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Effects of human mesenchymal stem cells in asthmatic airway inflammation

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Effects of human mesenchymal stem cells in asthmatic airway inflammation

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국 문 초 록

서론: 천식은 기도 폐쇄와 기도 과민성을 특징으로 하는 만성 염증성 기도 질환으로, 천식에서 제대혈 및 제대 유래 중간엽 줄기세포의 치료 효과는 아직까지 명확하게 알려져 있지 않다. 이 연구는 천식 실험동물모델에서 인체 유래 (제대혈 및 제대) 중간엽 줄기세포의 알레르기 염증반응 조절 효과를 선천면역 및 적응면역에 관련된 세포들의 반응을 통해 확인해 보기 위해 수행되었다.

방법: 천식 동물 모델인 BALB/c 마우스의 복강으로 난알부민을 투여하여 감작시킨 이후, 난알부민 기도투여로 알레르기성 천식을 유도하기 전 또는 후에 꼬리 정맥으로 제대혈 또는 제대유래 중간엽 줄기세포를 주입한다. 이후 마우스의 혈청, 기관지폐포세척액, 폐조직, 비장세포에서 줄기세포 투여에 따른 염증세포 분획, 조직학적 변화, 혈청 특이 알레르겐 항체, 염증 사이토카인 분비, 호산구, T세포와 조절 T 세포, 선천성 림프구 세포, 수지상세포와 대식 세포를 생체 내 실험(*in vivo*)을 통하여 확인하였다. 또한 제대유래 중간엽 줄기세포의 주변 분비 작용(paracrine effect)을 확인하고자 M2 대식세포주를 이용하여 생체외(*in vitro*)실험을 진행하였다.

결과: 제대혈유래 중간엽 줄기세포는 기도과민성을 감소시키고 조직에서 호산구 침윤, 점액과 술잔세포의 과형성을 유의하게 감소시켰다. 기관지폐포세척액에서 호산구를 비롯한 염증세포들을 감소시키고 혈청에서 난알부민 특이 IgE와

IgG1 항체를 의미 있게 줄였다. 또한 제대혈유래 중간엽 줄기세포는 기관지폐포세척액과 폐/비장 조직에서 Th2 사이토카인(IL-4, 5, 13)을 의미 있게 감소시키며 조절 사이토카인(IL-10, TGF-β)을 의미 있게 증가시켰다. 아울러 폐 림프절 및 비장 조직에서 조절 T세포의 증가도 관찰되었다. 제대유래 중간엽 줄기세포는 폐조직에서 type 2, 3 선천성 림프구 세포, 수지상세포와 대식세포의 수를 의미 있게 감소시켰다. 대식세포 중 폐포 대식세포는 제대유래 중간엽 줄기세포 치료 후 의미 있게 증가한 반면, 간질성 대식세포는 감소하였으며 간질성 대식세포군 들 중 하위 유형인 M1, M2, M2a, M2c도 간질성 대식세포와 비슷한 양상을 보였다. 생체외 실험을 통해 제대유래 중간엽 줄기세포의 주변 분비 작용도 확인하였다.

결론: 인체 유래 중간엽 줄기세포는 Th1/Th2 세포 면역 편향, 조절 T 세포 등 적응면역계, 그리고 선천성 림프구 세포와 대식세포 등 선천면역계의 변화를 통해 천식 알레르기 염증반응을 조절하는 역할이 있음을 확인할 수 있었다. 이를 통해 선천면역계 및 적응면역계에 모두 관여하는 제대 및 제대혈 유래 중간엽 줄기세포가 천식 치료제로 효과적임을 확인할 수 있었다.

주요어: 천식, 중간엽 줄기세포, 제대, 제대혈, T림프구, 대식세포

학번: 2017-39143

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Introduction

Asthma is a chronic airway disease characterized by persistent inflammation and airway remodeling.[1] Traditional asthma management strategies focus on two types of medicines: controllers, to treat the underlying airway inflammation, and relievers, to relax the airway smooth muscles. Despite these treatments, asthma remains uncontrolled in a considerable number of patients.[2,3] Therefore, alternative treatment options are warranted for asthma management.

In the late 1960s, Friedenstein and colleagues identified a population of fibroblast-like cells that formed clonal colonies, which were derived from bone marrow *in vitro* culture.[4] Their findings allowed for the discovery of a specific type of cells, referred to as mesenchymal stem cells (MSCs). MSCs are defined as multipotent cells, which are recognized as non-hematopoietic adult stem cells originating from the mesoderm layer, with the capacity of self-renewal and differentation.[5,6] Human MSCs (hMSCs) are well tolerated without serious adverse events following allo- and xeno-transplantation, and hMSCs can differentiate into various connective tissue lineages, including adipocytes, chondrocytes, osteoblasts, myocytes, and neuronal cells.[7,8] hMSCs can contribute to regenerate damaged tissues from several disease states, such as cardiovascular diseases, liver injury, renal failure, musculoskeletal disorders, and neurological defects.[9-11] In addition to their repairing damaged and inflamed tissues, hMSCs can also regulate innate and adaptive immune responses.[9-11] Previous data have demonstrated that the action of hMSCs includes paracrine activity, cell-to-cell contact, mitochondrial transfer, and the release

of extracellular vesicles.[12] Due on their multipotency, hMSC therapy has been investigated extensively as an innovative drug type, in order to treat multiple pathologies lacking appropriate treatments.[13] Notably, hMSCs exhibit potent immunomodulatory properties in murine models of acute and chronic asthma.[14] Moreover, hMSCs have beneficial effects on eosinophil-dominant allergic airway inflammation and neutrophil-predominant asthma, which is characterized by the corticosteroid insensitivity or resistance to corticosteroids.[15-25]

Umbilical cord blood (UCB) and umbilical cord (UC)- derived MSCs have been the most widely used types of MSCs, along with bone marrow-derived MSCs (BM-MSCs) and adipose tissue-derived MSCs (AD-MSCs). Human UCB-MSCs and UC-MSCs are easily collected without invasiveness or pain, and the procedure is relatively free of other ethical and technical issues.[26] Although human BM-MSCs and AD-MSCs have been shown to attenuate the severity of asthmatic airway inflammation, the immunomodulatory outcomes of UCB-MSCs and UC-MSCs remain undetermined. Therefore, we hypothesized that UCB-MSCs and UC-MSCs could exhibit therapeutic effects on eosinophil-dominant airway inflammation through the activation of both innate and adaptive immune cells. To verify this hypothesis, we evaluated the *in vivo* and *in vitro* immunomodulatory effects of UCB-MSCs and UC-MSCs via interaction with innate and adaptive immune cells in an ovalbumin (OVA)-induced murine allergic asthma model.

Methods

Literature review of stem cells in animal asthma models

Prior to conducting the *in vivo* study, we reviewed studies using *in vivo* animal models of asthma in which the effects of MSCs administration were investigated. We searched PubMed to identify English-language articles published in 2010 – 2016. A manual search was also conducted using Google. The following relevant terms were included in our search: stem cells, stromal cells, bone marrow cells, stem cell transplantation, asthma model, laboratory, and experiment. A total of 31 studies were selected for full text review.[16-21,23,27-50]

Preparation of hMSCs

The isolation and culture of UCB-MSCs in experiment I were performed as previously described.[51,52] After obtaining written and informed consent, UCB-MSCs were collected from the umbilical vein of neonates immediately after birth and prepared based on proper manufacturing protocols at MEDIPOST Co., Ltd (Seoul, Korea). UCB-MSCs were collected using a 16-gauge needle, and these cells were joined to a UCB-MSCs collection bag containing 23 mL of citrate-phosphate dextrose adenine (CPDA)-1 anticoagulant (Greencross Co., Yongin, Kyunggi-do, Korea). UCB-MSCs were harvested within 24 hours of collection, and cell viability was always higher than 90%. Mononuclear cells were isolated through centrifugation using a Ficoll-Hypaque gradient (density: 1.077 g/cm^3; Sigma-Aldrich, St Louis, MO, USA). The separated mononuclear cells were washed, suspended in minimum essential medium (a-MEM; GibcoBRL, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS) (HyClone, Logan, UT, USA). The cultures were maintained at 37°C in a humidified atmosphere containing 5% carbon dioxide, and the medium was changed twice a week. One to

three weeks later, when the monolayer of adherent cells reached confluence, the cells were trypsinized (0.25% trypsin; HyClone), re-suspended in culture medium (a-MEM supplemented with 10% FBS and 50 μg/mL gentamicin) and subcultured at a concentration of 5×10^5 cells/cm^2. We used cells from passage six for experiments. Flow cytometric analysis was performed to identify the cultured cells at each passage. We stained cells with antibodies against human CD13, CD14, CD34, CD45, HLA-DR (FITC; Becton Dickinson, San Jose, CA, USA), CD31, CD90, CD51/61, CD64 (FITC; Pharmingen, Los Angeles, CA, USA), CD29, CD44, and CD106 (PE; Pharmingen). Corresponding mouse isotype antibodies were used as controls. The labelled cells were analyzed using FACSCalibur flow cytometer and Cell Quest Pro software (Becton Dickinson, San Jose, CA, USA).

