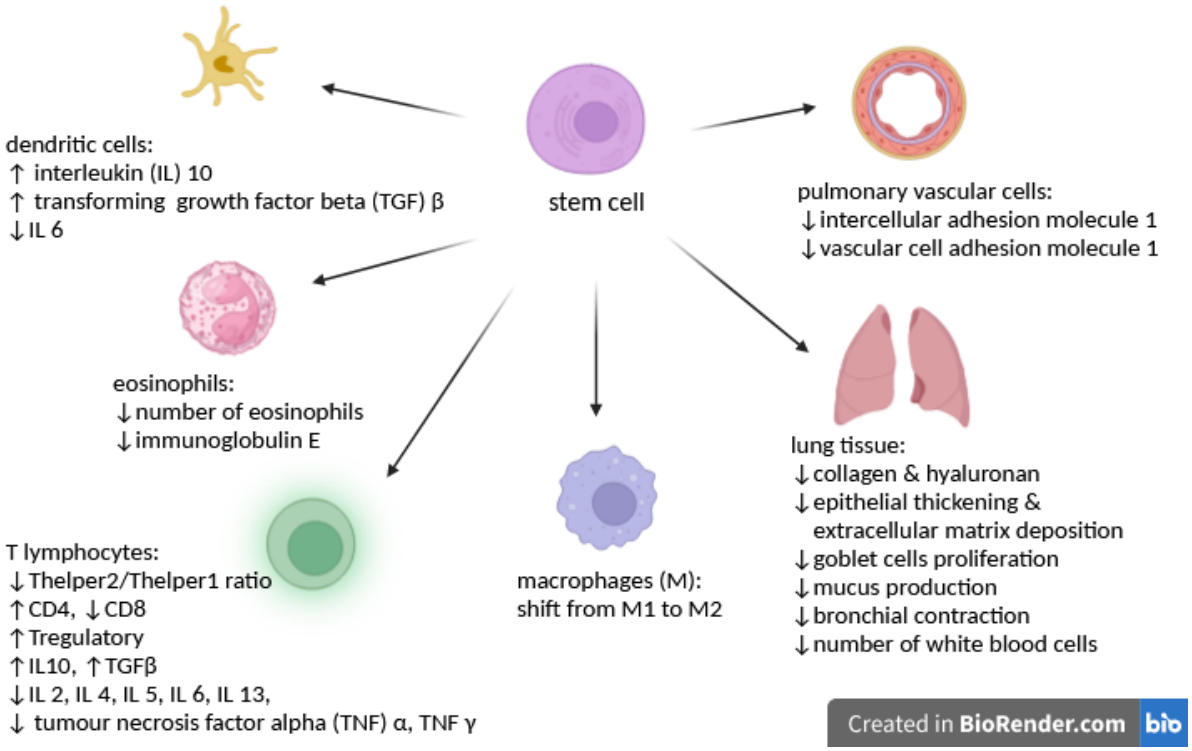

Figure1. Effect of stem cells on pulmonary asthmatic niche

Effect of Human MSCs in Experimental Asthma Models

Yijun Liu et al reported that immune polarization of human MSCs by interferon-gamma treatment causes remodeling of human MSC (hMSC) metabolic pathways leading to glycolysis, necessary for sustaining the secretion of immunosuppressive causes.[104]

Interestingly, despite species variation in MSC-mediated immunosuppression, human BMSCs have been demonstrated to repress airway inflammation in the chronic and acute asthma mouse model, even though no effect on lung function was demonstrated.[105] However, double hMSC treatment remarkably induces lung histological changes and eosinophilic airway inflammation. As a result, double hMSC therapy is not effective against asthma.[55]

Moreover, Mathias et al, have shown that 3days of intravenous administration of hMSCs isolated from bone marrow, adipose tissue, or umbilical cord to OVA-sensitized mice model, inhibited asthma hallmark features, including BALF eosinophilia and AHR decreased goblet cells secretion in the airway epithelium and Th2 cytokine release, and increased Tregs in the lung and alveolar macrophages. Importantly, by the reduction of alveolar macrophages by clodronate-encapsulated liposomes, the suppressive effects of MSC treatment on hallmarks of asthma were destroyed, indicating that alveolar macrophages have a critical role in mediating the effects of MSCs.[106] Song et al showed human BMSCs delivery via tail vein-induced alveolar macrophage polarization into M2 subtypes and, consequently decreased the hallmark features of OVA-induced asthma in mice, including AHR and eosinophilia, mediated by TGF-β–signaling pathway.[107] Goldestin et al have also described retro-orbital administration of hMSCs therapy inhibited chronic inflammation and extracellular matrix deposition and decreased hyaluronan in OVA-induced chronic asthma in mice.[108] Interestingly, Cruz et al reported that both extracellular vesicles and conditioned media from hBMSCs are more effective than that from mouse BMSCs in improving *Aspergillus* hyphae extract-provoked AHR and airway inflammation in a mouse model.[109] The mechanisms underlying therapeutic

