Allergologia et immunopathologia
Sociedad Española de Inmunología Clínica, Alergología y Asma Pediátrica
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ORIGINAL ARTICLE

OPEN ACCESS

Effect of transduced mesenchymal stem cells with IL-10 gene on control of allergic asthma

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Received 29 October 2022; Accepted 22 November 2022
Available online: 1 March 2023

KEYWORDS
Cell therapy;
Gene therapy;
Allergy;
Inflammation;
Cytokine

Abstract
Asthma is an important pulmonary disease associated with T helper lymphocyte (Th)2 dominant immune response, which can initiate allergic and inflammatory reactions. Interleukin (IL)-10 is the main immune suppressor cytokine, and mesenchymal stem cells (MSCs) have an immune-modulatory potential that can be transduced with the expression of the IL-10 gene to control pathophysiology of allergic asthma. Bone marrow's MSCs were isolated and transduced with the expression vector that contains the expressible IL-10 gene. Then, allergic asthma mouse model was produced and treated with manipulated MSCs. Methacholine challenge test; measurement of IL-4, IL-5, IL-8, IL-13, IL-25, and IL-33; and total and ovalbumin (OVA)-specific immunoglobulin (Ig)E levels were done. Hyperplasia of the goblet cell, secretion of mucus, and peribronchiolar and perivascular eosinophilic inflammation were evaluated in lung pathological sections. IL-25, IL-33, and total IgE levels; AHR; eosinophilic inflammation; hyperplasia of the goblet cell; and secretion of mucus could be controlled in M, MV, and MV-10 groups, and the control in the MV-10 group was strong compared to M and MV groups. MSCs have immune-modulatory capacity that can control allergic asthma pathophysiology, and this effect can be strengthened and reinforced by the expression of IL-10 gene.

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https://doi.org/10.15586/aei.v51i2.789

Introduction

Asthma is a complicated pulmonary disease and is influenced by genetic predisposition and environmental factors. The prevalence of asthma has increased in recent decades globally.[1,2] The main symptoms of asthma are breathlessness, wheezing, and coughing, which have higher incidence and prevalence in children and young individuals. There is no certain treatment for asthma and all drugs can control symptoms of asthma. Asthma is an airway disease that is associated with airway hyperresponsiveness (AHR), eosinophilic inflammation, airway remodeling, mucus hypersecretion, and goblet cell hyperplasia. Allergy and atopy constitute the main causes of asthma. Th2 dominant immune response is responsible and via type 2 cytokines can initiate allergic and inflammatory reactions in airways.[3-6]

IL-10 is an immune suppressor cytokine, which can regulate immune response and control responses. IL-10 displays pleiotropic effects in asthma and has a role in lessening allergic inflammation via inhibition of pro-inflammatory cytokines' production. IL-10 can greatly reduce the development of AHR and airway inflammation. IL-10 shows its effects through a heterotetramer receptor, which is composed of two of each of the receptor chains IL-10R1 and IL-10R2. The IL-10R1 mediates high affinity ligand binding and signal transduction, whereas the IL-10R2 is required for signaling only.[7,8] The IL-10/R signaling pathway plays a key role in the pathogenesis of asthma; therefore, the investigation effect of expressible IL-10 gene via cell therapy may be an important treatment for asthma.

MSCs therapy is one of the new treatments that have an immune-modulatory capacity and immunoregulatory potential on immune response–related diseases. However, the underlying molecular mechanism of MSCs-mediated immunomodulation has not been fully clarified. MSCs can be harvested from various tissues such as bone marrow, peripheral blood, inner organs, adipose tissue, as well as from neonatal tissues (e.g., umbilical cord, amniotic membrane, amniotic fluid, and placenta).[9,10] MSCs through a modulatory effect on the immune system may control inflammation in asthma, and this effect may be increased via the expression of the IL-10 gene insertion. In this study, immune-modulatory effect of transduced MSCs with the expression of the IL-10 gene on allergic asthma pathophysiology was evaluated and the MSC-derived immunomodulation by highlighting the IL-10 effect in asthma was studied.