All human UC-MSCs used in experiment II were cultured and prepared by Professor In-kyu Kim's laboratory (College of Medicine, Seoul National University, Seoul, Korea). All procedures involving human UC were conducted in accordance with the approved guidelines of the Seoul National University College of medicine and Hospital Institutional Review Board (IRB No. C-1708-083-878). Human UC tissues were obtained immediately from full-term births with informed consent and the approval from the parents. These collected tissues were washed repeatedly with phosphate buffered saline (PBS) to remove the vessels and amnion. Then, Wharton's Jelly (WJ) tissues within human UC were isolated and minced. These explants were digested for 3 hours at 37°C using an enzyme mixture (130-105-737, Miltenyi Biotec, Germany). They were then filtered through a 100 μM cell strainer (BD Biosciences, Franklin Lakes, NJ, USA) and pelleted using low-speed centrifugation at 200 × *g* for 10 min. The isolated WJ-MSCs were cultured in CellCor™ CD medium (Xcell Therapeutics, Seoul, Korea)

supplemented with 2% human platelet lysate (hPL, StemCell Technologies, Vancouver, BC, Canada) in a 37°C incubator with a humidified atmosphere containing 5% carbon dioxide. Once these cells reached about 90% confluency, they were harvested and used for subsequent studies.

Murine asthma model with or without MSCs treatment

For experiment I, 6-week-old female BALB/c mice (16–18 g) were purchased from SLC Inc. (Hamamatsu, Kotoh-cho, Japan) and maintained under specific pathogen-free conditions. The experiment I was approved by the Institutional Animal Care and Use Committee at the Institute of Laboratory Animal Resources at Seoul National University (SNU-130829-3-2). A total of 18 BALB/c mice were divided into 3 groups: Group A (control group, n = 6), Group B (OVA-induced asthma + PBS, n = 6), and Group C (OVA-induced asthma + UCB-MSCs, n = 6). BALB/c mice in Groups B and C were treated intraperitoneally with 75 μg of OVA (Grade V, Sigma-Aldrich, St. Louis, MO, USA) and 2 mg of aluminium hydroxide (Alum, Pierce, Rockford, IL, USA) in 200 μL of PBS on days 1 and 14. The mice in Group A were treated with saline and aluminium hydroxide only. Following sensitization, UCB-MSCs were administered via the tail vein (5×10^5 cells/100 μL) in Group C mice before the initial OVA challenge. As the control, PBS was administered to mice in Groups A and B before the initial OVA challenge. All mice were challenged intranasally with 50 μg of OVA in 20 μL of PBS on days 29, 30, and 31 (**Figure 1**).[53]

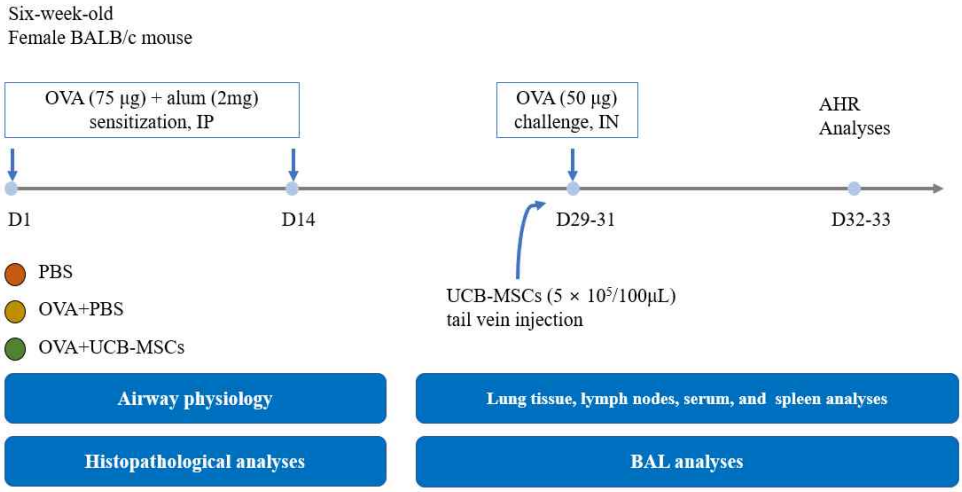


Figure 1. Experimental protocol of experiment I. BALB/c mice were divided into 3 experimental groups and used in this study: Group A (control group), Group B (OVA-induced asthma + PBS), and Group C (OVA-induced asthma + UCB-MSCs). PBS, phosphate-buffered saline; UCB-MSCs, human umbilical cord blood-derived mesenchymal stem cells; OVA, ovalbumin; alum, aluminium hydroxide; IP, intraperitoneal; IN, intra-nasal; AHR, airway hyperresponsiveness; BAL, bronchoalveolar lavage.

Additional experiment II was performed using female 6-week-old BALB/c mice to identify the effect of UC-MSCs on innate immune cells, such as innate lymphoid cells (ILCs), dendritic cells (DCs), and macrophages. Mice were purchased from Orient Bio (Anyang, Korea). A total of 16 BALB/c mice were divided into 4 groups: Group A (control group, n = 4), Group B (UC-MSCs treatment, n = 4), Group C (OVA-induced asthma, n = 4), and Group D (OVA-induced asthma + UC-MSCs, n = 4) (**Figure 2**). Mice were sensitized with 100 μg of OVA (Grade V,

Sigma-Aldrich, St. Louis, MO, USA) and 2 mg of aluminum hydroxide (Alum, Pierce, Rockford, IL, USA) via intraperitoneal injection on days 0 and 7. Allergen challenge was performed via intraperitoneal injection of 50 μg of OVA on days 14, 15, 16, 21, 22, and 23. A total of 1×10^5 UC-MSCs/100 μL were applied intravenously on day 17. Experiment II was approved by the Institutional Animal Care and Use Committee of Seoul National University (SNU-200302-2-3).

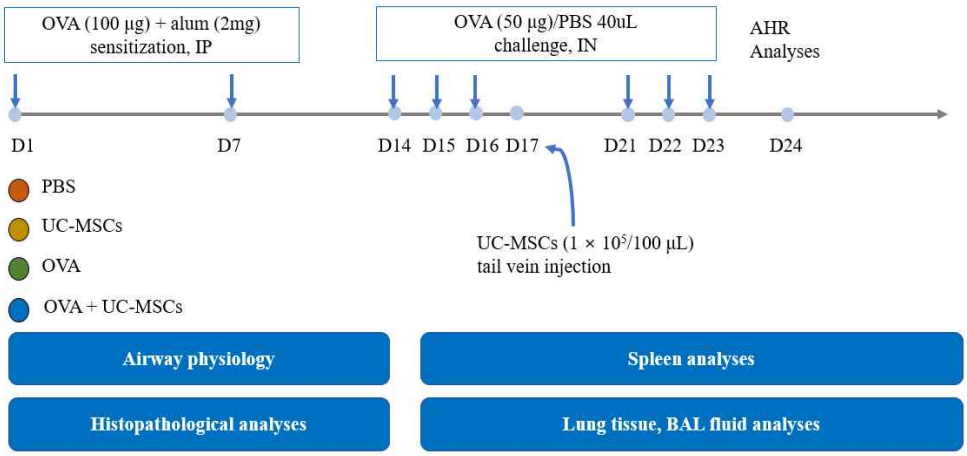


Figure 2. Experimental protocol of experiment II. BALB/c mice were divided into 4 experimental groups and used in this study: Group A (control group), Group B (UC-MSCs treatment), Group C (OVA-induced asthma), and Group D (OVA-induced asthma + UC-MSCs). PBS, phosphate-buffered saline; UC-MSCs, human umbilical cord-derived mesenchymal stem cells; OVA, ovalbumin; alum, aluminium hydroxide; IP, intraperitoneal; IN, intra-nasal; AHR, airway hyperresponsiveness; BAL, bronchoalveolar lavage.