effects of human BMSCs in animal asthma models are not exactly known but are attributed to paracrine mechanisms.

Clinical Trials

Most of the reports on the stem cells' therapeutic effects in asthma are the results of experiments which are conducted on laboratory animals. Up to June 2024, only 4 preclinical studies about the MSCs' therapeutic effects on asthma patients have been registered on ClinicalTrials.gov, among which two trials are currently recruiting patients. One of these trials is the phase I clinical trial (NCT05147688) at the Medical Surgical Associates Center, started in 2021, in which human MSCs obtained from the umbilical cord (hUCMSC) are injected intravenously into patients suffering from lung diseases, including asthma. In a separate phase I clinical trial (NCT05035862) at Children's Healthcare Atlanta in 2021, human MSCs obtained from the umbilical cord and IFN-γ-primed human-BM-MSCs were administered intravenously to patients with moderate to severe asthma. The symptoms and side effects caused by cell injection, including cough, shortness of breath, wheezing, respiratory failure, and allergic reactions are monitored 3 to 7 days after administration. In these 2clinical trials, lung function, airway inflammation, and drug side effects will be evaluated over a 4-year period.

In a phase II clinical trial conducted in 2014 at Punta Pacifica Hospital in Panama City (NCT02192736), trophic factors derived from mesenchymal cells allogeneic human MSCs obtained from the umbilical cord (human-UC-MSCs) were injected intranasally to patients suffering from asthma, on a daily basis, for 4 weeks.

The results of this research are unclear. Finally, in another phase I clinical trial (NCT03137199), which was conducted in 2017 at the University of Miami, School of Medicine, the allogeneic human MSCs effect obtained from bone marrow (human-BM-MSCs) on lung function and inflammation of the airway in patients with asthma through intravenous injection for 48 weeks was scrutinized. After some time, the study was stopped.

Moreover, a 68-year-old man with refractory asthma symptoms who participated in a phase 1 clinical trial received an intravenous infusion of 100 million human MSCs derived from the umbilical cord. Then, the follow-ups were done 2 and 6 months after the treatment. Throughout several months of follow-up, no adverse effects related to the treatment were identified. Within 2 months after treatment, asthma symptoms and the amount of drugs used to reduce asthma symptoms decreased by more than 70%.[110] Owing to the significant potential of stem cells in treating asthma, more human trials are needed to bring stem cell-based treatments closer to a therapeutic reality.

Proposed Pathways

Multiple ways have been suggested for the beneficial impacts of MSCs on improving cardinal hallmarks of asthma, such as differentiation into specific cell types, cell fusion, mitochondrial transfer, exosomes, and paracrine factors.[111] Extensive studies have investigated the paracrine factors effects of transplanted MSCs, including molecules and EV in asthma. Additionally, several factors, such as administration route, dose, source of MSCs, the timing of administration, and durability of MSCs may affect the outcome.[90,112] Yu X et al, revealed that MSCs are able to differentiate into epithelial pulmonary cells, repair the damaged epithelium, and restore its integrity and structure.[19] MSCs have been suggested as promising agents for treating allergic disorders because they can differentiate into different cells and repair damaged cells with this process. Nevertheless, these cells have many limitations, including the danger of aneuploidy, rejection of the immune system, and tumorigenesis. Thus, attention must be paid to EVs released from MSCs in solution. These vesicles are as effective as MSCs in suppressing inflammation of the airways by reducing Th2 cytokine production and increasing Treg activity, and they also eliminate the risks associated with the use of MACs.[72]