Material and Methods

Culture of MSCs

Bone marrow MSCs were isolated in mice according to a previous study.[11] Tibia and femur bones were dissected and scrubbed to remove the residual tissues that were transferred to complete an MEM medium. After flushing out the bone marrow, it was cultured for 5 days. Bone marrow-MSC cells, after the third passage, were stained and labeled with specific antibodies for identification and analysis.

Transduction of MSCs

Transduced MSCs were produced according to previously studied methods.[10,12,13] Expression vector containing a fully sequenced open reading frame was prepared, and two types of this vector were used for the transduction of MSCs: the expression vector and the expression vector that contained the expressible IL-10 gene. One group of MSCs was transfected with the expression vector and the other group was transfected with the expression vector containing IL-10. For the separation of vector transduced and un-transduced cells, MSCs were incubated with a differentiation medium, supplemented with Blasticidin (the vector containing blasticidin resistance gene), and transduced cells can survive in this medium.

Approving of IL-10

The expression protein level and concentration of IL-10 in the cell supernatant were measured by IL-10-specific enzyme-linked immunosorbent assay (ELISA) kit to approve the expression of IL-10 and protein production.

Animal treatment

The 40 male BALB/c mice (6- to 8-week-old) were kept in the animal house under standard conditions and divided into four groups (n = 10), which include allergic asthma group (A), allergic asthma group that was treated with nontransduced MSCs (M), allergic asthma group that was treated with transduced MSCs with expression vector (MV), and allergic asthma group that was treated with transduced MSCs with expression vector containing expressible IL-10 (MV-10) via intra-bronchial administrated on day 25 (2.5×10^5 cells). To produce the allergic asthma mouse model, the animal was sensitized and challenged with OVA according to previous studies.[3,4,10] OVA plus alum were used via intraperitoneal injection for sensitization on days 1 and 14. Thereafter, OVA solution was used via nebulizing for challenging (Figure 1) on days 24, 26, 28 and 30.

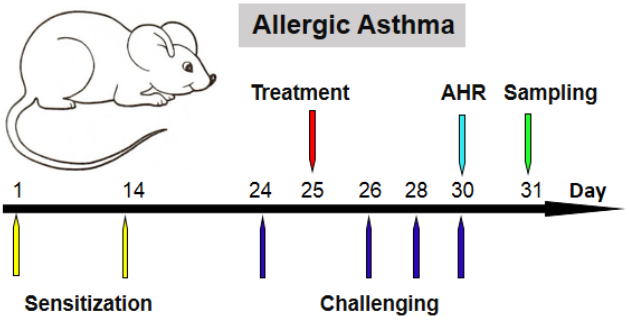

Figure 1 For production of the mice model for asthma and treatment, for sensitization, OVA with alum were used via the intra-peritoneum on days 1 and 14, and for challenging, OVA solution was used via nebulizing on days 24, 26, 28, and 30. MSCs were administrated via intra-bronchial on day 25.

AHR Measurement

On day 30, the Methacholine (MCh) challenge test was done to determine the Penh value and assess AHR[4,10] under anesthetization with ketamine and xylazine. The mice were initially exposed to PBS aerosol and different MCh concentrations (1, 5, 10, 15, 20, 25, and 30 mg/mL).

Cytokines Measurement

On day 31, the mice were euthanized with CO_2 and bronchoalveolar lavage fluid (BALf) was collected from mice via intubation, and the levels of IL-25 and IL-33 in BALf supernatant were measured using Multi-Plex ProTM Mouse Cytokine, Chemokine, and Growth Factor Assays.

IgEs Measurement

On day 31, blood samples were taken and the total IgE level was measured in the blood serum of mice by anti-Mouse IgE ELISA kit.

Histopathology

On day 31, lung tissues were isolated and fixed. Then, pathological sections were produced and stained with Hematoxylin and Eosin, and Periodic acid Schiff-Alcian blue stains. Afterward, hyperplasia of the goblet cell, hyper secretion of mucus in the bronchi, and peribronchiolar and perivascular inflammation were evaluated by using light microscopy.