Measurement of airway hyperresponsiveness

For experiment I, airway hyperresponsiveness (AHR) to methacholine exposure was measured by a barometric plethysmographic chamber (OCP 3000; Allmedicus, Anyang, Korea) one day after the final OVA challenge. The 3-minutes enhanced pause (Penh) value was also measured. For experiment II, AHR was measured by a Buxco® FinePointe system (Buxco, Wilmington, NC, USA). Mice were anesthetized with pentobarbital sodium (50 mg/kg), and lung resistance (RL) was measured for 3 minutes. The measured RL values were subtracted from the baseline values and then converted to a percentage.

Analysis of bronchoalveolar lavage fluid

Twenty-four hours after assessing AHR, the mice were sacrificed, and bronchoalveolar lavage (BAL) fluid was collected. BAL fluid collection was performed by inserting a cannula into the bronchi and infusing 2 mL of PBS. BAL fluid was recovered by aspirating using a syringe. Cellular components were separated from the lavage fluid, and microscope slides were prepared using cytospin 3 (Thermo Scientific, Pittsburgh, PA, USA). Cells were stained with Diff-Quik staining kit (Sysmex Co., Kobe, Japan). A total of 300 cells per slide were assessed using light microscopy based on the specific morphological criteria to quantify the cells of each inflammatory cell type, such as macrophages, neutrophils and eosinophils, and lymphocytes.

Histopathological analysis

Lung tissues were obtained from the middle zone of the left lung to evaluate the pathological changes in lung parenchyma. Samples were fixed in 4% neutral buffered formalin, processed, paraffin embedded, and sectioned at 3-mm intervals. Two different staining methods were used for light microscopic evaluation. Hematoxylin and eosin (H&E) stain was used for recognizing peribronchial and perivascular inflammation, and the thicknesses of the epithelium and the subepithelial smooth muscle layers. Mucous secreting goblet cells were detected by Periodic acid-Schiff (PAS) staining. All sample slides used for comparison were observed under the same magnification. The degree of peribronchial and perivascular inflammation was evaluated using a subjective scale of 0 – 3, as described previously.[54,55] A value of 0 represents no detectable inflammation, a value of 1 represents occasional cuffing with inflammatory cells, a value of 2 indicates that most bronchi or vessels are surrounded by a thin layer (1 – 5-cells thick) of inflammatory cells, and a value of 3 indicates that most bronchi or vessels are surrounded by a thick layer (> 5-cells thick) of inflammatory cells. To grade the extent of goblet cell hyperplasia, a subjective five-point scoring system (PAS score) was used as described elsewhere.[56,57] The scoring criteria were as follows: 0, < 0.5% PAS-positive cells; 1, < 25%; 2, 25% – 50%; 3, 50% – 75%; and 4, > 75%. Eight random fields were counted for each slide, and the mean score was calculated. Analyzes were performed by a pathologist in a blinded fashion, and the slides were presented in random order for each examination.

Analysis of cytokine expression

To measure the cytokine responses in the lung tissue, lung lysates from all the mice used in this study were prepared and assayed. The total RNA (2 μg) was reverse transcribed into cDNA using a single-strand cDNA synthesis kit (Promega, Madison, WA, USA). Gene expression was measured using an ABI 7500 real-time polymerase chain reaction system (Applied Biosystems, Foster, CA, USA), cDNA, gene-specific primers, and SYBR Green master mix. The expression of each gene was normalized against that of β-actin and presented as fold change compared with untreated cells. Mouse primer sequences used for quantitative real-time PCR (qRT-PCR) analysis were as follows:

Interleukin (IL)-4:

(Forward primer) 5‘-ACTTGAGAGAGATCATCGGC-3’

(Reverse primer) 5‘-ACTTGAGAGAGATCATCGGC-3’

Interleukin-5:

(Forward primer) 5‘-ACCACAGTGGTGAAAGAGAC-3’

(Reverse primer) 5‘-CATCGTCTCATTGCTTGTCA-3’

Interleukin-13:

(Forward primer) 5‘-CCTGGCTCTTGCTTGCCT T-3’

(Reverse primer) 5‘-GGTCTTGTGTGATGTTGCTCA-3’

The protein levels of IL-4, IL-5, and IL-13 in the spleen and those of IL-4, IL-10, IL-13, and transforming growth factor (TGF)-β in BAL fluid were evaluated using a sandwich enzyme-linked immunosorbent assay (ELISA) (R&D Systems Inc., Minneapolis, MN, USA) according

to the manufacturer's protocol. Erythrocyte-depleted splenocytes were obtained to measure the cytokine concentrations from the supernatant after re-stimulation with OVA (100 μg/mL) for 72 hours.

Analysis of serum OVA-specific antibody

After forty-eight hours from the final OVA challenge, blood samples were obtained via cardiac puncture, and the concentration of OVA-specific antibodies (Immunoglobulin (Ig) G1, IgG2a, and IgE) was measured using sandwich ELISA (R&D Systems Inc., Minneapolis, MN, US). For thus, 5 μg/mL grade V OVA (Sigma-Aldrich, St. Louis, MO, USA) was used for coating and anti-mouse isotype-specific antibodies (Southern Biotechnology Associates, Birmingham, AL, USA) were used for detection.

Flow cytometric analysis

In experiment I, single-cell suspensions of lung-draining lymph nodes, and spleen were prepared from non-treated and UCB-MSCs-treated samples to evaluate whether injected UCB-MSCs affected regulatory T (Treg) cell expansion. A phenotypic analysis of Treg cells was performed by three-color flow cytometry, in which cells were stained with anti-mouse CD4-FITC, anti-mouse CD25-PE, and anti-mouse Foxp3–PE–cyanine 5. For the Foxp3 antibody, an isotype-matched control was used to determine the background fluorescence. Treg cells were identified as $CD4^+CD25^+Foxp3^+$ triple-positive and expressed as the percentage of total $CD4^+$

T cells. Data were collected using an LSRII flow cytometer (BD Biosciences, San Jose, CA, USA) and analyzed with FlowJo software (Tree Star, Ashland, OR, USA).

In experiment II, single-cell suspensions from lung tissues were treated with FcγR-blocking monoclonal antibody, and the cell surface, intracellular cytokines and transcription factors were stained accordingly. Target cells were analyzed using LSR Fortessa X-20 (BD Biosciences, San Jose, CA, USA) and FlowJo software (TreeStar, Woodburn, OR, USA). After single-cell and lymphocyte gating, T helper (Th) cells were defined as $CD45^+CD3^+CD4^+$ cells. Treg cells were defined as those that expressed Foxp3, while cytokine-secreting effector Th cells were defined as those that expressed IL-5, IL-13, interferon (IFN)-γ, and IL-17A. To stain ILCs, lineage-negative cells (Lin^-) were sorted, and we also used CD90.2 as a surface marker. ILC1s and ILC3s were defined as $CD45^+Lin^-CD90.2^+INF\text{-}\gamma^+$ and $CD45^+Lin^-CD90.2^+IL\text{-}17^+$ lymphoid cells, respectively. ILC2s were defined as $CD45^+Lin^-CD90.2^+IL\text{-}5^+$ and $CD45^+Lin^-CD90.2^+IL\text{-}13^+$ lymphoid cells. After eosinophils were gated out, macrophages were identified as $CD45^+F4/80^+$ cells, and DCs were identified as $CD45^+F4/80^-CD11c^+$ cells. Based on CD11c and CD11b expression, macrophages were divided into $CD11C^+CD11b^-$ cells (alveolar macrophages, AMs) and $CD11b^+$ cells (interstitial macrophages, IMs). From the IMs, M1 and M2 macrophages were defined as $CD206^-CD86^+$ cells and $CD206^+CD86^-$ cells, respectively. In addition, by using CD206, CD86, and MHCII, M2 subtypes were subdivided into M2a ($CD206^+CD86^-MHCII^+$), M2b ($CD206^-CD86^+MHCII^+$), and M2c ($CD206^+CD86^-MHCII^-$) macrophages. Siglec-F is a marker specific for murine lung-resident alveolar macrophages. Ly6C and MHC were used in our study to determine the expression of activation markers on macrophages and DCs.