Immunosuppression

Several research groups demonstrated the immunosuppressive and/or anti-inflammatory effects of MSCs, which enable them to diminish inflammation and remodeling of airways and, ameliorate lung function in asthma. It has been found that the paracrine function of MSCs can be mediated through EV.[28] Mesenchymal stromal cells derived exosomes reduce IL-6 release but increase TGF-β and IL-10 release in tolerogenic dendritic cells. The maturation of bone marrow dendritic cells was suppressed by these exosomes and they may be major modulators of the immune responses by dendritic cells.[56] Placenta-derived MSCs were cultured with isolated blood mononuclear cells from asthmatic children and reduced IL-5, $CD8^{+,}$ and $CD4^{+}$ T cells.[57] Human bone-marrow-derived MSC exosomes, upregulated TGF-β1 and IL-10

from mononuclear cells of patients with asthma.[58] Genç et al, showed that dental follicle MSCs decreased the proliferation of $CD4^+$ T cells and improved Treg frequency in mononuclear cells of asthmatic patients. In addition decreased GATA3 and IL-4 expression and increased IL-10, IFN-γ and T-bet expression.[59]

Habibian et al showed that MSCs remarkably reduced the total white blood cell count, eosinophils, and the IgE concentration in the serum of mice sensitized with OVA. They also concluded that these cells elevated Treg cytokines levels and suppressed expressions of Th2 and Th17 cytokines. Consequently, MSCs have a good therapeutic potential for allergic asthma.[21,94] Moreover, it has been shown that allergic asthma is associated with an elevated Th2/Th1 ratio, by which increased Th2 cell activity results in eosinophilic infiltration and accumulation and subsequent degradation of elastin fibers in the lung parenchyma.[113] However, MSCs are capable of interfering with allergic airway inflammation by increasing Tregs, promoting a Th1 phenotype.[70] Accordingly, reduced cytokines of Th2 (IL-5, IL-4, and IL-13) accompanied by inhibition of lung Myeloid dendritic cells, IgE production, and activity of IgE-dependent mast cells, inhibited eosinophilic infiltration and mucus production.[26,114]

Furthermore, MSCs enhance the generation of anti-inflammatory cytokines, including TGF-β and IL-10 which, together with Tregs, inhibit ongoing Th2-mediated inflammation in the lung in asthmatic animals.[26,47,58,114] These MSC-mediated effects lead to a substantial depletion of airway inflammation, AHR, and notably enhanced lung function. Along with anti-inflammatory properties, MSC delivery averts airway remodeling in asthma animal models by reducing lung parenchyma collagen deposition, thickening of the basement membranes in the airways, and fibrosis.[24]

Paracrine Effects of MSCs

Some of the therapeutic effects of MSCs, especially their anti-inflammatory properties, are attributed to soluble factors. Factors for instance prostaglandin E2 (PGE2), IL-10, and TGF-β.[34] PGE2 binds to E-prostaglandin 2 (EP2) and EP4 receptors, which are expressed on the immune cells' surface, and exert anti-inflammatory effects. This compound leads to the differentiation of dendritic cells to an anti-inflammatory phenotype, suppresses the mast cells' inflammatory response (MC), and increases the production of M2 macrophages.[34,115] In a mouse asthma model, injecting MSCs derived from mouse adipose tissue increased lung TGF-β and PGE2 and improved lung function.[116] Inhibits the production of PGE2, TGF-β, and MSC-induced Tregs, and abolishes the immunosuppressive effects of mouse adipose tissue-derived MSCs in asthma.[117]

IL-10 is an immunomodulating agent with significant anti-inflammatory properties. This cytokine acts by inhibiting several immune responses. IL-10 reduces T-cell proliferation and Th17 cell production while simultaneously increasing Treg production.[118] Studies have shown that transplanting human placenta-derived mesenchymal stem cells (PL-MSCs) into asthmatic mice leads to a significant increase in IL-10, Treg, and FoxP3 levels in peripheral blood, lymph nodes, and lung tissue. This process leads to the inhibition of IL-17 and inflammation.[23] TGF-β is essential for the suppression of allergic responses caused by asthma induced in mouse models. This factor inhibits Th2 differentiation and increases Treg production.[34] The secretion of TGF-β from MSCs and their binding to immune cell receptors decreases the IL-4 production and inhibits inflammation. Knockout mice that are unable to secrete TGF-β do not show positive effects.[92] There are also contradictions in this case. Zhonga et al showed that by reducing the production of TGF-β through inhibiting the Smad2/Smad3 signaling pathway, the level of inflammation subsides in mouse models with allergic asthma.[119] Researchers declared that TGF-β is a profibrotic growth factor that plays an important role in remodeling in chronic asthma. This factor causes the production of extracellular matrix (ECM) through the Smad2/Smad3 signaling pathway.[120] Nevertheless, the function of TGF-β has not been explained in vivo completely and needs further investigation.