Statistical Analysis

All tests were repeated thrice and results were presented as a mean with standard deviation (SD). The SPSS (version 22) was performed for statistical analyses and less than 0.05 of the p-value was supposed to be significant. The graphs were drawn by using GraphPad prism. Correlation analysis was carried out using Pearson's method and to analyze data, the paired *t*-test was used.

Result

Manipulated MSCs

Harvested MSCs in medium with blasticidin were selected as transduced MSCs with vectors. The transduced MSCs with the IL-10 vector had notable protein expression of cytokine than normal bioactivity of vector in manipulated MSCs.

AHR

The Penh value in the asthma group was increased in response to the MCh. Treatment with M, MV, and MV-10 could harness the Penh value significantly ($p < 0.05$) in all MCh concentrations compared with the asthma group. The M and MV groups had no significant difference ($p > 0.05$) between them (Figure 2), but the MV-10 group ($p < 0.05$) had significantly decreasing Penh value compared to M and MV groups in all MCh concentrations.

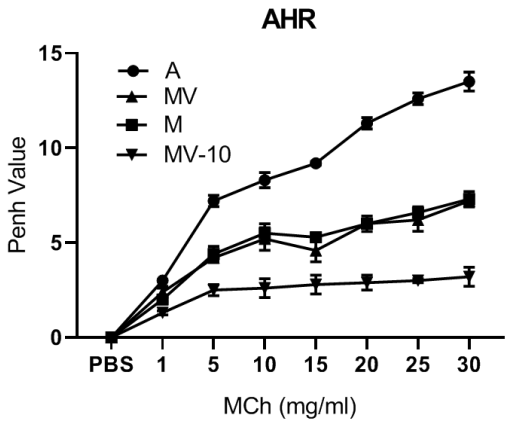

Figure 2 MCh challenge test was done on day 30 to assess AHR. The Penh value in the asthma group was increased in response to MCh, and treatments with M, MV, and MV-10 could decrease the Penh value in all MCh concentrations.

Cytokines

IL-25 and IL-33 levels in BALf of the asthma group (163.6 ± 12.6 and 314.8 ± 21.5 pg/mL, respectively) were decreased by treatment in M (144.1 ± 11.2 and 227.4 ± 20.4 pg/mL, respectively), MV (143.9 ± 7.5 and 219.3 ± 17.3 pg/mL, respectively), and MV-10 (69.3 ± 8.3 and 78.3 ± 19.9 pg/mL, respectively) groups (Figure 3). Decreasing of the IL-25 was significant ($p < 0.05$) in the MV-10 group compared with the asthma group and decreasing of the IL-33 was significant ($p < 0.05$) in all three treated groups compared with the asthma group.

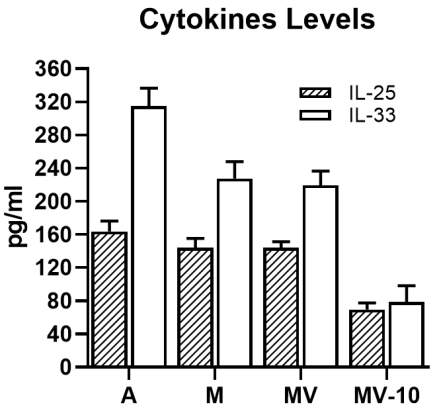

Figure 3 The levels of IL-25 and IL-33 in BALf supernatant (that was collected on day 31) were measured. Cell therapy could control cytokine levels, decreasing of the IL-25 was significant in the MV-10 group, and decreasing of the IL-33 was significant in all three treated groups compared with the asthma group.

IgE

Total IgE level as the main allergic factor in serum of the nontreated asthmatic mice was 3096±149 ng/mL. Treatment with M, MV, and MV-10 could significantly ($p < 0.05$) decrease the levels of total IgE (1434±162, 1394±177, and 612±151 ng/mL, respectively) compared to the asthma group.