Real time quantitative PCR to assess macrophage polarization

Mouse lung cells from the four groups described above were isolated, and qRT-PCR was carried out to assess the levels of CD206, Arg-1, and IL-13.

In addition, macrophages from BAL fluid were cultured in 48-well plates (1×10^4 cells/well) for 6 hours, and then stimulated with 20 ng/L recombinant IL-4 and IL-13 (R&D Systems, Minneapolis, MN, USA). Four groups were set up for the experiments: Group A (control group), Group B (UC-MSCs treatment), Group C (IL-4/IL-13 stimulation), and Group D (IL-4/IL-13 stimulation with UC-MSCs). Macrophages were stimulated with recombinant IL-4 and IL-13, and then treated with UC-MSCs (1×10^4 cells/mL) for 18 hours (**Figure 3**).

To investigate the paracrine activity of UC-MSCs, *in vitro* transwell experiments were performed using Transwell Permeable Support (Costar; Kennebunk, ME, USA). Macrophage cells from BAL fluid were stimulated with IL-13 and treated with UC-MSCs. Four groups were set up for the experiments: Group A (PBS group), Group B (IL-13-stimulated group), Group C (IL-13-stimulated group directly treated with UC-MSCs), and Group D (IL-13-stimulated group indirectly treated with UC-MSCs).

Messenger RNA expression levels were measured using a 7500 real-time PCR system (Applied Biosystems, Foster City, CA, USA) after converting the extracted RNA to cDNA using a SensiMix II probe kit (Bioline, London, UK). The expression levels in each sample were standardized against GAPDH expression using the ΔΔCt method, and the relative expression level compared with the control sample was calculated. The following primer sequence of

CD206, Arg1 and IL-13 was used and verified by PrimerBank (Harvard, MA, USA).

CD206:

(Forward primer) 5‘-TATCTCTGTCATCCCTGTCTCT-3’

(Reverse primer) 5‘-CAAGTTGCCGTCTGAACTGA-3’

Arg-1:

(Forward primer) 5‘-GAATGGAAGAGTCAGTGTGGT-3’

(Reverse primer) 5‘-AGTGTTGATGTCAGTGTGAGC-3’

IL-13:

(Forward primer) 5‘-GATCTGTGTCTCTCCCTCTGA-3’

(Reverse primer) 5‘-GTCCACACTCCATACCATGC-3’

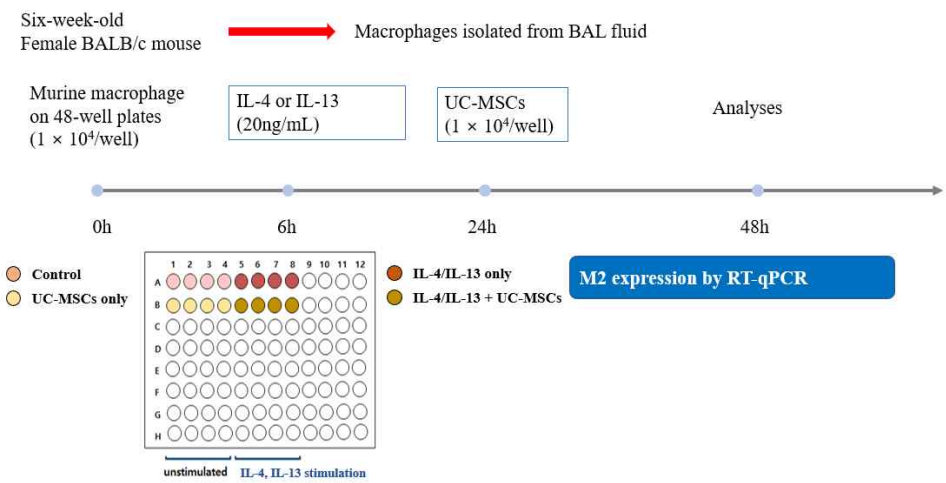


Figure 3. Experimental protocol of the paracrine activity of UC-MSCs. Murine macrophages

isolated from BAL fluid were divided into four experimental groups and used in this study: Group A (control group), Group B (UC-MSCs treatment), Group C (IL-4/IL-13 stimulation) and Group D (IL-4/IL-13 stimulation with UC-MSCs).

Statistical analysis

Data are displayed as individual data with the mean ± standard error of the mean (SEM). Comparisons of nonparametric variables were performed using Kruskal-Wallis tests and then post hoc analyses using Mann-Whitney U-tests. All experiments were repeated at least three times. A value of $p < 0.05$ was considered significant. All statistical analyses were performed using Stata 14.1 software (StataCorp LP, College Station, TX, USA) and GraphPad Prism 5.0 software (GraphPad, La Jolla, CA, USA).

Results

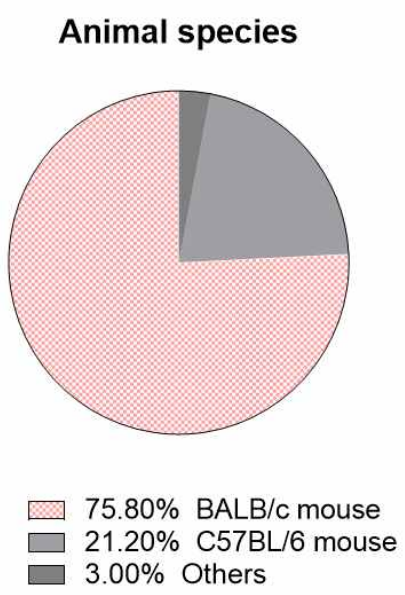
1. **Literature review of stem cells as anti-asthmatic therapeutics in animal asthma models**

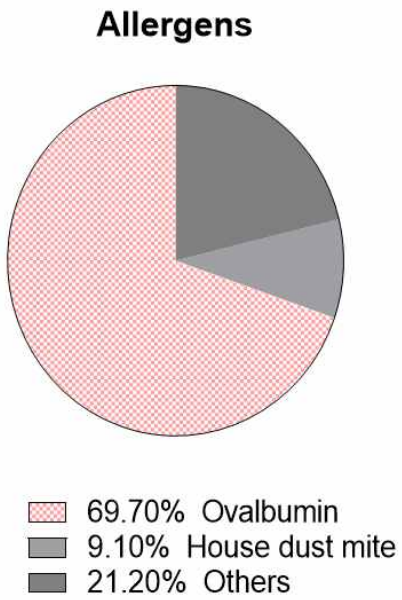
As shown in **Figure 4 and Table 1**, there were variations in animal species, protocols, allergens, and dose of MSCs used. The heterogeneity of study designs mirrors the diversity of environments present in MSCs studies. BALB/c mice were the most frequently used type of asthma model (25 studies, 75.8%), followed by C57BL/6 mice (7 studies, 21.2%). The number

of animals in each group ranged from 4 to 25 with an average of 8 mice. Ovalbumin (23 studies, 69.7%) was the most common allergen used, followed by house dust mite (3 studies, 9.1%), *Aspergillus* fungal extract (3 studies, 9.1%), and other allergens including ragweed, toluene diisocyanate, ammonium persulfate, and cockroach extract. Stem cells derived from the mice were the most commonly used (21 studies, 63.6%), followed by human stem cells (12 studies, 36.4%). Among the hMSCs used, BM-MSCs, AD-MSCs, UC-MSCs, and both induced pluripotent stem cells (iPSCs)/BM-MSCs were used in 9, 1, 1, and 1 study(ies), respectively. Stem cell administration schedules can be largely divided into four types: administration after allergen challenge, during allergen challenge, before allergen challenge, and before allergen sensitization. Administration after allergen challenge was most commonly used (14 studies, 42.4%), followed by treatment before allergen challenge (11 studies, 33.3%), during allergen challenge (5 studies, 15.2%), before allergen sensitization (2 studies, 6.1%), and before allergen sensitization and challenge (1 study, 3%). Among the used routes of administration, intravenous (IV) administration was the most common route of stem cell administration (24 studies, 72.7%). Intratracheal (IT) stem cell injection was used in 7 studies (21.2%). Doses of administration ranged from 1×10^5 to 4×10^6 cells in IV injection, and from 1×10^5 to 2×10^6 cells in IT injections. Despite numerous reports using MSCs in preclinical models and clinical trials, it is not clear whether MSC treatment acts through a regular dose/response pattern.[58] Recent studies have shown that different doses of MSCs have different effects in a non-dose dependent manner.[59] However, another study reported that the effect of MSCs on systemic cytokine levels and mRNA expression in the liver and lungs was directly dose-dependent.[60] Moreover, high doses of MSCs were required to elicit systemic effects and low doses were sufficient to elicit

local effects.[60] Another study revealed that MSCs treated in the early period in acute myocardial infarction had a significant dose-dependent effect.[61]

In this review, various effects following MSCs transplant have been reported, including the reduction of eosinophilic inflammation in BAL fluid, and peribronchial and perivascular inflammation in lung tissues. MSCs were generally effective in reducing Th2 cytokine levels and airway hyper-responsiveness.