Cruz et al, reported that systemic delivery of mouse or human MSC-secreted EV or CM is as effective, if not greater, than the MSCs themselves in improving, lung inflammation and AHR in a mouse model of severe refractory clinical asthma induced by *Aspergillus* hyphal extract.[121] The results of clinical trials and experiments have shown that EV derived from MSCs, including cytokines, chemokines, growth factors, and protease vesicles, are capable of immunoregulatory and repairing and improving damaged lung tissue in lung diseases, including asthma, via paracrine mechanisms.[122]

The Paracrine Effect of Condition Media

Given that some MSC therapeutic effects are facilitated by the secretion of numerous soluble factors,

a new cell-free remedy like CM of MSC could be considered as a substitute approach that may warrant more efficient outcomes without any concern regarding cell transplantation, such as tumorigenesis, immune compatibility, and emboli.[123] Moreover, CM can be stored, handled, and transported easily during clinical procedures.

Several studies verified the efficiency of MSCs-derived CM injection in improving airway inflammation in allergic asthma.[51,63,69] Keyhanmanesh et al reported that regardless of the ineffectiveness of a single dose of MSC-derived CM, 3 times intravenous injections could decrease the IL-4 level in serum, increase the IFN-γ/IL-4 ratio and level of INF-γ, and decrease pathological lesions in the lungs, including shedding of epithelial cell, alveolar hemorrhage, and hyperplasia of goblet cell in OVA-sensitized rats. These outcomes were accompanied by upregulation of INF-γ mRNA expression and T-bet and down-regulation of GATA-3 mRNA expression and IL-4 in the lung tissue.[51] Another study from this group revealed that intratracheal injection of BMSCs and/or CM, notably MSCs, diminished Th2-mediated airway inflammation, indicated by downregulation of IL-5 mRNA expression and upregulation of IL-12 and INF-γ mRNA, and interstitial hemorrhage, in part, by diminishing the endothelial adhesion molecules, intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) expression in the asthmatic rat lung tissue.[63] In contrast, a study reported that intravenous injection of CM did not affect airway inflammation in OVA-induced asthma in rats.[124] Similar reports have been provided using EV and EV-mimetic nanovesicles obtained from these cells.[125]

Another mechanism of asthma therapy by MSCs is the interaction between alveolar macrophages and MSCs in the damaged lungs, resulting in alveolar macrophages polarization into anti-inflammatory M2 subtypes, thereby suppressing ongoing mucus hypersecretion and inflammation, and promoting asthmatic lung regeneration.[62,107]

Interestingly, it has been reported that pre-stimulation of BMSCs with serum or BALF obtained from asthmatic mice could enhance their therapeutic effects in experimental allergic asthma. Abreu et al, have shown that BMSCs pretreatment with asthmatic mice serum enhances their immunomodulatory properties and potentiates their therapeutic effect, thereby attenuating lung inflammation and remodeling and ameliorating lung function in house dust mite-induced asthma.[126] Intratracheal administration of MSCs-derived exosomes in mice challenged with OVA reduced inflammation, improved histological changes, suppressed M1 polarization, and increased M2 polarization in asthmatic lungs.[127]

In response to injury, MSCs also have the capacity for mitochondrial transfer, which promotes tissue regeneration. This effect is mediated by interaction with the beside cell and making a gap junctional channel, microvesicles, formation of tunnel tube, cell fusion, and uptake of mitochondria directly, by which its mitochondria are transferred to the impaired cells.[29] Several signals trigger mitochondria transfer to the damaged cells from MSCs, including oxidative stress, inflammation, and mitochondrial dysfunction.[29,128] Since the mitochondria restore the bioenergetics needs of damaged cells, it can be regarded as an innovative approach for treating several disorders, namely lung diseases.