Histopathology

Inflammation around the bronchi and vessels, hyperplasia of the goblet cell, and secretion of mucus to the airways were decreased significantly ($p < 0.05$) in M, MV, and MV-10 groups compared with the nontreated asthma group (Figure 4). Inflammation around the bronchi, hyperplasia of the goblet cell, and secretion of mucus were significantly decreased in the MV-10 group compared to M and MV groups. Decreasing of perivascular inflammation in MV-10 compared to M and MV groups was not significant ($p > 0.05$).

Discussion

One of the main methods in asthma treatment is the use of immunosuppressive agents via gene and cell therapy. To treat asthma, optimizing and manipulation of immunemodulatory cells (such as MSCs) via effective biofactors (including IL-10) could be the main method, which was evaluated in this study.

Signaling pathway of the IL-10/IL-10-receptor is central to the inflammation leading to asthma, and signal transducer and activator of transcription (STAT)3 is the main agent in this pathway. Counts of eosinophils and serum IgE were differently regulated by the IL-10 genotype in asthmatic and normal subjects, and IL-10 negatively or positively influences IgE by inhibiting the isotype switch through IL-4 and IL-13 inhibition or by stimulation of IgE release from B lymphocytes (B) cells.[7,14] The STAT3 gene expression is induced by IL-6, IL-10, and IL-13. STAT3 was implicated in the pathogenesis of asthma and STAT3-dependent pathways induced by IL-13 in lung were inhibited by the inhaled corticosteroid. STAT3 plays a role in the