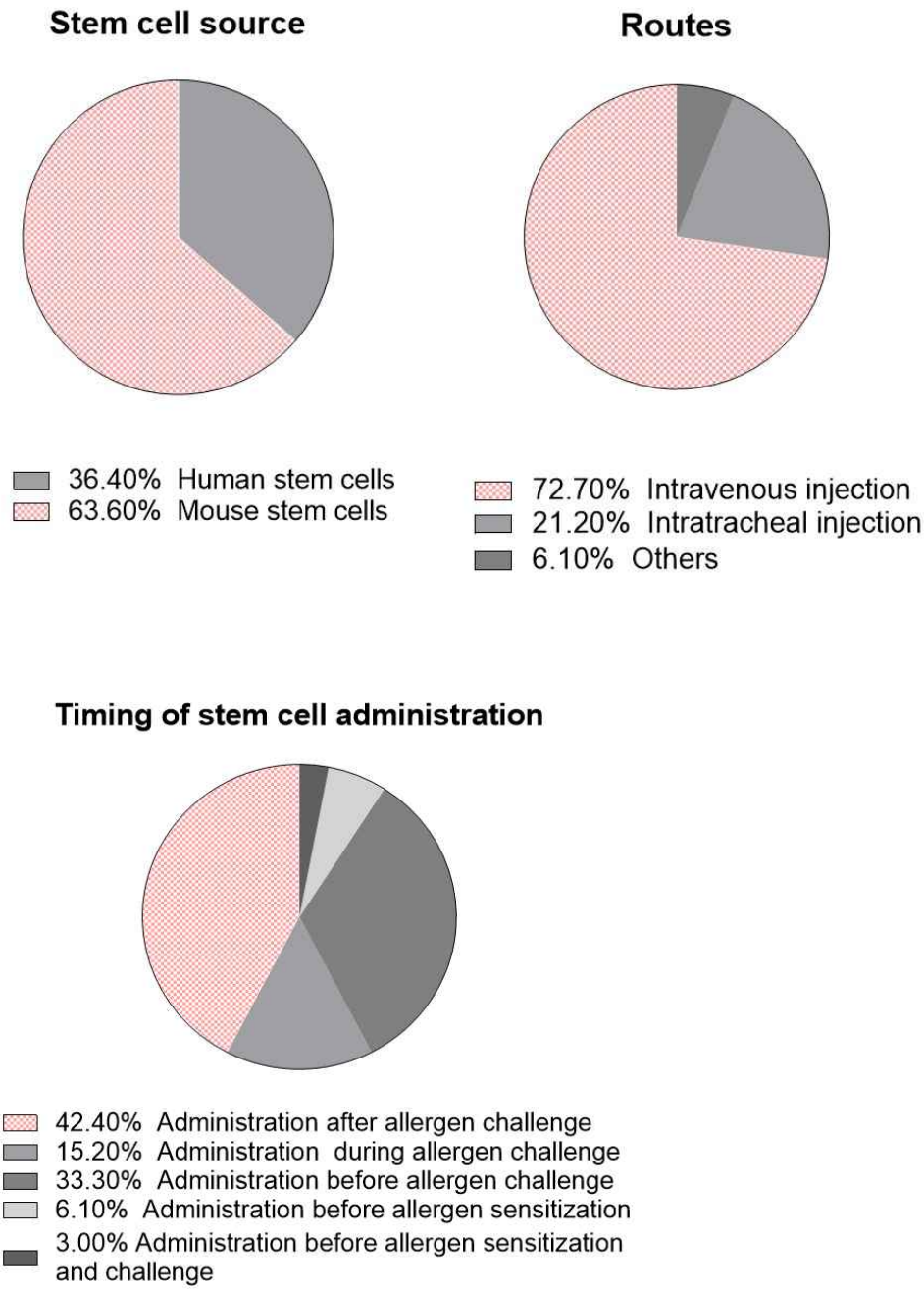


Figure 4. Animal models and various methods used for stem cell treatment of asthma.

2. Experiment I assessing the effect of UCB-MSCs on the adaptive immune responses in asthma

AHR to methacholine

Methacholine doses significantly induced the AHR in the OVA groups, compared with the control group. However, BALB/c mice treated with UCB-MSCs showed a significant decrease in the AHR, following methacholine challenges (**Figure 5**).

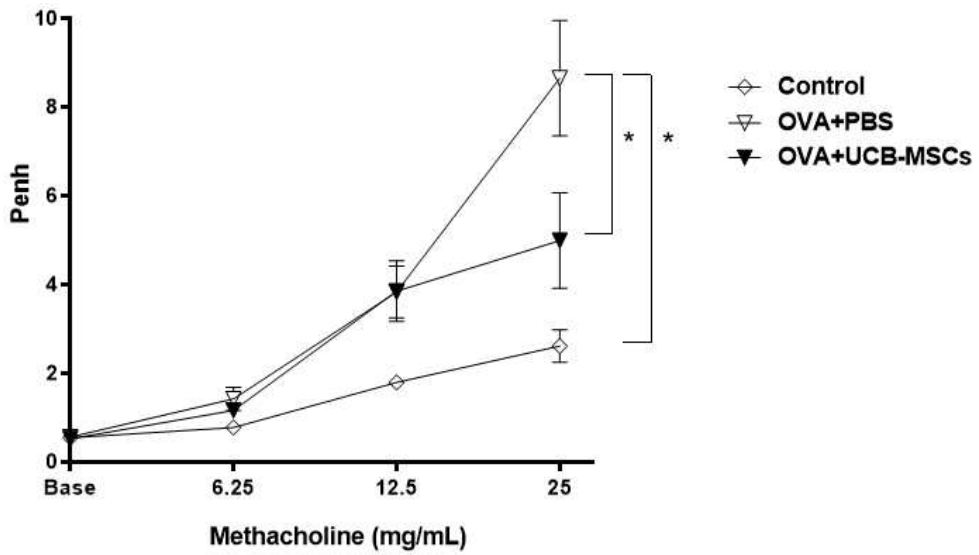


Figure 5. Effects of UCB-MSCs on airway hyperresponsiveness (AHR) to methacholine in

OVA-induced asthma. Methacholine AHR was significantly increased in OVA-treated mice and reduced in UCB-MSCs-treated mice that were challenged with OVA. Data shown are from a representative experiment. Penh values were corrected by subtracting the pre-challenge from the post-challenge values. * $p < 0.05$.

BAL and histologic findings

Lung histology and cellularity in BAL samples were assessed to evaluate whether UCB-MSCs could modify allergen-driven airway inflammation in mice. As shown in **Figure 6**, OVA exposure mice had significantly increased the number of total cell (653×10^3/mL) and inflammatory cells, including eosinophils (320×10^3/mL), macrophages (276×10^3/mL), lymphocytes (30×10^3/mL), and neutrophils (27×10^3/mL), compared with PBS-treated mice. There were significant decreases in the number of inflammatory cells of UCB-MSCs-treated mice, especially post-treatment eosinophils (148×10^3/mL), which were significantly reduced by over 50% compared with OVA-challenged mice. Microscopically, the epithelium, subepithelial smooth muscle layers, and basement membrane stained with H&E were thickened in those cells from OVA-challenged mice. The treatment with UCB-MSCs decreased the thickness of the epithelium, subepithelial smooth muscle layers, and basement membrane (**Figure 7**). Lung tissue stained with PAS revealed that the mucus production was markedly decreased after UCB-MSCs treatment (**Figure 8**). The microscopic findings described above were further confirmed via subjective scoring of the inflammation and PAS-positive cells. Both the inflammation and PAS scores showed a significant reduction after treatment with UCB-

MSCs, compared with those of the group treated with OVA alone (**Figure 9**).