Islam et al, in an in vivo study, reported that mouse BMSCs interacted with alveolar epithelium cells, forming connexin-43 GJC for transferring their mitochondrial, restoring epithelial bioenergetics and protecting from acute lung injury by lipopolysaccharide. Interestingly, BMSCs expressing dysfunctional connexin-43 were unable to attach to the epithelium of the alveolar and transfer their mitochondria, indicating the crucial role of connexin-43 in this process.[129] Another study showed the importance of Miro1, a mitochondrial Rho-GTPase, in the regulation of MSCs mitochondrial transfer to bronchial epithelial injury induced by rotenone in mice.[61] Li et al, too demonstrated the BMSC protective effect against smoking causes COPD by mitochondrial transferring mechanism in a rat model.[130] Recently, Yao et al reported that intratracheal transplantation of iPSC-MSC reduced inflammation of the airway and mitochondrial dysfunction of epithelial cells induced by OVA in mice through connexin-43-mediated mitochondria transfer.[131]

MSCs and Oxidative Stress

Additionally, oxidative stress is involved in asthma pathophysiology, causing airway inflammation, goblet cell hyperplasia, mucus hypersecretion, and airway remodeling.[132,133] On the other hand, MSC transplantation is reported to reduce oxidative stress in asthma by diminishing excessive reactive oxygen and nitrogen species generation. Malaquias et al, have shown

that intravenous hBMSCs administration in a paracrine manner attenuated nitrotyrosine and maintained the oxidant/antioxidant balance in the lung tissue of asthmatic animals.[134] Furthermore, Li et al have demonstrated that induced pluripotent stem cells attenuated oxidative stress-induced dysfunction of mitochondria in smooth muscle cells of the human airway in vitro and reduced airway inflammation, AHR, and mitochondrial dysfunction in the lung tissue of mice in vivo.[27] Fereshteh Dalouchi et al, Reported that MSC-CM can improve pathological conditions, for example, fibrosis, oxidative stress, and airway inflammation in allergic asthma induced by OVA.[135]

Effects on MicroRNAs

Several studies have confirmed microRNA involvement in the expansion of allergic inflammation of the airway. Therefore, it seems that targeting microRNAs in the airway can be a promising treatment for allergic asthma.[136,137] Human MSCs have been shown to decrease miR-155-mediated inflammatory responses induced by stretch in epithelial cells of human bronchial.[138] Tang et al, reported that injection of BMSCs intravenously reduced mmu-miR-21a-3p and mmu-miR-449c-5p, but increased mmu-miR-496a-3p expression levels in OVA-sensitized female mice.[137] Li et al have also shown that iPSC-MSCs keep safe epithelial cells of human bronchial from hypoxia through up-regulation of miR-21.[139] A recent study showed that BMSC transplantation attenuated asthmatic symptoms and pathological changes through the downregulation of miR-133 and miR-155 in an OVA asthma rat model.[28] EV of MSCs prevented Group 2 innate lymphoid cell-dominant airway inflammation at least partially via miR-146a-5p, indicating that EV could represent a novel cell-free approach for the treatment of allergic disorders.[140]

Effects on Apoptosis and Autophagy Pathways

Evidence shows that mitochondrial dysfunction induces apoptosis cell death and airway epithelial cell damage, resulting in allergic airway inflammation.[141,142] MSCs also produce and secrete proteins that directly inhibit apoptosis or neutralize the apoptotic pathway, eliminating cellular injury.[143,144] Therefore, to manage and treat asthma, one of the existing strategies can be targeting the apoptosis of epithelial cells through mitochondria.

Transplantation of BMSCs (intravenous route) has been demonstrated to reduce microvascular permeability and inflammation induced by ischemia-reperfusion lung injury mice model by enhancing autophagy-related signaling pathway.[145] Zhou et al also discovered that MSC transplantation reduces LPS-induced acute lung injury by increasing autophagy of pulmonary endothelial cells and downregulation of miR-142a-5p.[146] Paliwal et al, have shown that transplantation of iPSC-MSCs reduced allergic airway inflammation by renovating bronchial mitochondrial function and downregulation of apoptosis markers (activated caspase 3 and caspase 9) in the lung of OVA-sensitized mouse model.[29] In addition, a significant reduction of apoptosis and mitochondrial reactive oxygen species has been observed in laboratory results on human airway smooth muscle cells treated with iPSC-MSCs.[147]