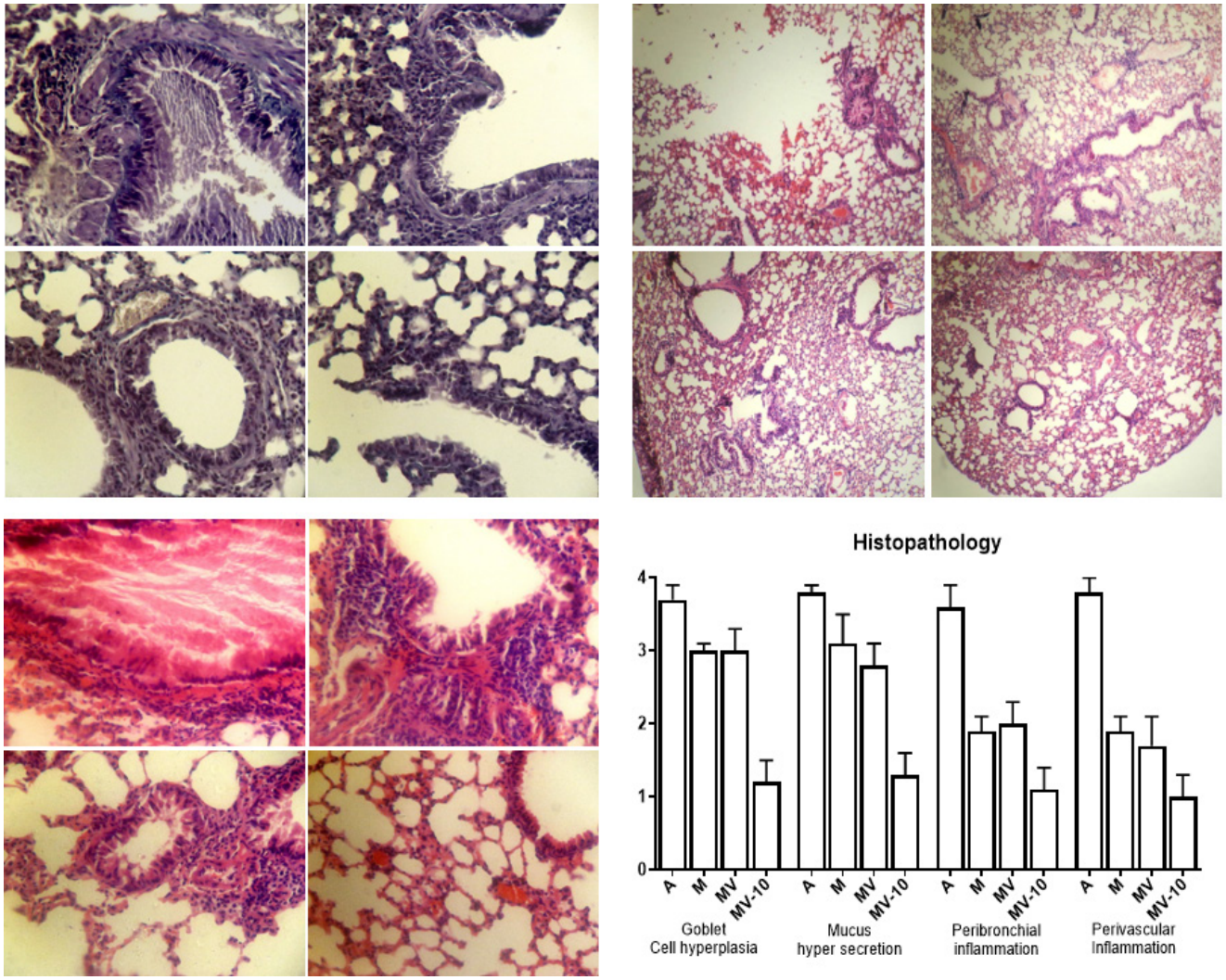

Figure 4 Lung tissues' pathological sections were stained and evaluated by using light microscopy. Inflammation, hyperplasia of the goblet cell, and mucus secretion were decreased in treated groups compared with the nontreated asthma group.

surfactant regulation of lung and inflammatory response in lung fibrosis.[15-17] In a 2022 study, another allergic harnessing cytokine was used as anti-asthma therapy, and IL-35-transduced MSCs were used to treat asthma, and it was presented that this therapy via the formation of regulatory T (T-reg) cells as the mechanism of action could control asthma pathophysiology.[10]

The immune response in allergy and parasite infections shares many important features, such as Th2 response (IL-4, IL-5, and IL-13 upregulation), IgE production, and mast cell and eosinophil activation. Th1/Th2 imbalance is the main problem in asthma and modulation of the immune response could control allergic asthma. Also, immunomodulatory network generated by IL-10 upregulation ultimately leads to the prevention of allergic inflammation in airways.[5,18] Therefore, modifying the immune system in atopic predisposed individuals is critical for primary prevention and new therapies for allergic asthma.

Recently, MSCs were used to treat or prevent allergic asthma by their immuno-modulatory effects that were increased. MSCs as multi-potent cells abundantly exist in bone marrow and adipose tissue, and intravenous administration of MSCs could reduce the allergen-specific Th2 response via suppressing the maturation and migration of lung dendritic cells (DCs).[19,20] It was demonstrated that administration of MSCs could suppress allergic asthma by increasing T-regs numbers and IL-10-producing macrophages.[21,22] In this study, inflammation around the bronchi and vessels, hyperplasia of the goblet cell, and secretion of the mucus to the airways were decreased by MSCs and MSCs-IL-10 treatment in asthmatic mice.

MSCs display an immunomodulation role in lung and could upregulate IL-10 in asthma patients. Thus, MSCs can promote the immunosuppressive effect. On the other side, lung interstitial macrophages could produce immunosuppressive cytokine IL-10, which can be enhanced by intranasal delivery of MSCs. MSCs-induced macrophages may be developed from splenic reservoir monocytes, which can inhibit allergic asthma and modulate allergic immune responses by enhancing IL-10 production.[19] IL-25 and IL-33, as important allergic cytokines, were decreased in all three treated groups (by MSCs and MSCs/IL-10) compared with the asthma group. These two cytokines IL-25 and IL-33, are upper hand of main allergic type 2 cytokines (IL-4, IL-5 and IL-13), therefore controlling of IL-25 and IL-33 can lead to decreasing of IL-4, IL-5 and IL-13 and can prevent symptoms of allergic asthma and asthma pathophysiology. Therefore, when IL-25 and IL-33 can be controlled and decreased, these changes can reduce the production of type 2 cytokines, which leads to the control of allergic response and can harness allergic asthma immunopathological events.