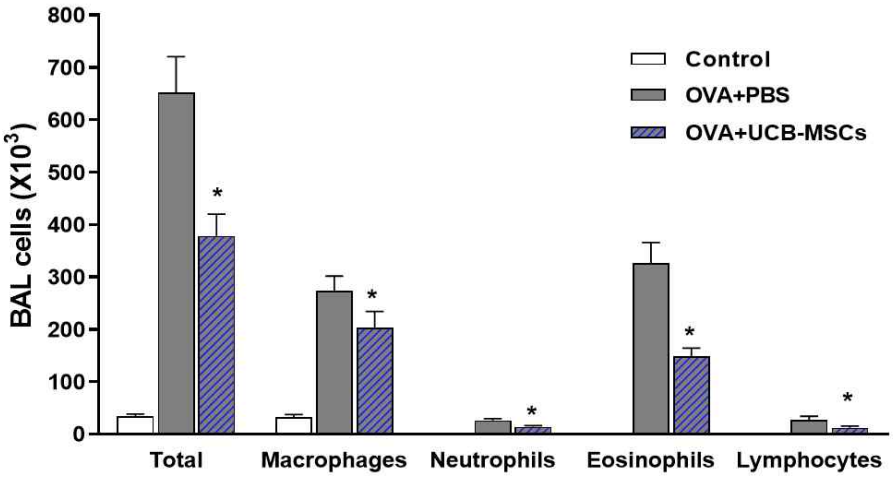


Figure 6. Effect of UCB-MSCs on the cell counts in bronchoalveolar lavage (BAL) fluid from asthmatic mice. Inflammatory cells and total cells were counted in BAL fluid. The number of inflammatory cells was significantly increased in OVA-challenged groups, but was markedly reduced upon UCB-MSCs treatment, especially eosinophil counts. The data are presented as the mean ± SEM. * $p < 0.05$

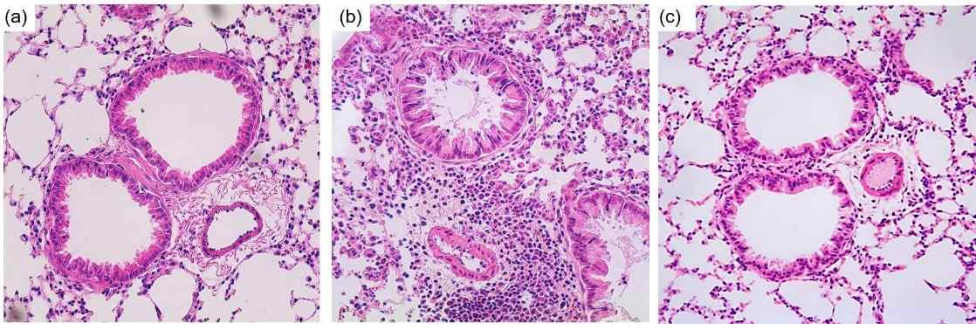


Figure 7. Effect of UCB-MSCs on inflammatory cells in lung tissues stained with H&E (a: PBS; b: OVA-induced asthma + PBS; c: OVA-induced asthma + UCB-MSCs. a – c, magnification × 200). OVA-sensitized mice treated with UCB-MSCs exhibited reduced airway inflammation, and displayed the thickness of the epithelium, subepithelial smooth muscle layers, and basement membrane.

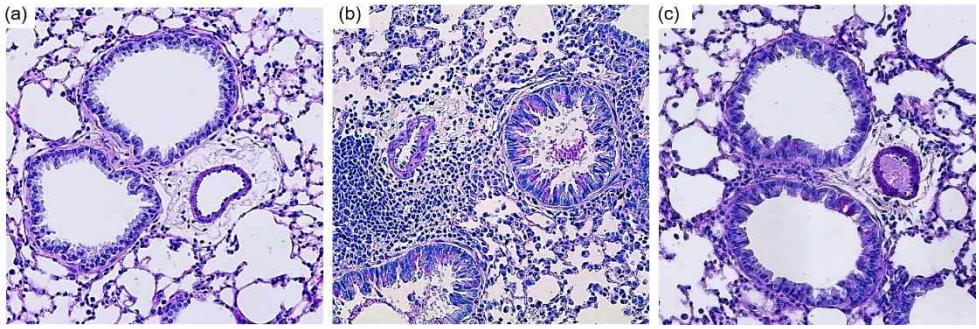


Figure 8. Effect of UCB-MSCs on lung tissues stained with PAS stain (a: PBS; b: OVA-induced asthma + PBS; c: OVA-induced asthma + UCB-MSCs. a – c, magnification × 200). OVA-sensitized mice treated with UCB-MSCs displayed a reduced mucus production.

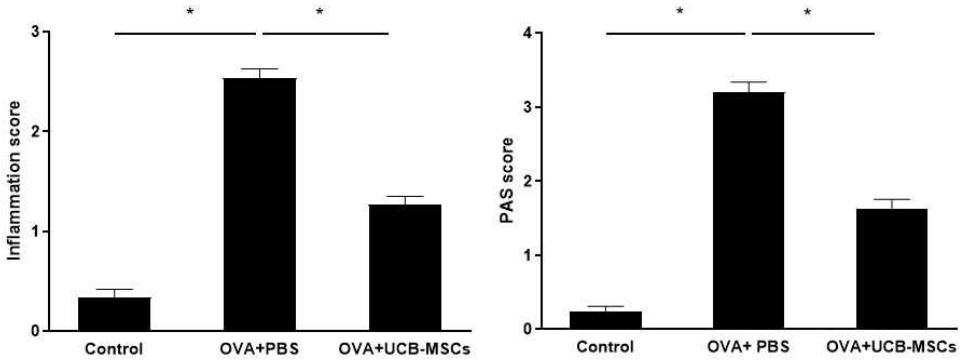


Figure 9. Effect of UCB-MSCs on lung inflammation and PAS-positive cell scores. Lung inflammation and goblet cell hyperplasia were significantly decreased in the UCB-MSCs-treated OVA-challenged mice compared with non-treated OVA-challenged mice. The data are presented as the mean ± SEM. * $p < 0.05$.

Measurement of OVA-specific immunoglobulin

The concentrations of OVA-specific IgG1, G2a, and IgE in the serum were measured to evaluate whether UCB-MSCs affected the production of serum OVA-specific antibodies. Using an ELISA kit, IgG1 (optical density (OD), 0.36 ± 0.05) and IgE (OD, 0.13 ± 0.03) levels were increased in the OVA groups compared with the controls. Notably, IgG1 (OD, 0.23 ± 0.03) and IgE (OD, 0.08 ± 0.01) levels were significantly lower in mice treated with UCB-MSCs. However, there were no significant differences in IgG2a levels under any conditions (**Figure**

10).

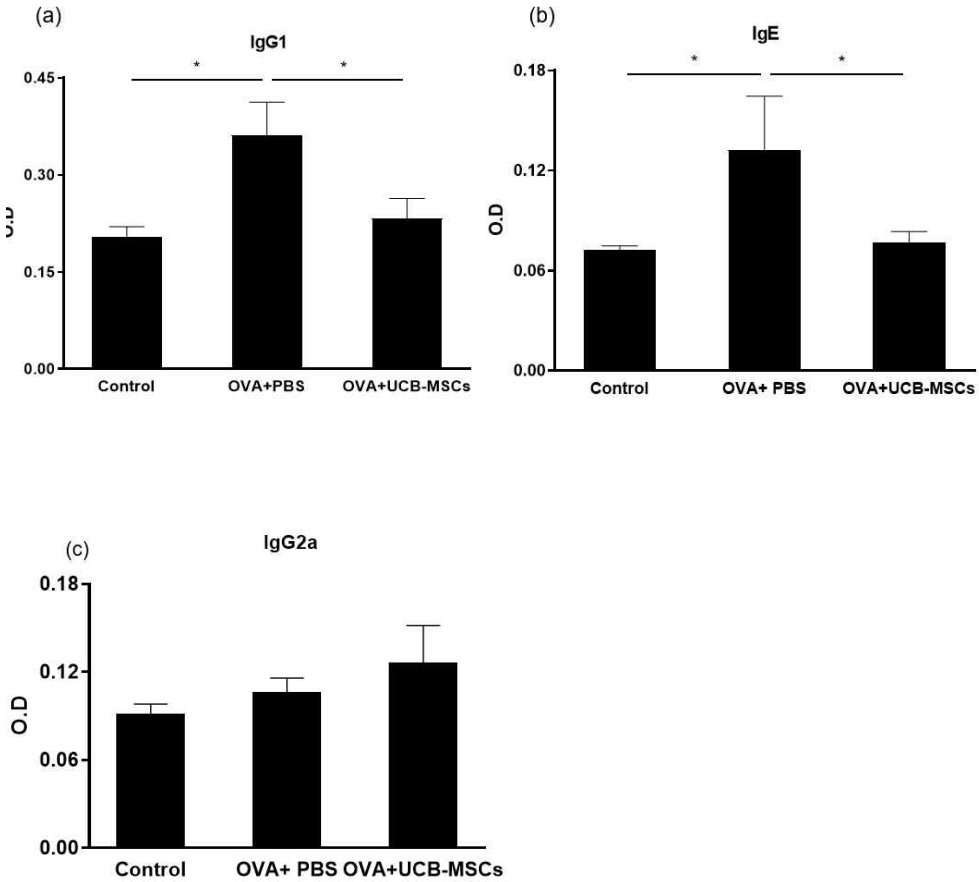


Figure 10. Levels of OVA-specific immunoglobulins in the serum. The UCB-MSCs-treated OVA group showed a significant decrease in IgG1 and IgE expression compared with the non-treated OVA group (a, b), but there were no significant differences regarding the IgG2 level (c). The data are presented as the mean ± SEM. * $p < 0.05$