Molecular Targets of MSCs in Asthma

Evidence confirmed that phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt) and Notch signaling are the central molecular targets of MSCs in asthmatic lungs.[77,148] Previous studies have revealed that the Notch pathway regulates the development, differentiation, and stimulation of T cells, and induces Th2 differentiation.[60,149] Lin et al found that intravenous transplantation of MSCs suppresses airway remodeling and lung inflammation by modulation of the PI3K/Akt signaling pathway in chronic asthma induced by OVA in rats.[77] Human placenta MSCs engraftment suppresses airway inflammation response in OVA-sensitized rats by blocking Notch signaling in the lungs.[25] Recently, it has been reported that systemic administration of iPSC-MSCs and BMSCs before the allergen challenge, inhibited inflammation of the chronic allergic airway, remodeling, and fibrosis by blocking the TGF-β1/standardized mean difference (Smad) pathway in mice model of asthma.[119] In the asthmatic rats lung, MSCs inhibit inflammation, remodeling, and airway epithelial-mesenchymal transition. This proceeding is partially attributed to the prevention of the Wingless/Integrated (Wnt)/β-catenin signaling pathway by MSCs-derived exosomes.[54]

Possible Benefits of c-Kit$^+$ Transplantation in Asthma

Another type of stem/progenitor cells are the c-Kit$^+$ cells, which possess typical features of stem cells, including multipotent, self-renewing, and clonogenic capabilities.[150,151] c-Kit receptor (c-Kit$^+$, also known as CD117) is a type III tyrosine kinase receptor for stem cell factor (SCF) and a marker of tissue stem cells and

BM-derived. This receptor is expressed only in 1% to 4% of BM-derived hematopoietic stem cells including EPCs, MSCs, and HSCs,[152,153] and contributes to the differentiation, proliferation, and survival of hematopoietic stem cells.[154,155] A clinical study confirmed the safety of the transplantation of c-Kit$^+$ cells in treating heart diseases.[156]

Although these cells have previously been reported at a frequency of 1 stem cell per 24,000 cells in the lung,[67] another study using genetic lineage tracing shows that a significant part of normal lung vascular endothelial cells are c-Kit$^+$ cells.[68]

The BM-derived c-Kit$^+$ cells are functionally heterogeneous, and only a limited group of these cells can home to the injured tissue.157 They have been shown to protect the cardiac function and heart tissue from ischemic injury by increasing endogenous cardiac progenitor cells and the cardiac angiogenic milieu.[158,159]

Most of the clinical trials and preclinical studies investigating the effect of c-Kit$^+$ cell delivery have been performed in the cardiac tissue in the treatment of ischemic heart disease.[150,158,160-162] Emerging evidence also shows that c-Kit$^+$ cells play a key role in alveolar maintenance and lung homeostasis so that mutation of c-Kit$^+$ in mice leads to the expansion of epithelial progenitors and abnormal lung architecture.[163] Evidence shows that lung c-Kit$^+$ cells exert stem cell features and contribute to pulmonary homeostasis and repair.[68,164] The c-Kit receptor contributes to lung development, vascular endothelial cell fate, and lung vascular development,[66,165] alveolar endothelial cells and CD133$^+$ epithelial progenitor cells express this receptor.[166,167]

Intratracheal delivery of BM-derived c-Kit$^+$ cells has also been shown to improve lung alveolarization and angiogenesis in neonatal hyperoxia-induced lung injury.[168] Furthermore, chronic hypoxia calls up c-Kit$^+$ cells from the BM in the circulation, resulting in the accumulation of c-Kit$^+$ cells in the remodeled artery wall of the pulmonary.[169,170]

Silencing of c-Kit with systemic injection of small interference RNA (siRNA) has been shown to attenuate the infiltration of lymphocytes and eosinophils into the lung tissue and BALF in OVA-induced allergic asthma model in mice.[171] Wu et al also found that administration of siRNA in experimental allergic asthma intranasally repressed the c-Kitgene expression in the lung tissue and reduced the production of SCF, IL-5, IL-4, and eosinophilic infiltration in BALF, but did not affect IFN-γ levels and decreased airway mucus secretion.[172]