The least two T-regs population, Th3 and Tr1, perform immunosuppressive function by secreting the anti-inflammatory cytokines, IL-10, or transforming growth factor beta (TGF-β). However, in asthma, there is a breakdown in Treg-regulatory mechanisms that result in the airway inflammation development. Therefore, Th2 cell responses' inhibition via T-reg induction is considered an effective strategy to treat allergic asthma.[23,24] Therefore, IL-10 can be the main branch of T-reg in immunosuppressive activity, which was used in our study to suppress allergo-inflammatory immune responses in asthma.

Th2 cells mainly cause eosinophilic inflammation asthma by secreting cytokines, such as IL-4, IL-5, and IL-13, which can induce asthma, the development of AHR, and mucus secretion in the airways. It is known that IL-4 induces the Ig switch in B cells to IgE production, and mast cell activation. IL-5 supports eosinophil development and migration. IL-13 reduces lung function, and induces AHR and mucus secretion in the airway.[23,25,26] Thus, for controlling allergic asthma, reducing these Th2 cytokines and downregulation of these to prevent airway obstruction and AHR is necessary. In our study, treatment with M, MV, and MV-10 could harness AHR and MCh challenge test. Also, total IgE level as the main allergic factor was decreased by treatment with M, MV, and MV-10 compared to the non-treated asthma group.

IL-10 as a pleiotropic cytokine is produced by different cell types, including lymphoid cells [B cells, T cells, and natural killer cell (NK)] and myeloid cells (eosinophils, neutrophils, macrophages, dendritic cells, and mast cells) with broad anti-inflammatory activity. Myeloid dendritic cells and macrophages express IL-10 upon activation of myeloid differentiation primary response (MyD)88 and TRIF-dependent toll-like receptor (TLR) pathways (such as TLR3 and TLR4). Moreover, tolerogenic dendritic cell [cluster of differentiation (CD)11c low/CD45RB high] produces large amounts of IL-10 that induces T-reg development. On the other hand, natural regulatory cell (nT-reg) produces IL-10 in response to IL-2, which is vital for immune homeostasis.[27,28] IL-10 plays a crucial role in the suppression and management of allergic diseases, especially in asthma. IL-10 in combination with immune regulating cells play a pivotal role in successful allergen immunotherapy.[29,30] The asthma pathogenesis is associated with reduced IL-10 production. It was demonstrated that IL-10-releasing and the IL-10-producing monocytes' numbers in severe persistent asthma patients were attenuated. Furthermore, the absence of IL-10 results in an increased eosinophilic inflammation of airway and increased synthesis of IL-5.[31]

MSCs through the immuno-modulatory effect can control inflammation and allergic asthma pathophysiology, and this effect was increased, strengthened, and reinforced via the expression of the IL-10 gene insertion. Allergic asthma symptoms were controlled by MSCs immuno-modulatory capacity, and immunosuppressive effect of IL-10 by the expressed gene could increase the controlling output of asthma pathophysiology. Therefore, manipulation of MSCs as immuno-modulatory cells via immunosuppressive biofactors (IL-10) could be the main method to control and cure asthma, which was observed in this study.

Ethics Approval and Consent to Participate

All methods of the current study were approved by the ethic committee of animal house of ix.med.vet.dep, 2022 (No. IX.MED.VET.DEP.REC.2022.4600051.0).

Consent for Publication

Not Applicable.

Availability of Data and Materials

Data are available on request from the corresponding author.

Conflict of Interest

There is no conflict of interest.

Funding

This study was funded by Henan Medical Science and Technology Project LHGJ20200230.

Authors' Contributions

CH, FS, YL, EMN, and SSA contributed to examination, manuscript drafting, and scientific revision. All the authors confirmed the final manuscript before submission.

Acknowledgements

Not Applicable.

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