Levels of cytokines measured using RT-qPCR and ELISA

The effect of UCB-MSCs on T cell-mediated immune response was evaluated by using qRT-PCR and ELISA. From the lung tissues, we found that OVA challenge significantly increased the proportion of IL-4, IL-5, and IL-13 mRNA expression by 4.2-, 0.09-, and 0.3-fold, whereas IL-4, IL-5, and IL-13 levels were reduced in the UCB-MSCs-treated OVA group by 1.3-, 0.04-, and 0.13-fold (**Figure 11**). The UCB-MSCs-treated OVA group exhibited a more significant increase in IL-10 (OD, 0.52 ± 0.01 in OVA-induced asthma + UCB-MSCs vs. 0.44 ± 0.03 in OVA-induced asthma + PBS) and TGF- β (OD, 0.11 ± 0.004 vs. 0.10 ± 0.001) levels of BAL fluid compared with the non-treated OVA group. The concentrations of IL-4 (OD, 0.37 ± 0.04 in OVA-induced asthma + UCB-MSCs vs. 0.55 ± 0.09 in OVA-induced asthma + PBS) and IL-13 (OD, 0.08 ± 0.003 vs. 0.09 ± 0.005) in BAL fluid were decreased in the UCB-MSCs-treated OVA group (**Figure 12**). IL-4 (93.18 ± 23.75 pg/mL) and IL-5 (285.04 ± 18.34 pg/mL) production in splenocytes was significantly increased in the OVA-treated group compared with the control, being significantly decreased upon treatment with UCB-MSCs (56.64 ± 5.38 and 148.73 ± 20.98 pg/mL) (**Figure 13**).

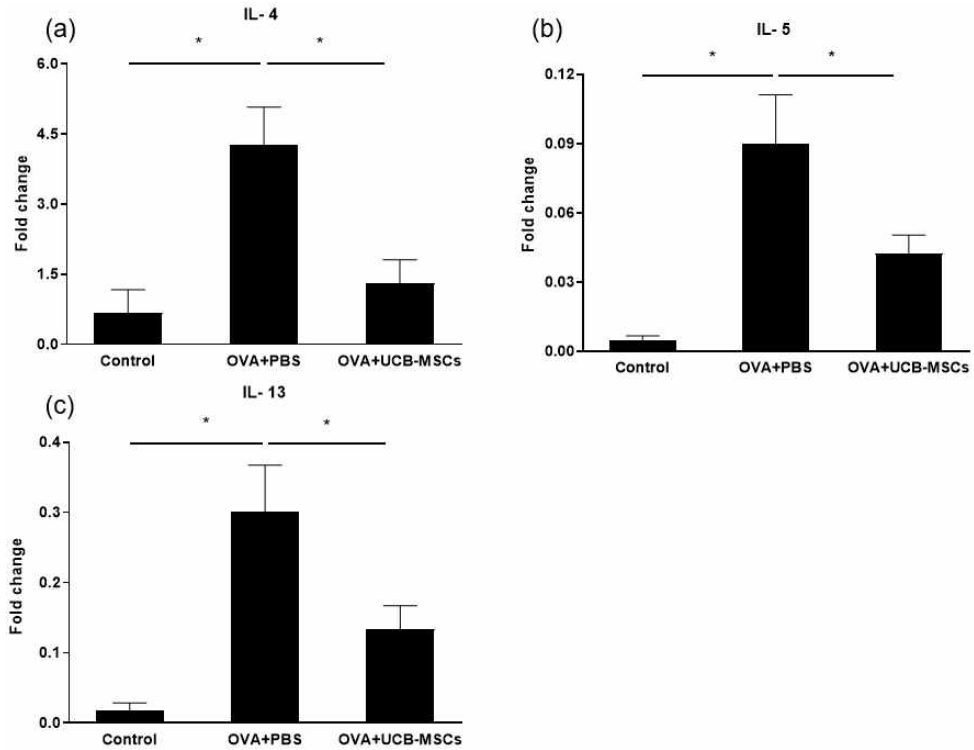


Figure 11. Messenger RNA expression of mouse lung tissues. The mRNA levels of IL-4, IL-5, and IL-13 were measured using RT-qPCR. OVA-challenged mice treated with UCB-MSCs had a reduced expression of IL-4 (a), IL-5 (b), and IL-13 (c) compared with non-treated OVA-challenged mice. The results are represented as relative fold change of expression after normalization to that of β-actin. The data are presented as the mean ± SEM. * $p < 0.05$.

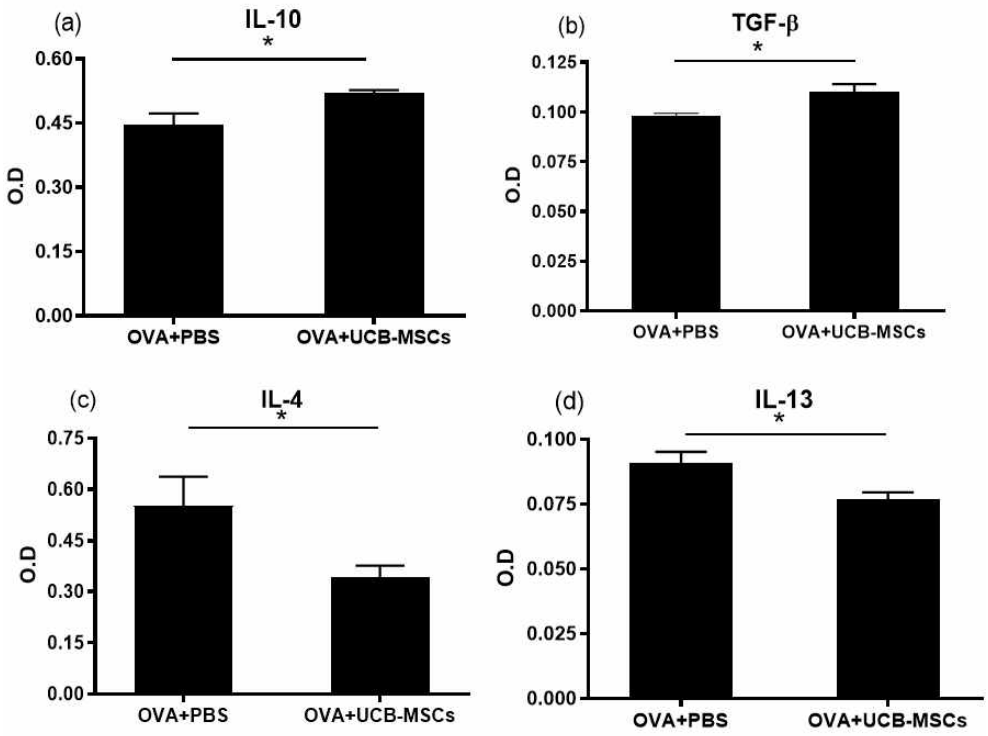


Figure 12. Comparison of the production of different cytokines in BAL fluid. ELISA assay showed that the IL-10 and TGF-β concentrations (a, b) in BAL fluid were increased in UCB-MSCs-treated OVA groups compared to non-treated OVA groups. The concentrations of IL-4 and IL-13 (c, d) were decreased in the group treated with UCB-MSCs. The data are presented as the mean ± SEM. * $p < 0.05$.