SCF plays a decisive role in eosinophil survival, activation, and chemotaxis of mast cells and basophils.[173,174] Previous animal and human studies showed that SCF levels in allergic asthma are increased.[164,175,176] Intratracheal transplantation of SCF induces IL-4 production, airway inflammation, and AHR in mice due to mast cell activation.[177] Moreover, Al-Muhsen et al have shown high expression of c-kit and SCF receptors in bronchial tissue and bronchial wash fluid of asthmatic patients.[50] A clinical trial also reported that inhibition of c-Kit by imatinib decreased mast-cell counts, AHR, and tryptase release and slightly increased forced expiratory volume in the first second (FEV1) in patients with severe refractory asthma (NCT01097694).[178]

Spaziano et al have found that c-Kit$^+$ cells were located rarely in the epithelial layer and mostly in the lung parenchyma of normal mice. Moreover, intratracheal delivery of c-Kit$^+$ cells decreased airway remodeling, manifested by decreased bronchial epithelium and airway smooth muscle layer thickness associated with a decrease in the goblet cell numbers and improved airway function in the OVA-induced asthma model. Furthermore, c-Kit$^+$ cells attenuated the immune response by decreasing the release of IL-13, IL-5, and IL-4, along with increased IL-10 in the BALF of asthmatic animals.[39] Systemic administration of c-kit$^+$ cells in asthmatic rats suppressed nitrosative stress and inflammation in lung tissue concur by decreasing pathological changes.[40] Intratracheal injection of c-Kit$^+$ cells reduced asthma-related pathologies, probably by controlling the expression of miR-133 and miR-126.[41] These cells also reduce inflammation in asthmatic mice by nitrosative stress decreasing the level of CD8$^+$ cells in the blood, inhibiting the ERK/NF-κB signaling pathway and differentiating into lung epithelial cells.[14] Intratracheal administration of c-Kit$^+$ cells in asthma models of rats ameliorates asthmatic damage by reducing the severity of collagen levels, suppressing the transcription of IL-4, and reducing total leukocyte number in lung tissue.[179] These effects confirm the immunosuppressive effect of c-Kit$^+$ cells, similar to the known function attributed to MSCs.

GMP Considerations for MSCs and MSCs-CM as Therapeutic Factors

Owing to their potent immunosuppressive and regenerative properties, MSCs and MSC-CM have attracted much interest from the medical and scientific

community. Lately, their safety and efficacy have been scrutinized for a wide range of clinical problems across the world. However, these agents are considered as an ATMP (Advanced Therapy Medicinal Products) in Europe. Therefore, they are controlled by a specific regulatory framework and cannot be used directly as pharmaceutical products in the clinic.[180]

Therefore, before the clinical application of MSCs and MSC-CM, several issues such as production protocols and quality control should be considered and they must be in accordance with GMP standards. In this process, several factors affecting the quality of cells and their secretions are scrutinized, and the sources of preparing cells, the method of proliferating cells, the number of cell passages, the duration of culture, and the method of separating secretions from cells are taken into account, so as to confirm the standardization and optimization of protocols. Observing GMP standards and compliance of protocols with this standard is necessary from the moment of isolating cells to their cultivation and injection into the patient, but this standardization has not been done yet.[181]

From among the most important GMP considerations and current challenges to achieve safe, compatible, and cost-effective MSC treatments, we can refer to the following:

MSCs can be derived from the patient himself (autologous) or from other donors (allogeneic). Using autologous cells will not have the risk of immune reactions, but in many cases, due to the impossibility of obtaining them from the patient himself, using ready-made allogeneic cells is more practical.[182] Generally, MSCs don't create an immune response in allogeneic environments, but there is evidence, proving otherwise.[183]

Other factors for instance gender, age, and medical history of donors should also be taken into consideration since they can affect the differentiation, proliferation, and therapeutic efficacy.[184,185]

In addition to the donor, the source of stem cell extraction is also of great importance. Bone marrow, adipose tissue, and umbilical cord are mostly used. The therapeutic potential of cells can also depend on the source of their preparation. In addition, this question comes to mind whether choosing the type of source is also related to the patient's medical condition or not.[186]

FBS is used as a supplement in the preparation of the culture medium, which, in addition to being expensive, has the risk of specific interspecific contamination. New commercial culture mediums that correspond with GMP are free of xenogeneic elements, which are also cost-effective. However, these cultures may also affect MSC function.[187]

MSCs that are in the culture medium are generally heterogeneous. It has been suggested that in order to produce more homogeneous and purer MSCs, specific antibodies can be used. Although this technology increases the purity and uniformity of the product, it requires reagents and technologies that are compatible with GMP, higher quality and safety control mechanisms, as well as higher cost.[188]

In new technologies, advanced and automatic cell culture systems have been built on a larger scale, which are designed for the cultivation of cells in high volumes, and allow the accurate control of variables such as pH, oxygen levels, pollution, and accumulation of metabolites. Preliminary studies have demonstrated that these systems do not affect the phenotype and function of MSCs.[189]

Thus, this technology will come with the production of MSC at the global level, but we will face challenges such as large investments to build advanced infrastructure.[190]

In order to ensure the quality and maintain the biological properties of MSCs, several tests should be designed and used as predictors of cell function so that they can be confirmed in phase III clinical studies. However, this issue would be challenging, since designing this system requires a complete understanding of the MSCs' action mechanisms while these mechanisms have not been fully recognized.[191]

Using MSCs, if it is in accordance with GMP standards, will eliminate the existing problems. By decreasing the immune response of the recipient, this technology improves genomic stability and reduces unwanted side effects of MSCs. However, there is still no agreement on the minimum quality control standards required for the therapeutic production of MSCs in accordance with GMP, and on the other hand, implementing this standard is quite complicated.

DISCUSSION

The characteristics of MSCs enable them to be effective in the therapy of several diseases, such as inflammatory disorders of the respiratory system. MSCs with immunomodulatory properties as special cells can reduce inflammation and repair damaged tissues in asthma.[192]

In experimental asthma models, the beneficial effect of treatment based on mesenchymal stem cells (MSCs) has been observed. When MSCs are administrated, homing and migration properties of the MSCs, automatically recruit them to the damaged area and repair injury tissue through regeneration and differentiation.[193,194] Additionally, MSCs can decrease lung tissue inflammation by increasing anti-inflammatory ones, reducing pro-inflammatory cytokines, and balancing the cytokine network.195,196 Therapeutic effects are exerted by different molecular pathways, including immunomodulation, mitochondrial donation, protection against apoptosis, oxidative stress, and paracrine factors.[13]

Currently, β-agonists and corticosteroids are the dominant drugs for asthma treatment. Still, these drugs sometimes are not effective, for example, in refractory and steroid-resistant neutrophilic asthma, in which corticosteroids cannot prevent the symptoms. Several studies in murine asthmatic models show that MSCs suppress airway inflammation in neutrophilic asthma, which may fill the gap in this field.[30] Besides some medicines can cause side effects, and their discontinuation can result in the relapse and reemergence of symptoms.

Mesenchymal stem cells (MSCs) are known as promising treatment options for asthma due to their easy access, few ethical issues, differentiation power, and immune modulation. Although in recent years, several reports have been published about the use of MSCs in the treatment of animal models of asthma, clinical trials are not yet sufficient. Before the safe, effective, and routine use of MSCs in clinical settings, the following issues must be resolved:

Recognizing the best source of MSCs in order to maximize therapeutic effects is of great importance.

The dosage and administration methods of MSCs have been reported differently and currently, there is no mutual agreement on this matter.

MSCs-based treatment requires a very large number of cells. The cells isolated numbers from the tissue are small and they must be cultivated for a long time so as to obtain a sufficient number. Long-term cultivation can lead to contamination of the culture medium, and reduction of cell quality, as well as the therapeutic potential.

Despite the low immunogenicity of MSCs, they cannot be considered completely safe. It has been reported that MSCs can cause inflammation in the body.[197] In case adverse effects for instance fever, headache, chills, and numbness have been observed after MSC transplantation.[35,198]

Through research, it has been determined that injecting MSCs into the non-inflammatory lungs of mice causes lung inflammation by increasing the expression of IFN-γ, damaging the endothelial cells, increasing the response of immune cells by producing IL-17 and finally apoptosis of MSCs and rejection.[199]

Another problem is that the uncontrolled differentiation of MSCs has become a safety issue. Long-term proliferation of these cells can lead to cytogenetic abnormalities and differentiation into tumor cells after injection into the body.[200].

Generally speaking, expressing a definite opinion about the clinical use of stem cells in the treatment of asthma is too early and requires a comprehensive review especially when it is needed to be in accordance with GMP standards.

STATEMENT OF ETHICS

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Data Availability

Upon reasonable request from the corresponding author via fmirershadi@yahoo.com.

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