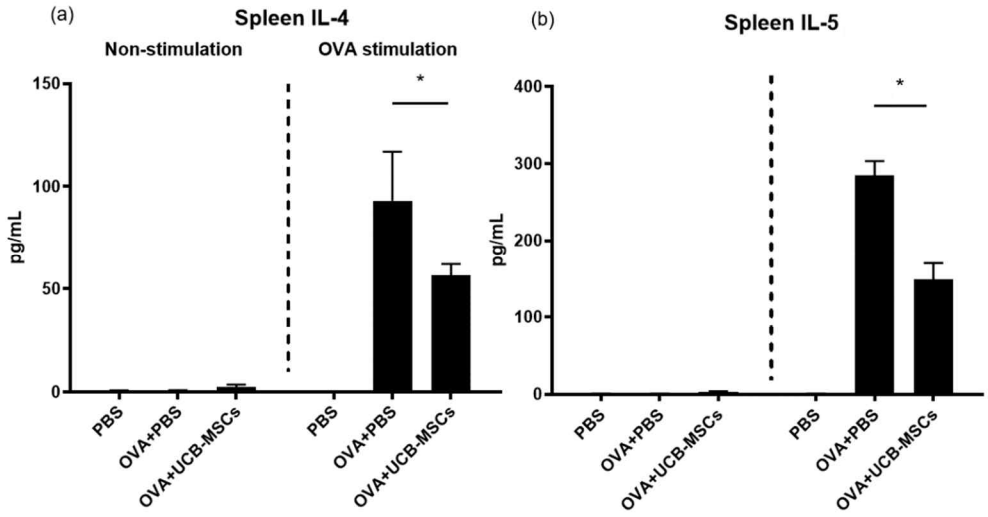


Figure 13. Cytokine production in spleen cell supernatants. ELISA assay showed that IL-4 and IL-5 concentrations (a, b) were significantly decreased in UCB-MSCs-treated OVA groups compared to non-treated OVA groups. However, there were no significant differences regarding the concentration of the two cytokines between the groups without OVA stimulation. The data are presented as the mean ± SEM. * $p < 0.05$.

Comparison of regulatory T cells in lymph nodes

The expansion of Treg population was assessed in draining lymph nodes of non-treated and UCB-MSCs-treated OVA groups. The proportion of $CD4^{+}CD25^{+}Foxp3^{+}$ Treg cells in the OVA group treated with UCB-MSCs was significantly higher than that in the non-treated OVA group (10.00% ± 0.39% in OVA-induced asthma + UCB-MSCs vs. 6.20% ± 0.23% in OVA-induced asthma + PBS). The percentage of Treg cells from the spleen was also observed to have an

increasing trend, following UCB-MSCs treatment (**Figure 14**).

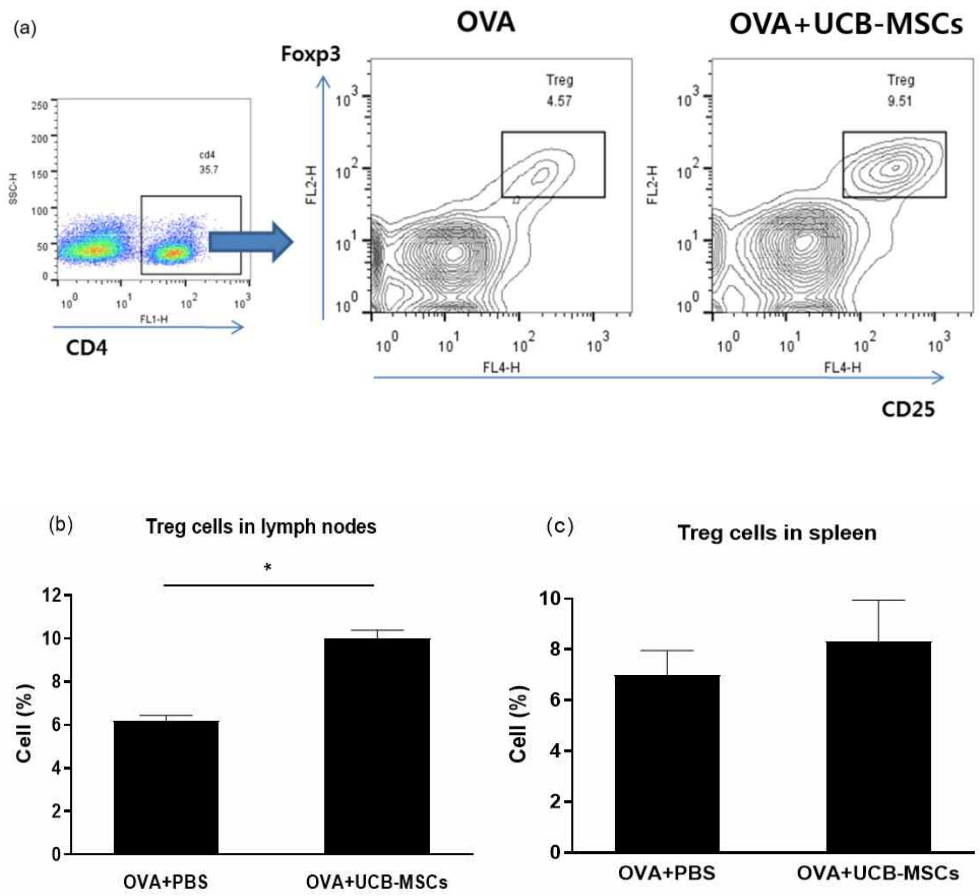


Figure 14. Frequency of $CD4^{+}CD25^{+}Foxp3^{+}$ Treg cells in draining lymph nodes and spleen during UCB-MSCs treatment. Representative flow cytometry plot showed the frequency of $CD4^{+}CD25^{+}Foxp3^{+}$ Treg cells, which were gated from $CD4^{+}$ T cells (a). An increased frequency of Treg cells in lymph nodes was significantly observed in UCB-MSCs-treated OVA cells (b). There was a non-significant trend toward an increased frequency of Tregs in the spleen

(c). The data are presented as the mean ± SEM. * $p < 0.05$.

3. Experiment II assessing the effect of UC-MSCs on the innate immune responses in asthma

AHR to Methacholine

Methacholine significantly induced AHR in OVA-treated mice compared to the control group, while there were no differences in any of the other 2 treatments groups (UC-MSCs-treated group and UC-MSCs-treated OVA group), compared with the PBS-treated mice (**Figure 15**).

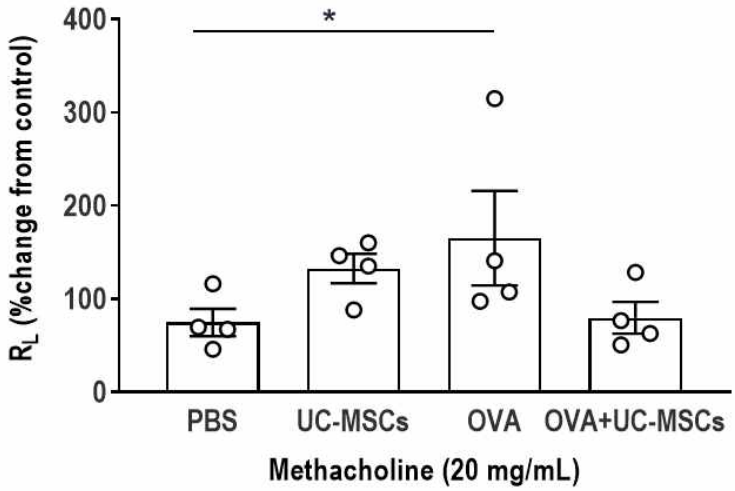


Figure 15. Effects of UC-MSCs on airway hyperresponsiveness (AHR) to methacholine

in OVA-induced asthma. Methacholine AHR was significantly increased in OVA-treated mice. UC-MSCs decreased AHR, although the difference was not statistically significant. * $p < 0.05$.

BAL and histologic findings

UC-MSCs treatment reduced airway inflammation of lung tissues and BAL fluid, which was enhanced by OVA challenge. The increase in the number of total cells (4.26×10^5/mL), eosinophils (2.01×10^5/mL), neutrophils (0.35×10^5/mL), and macrophages (1.71×10^5/mL) in BAL fluid was significantly observed in the OVA group, whereas the total number of inflammatory cells in BAL fluid (2.39×10^5/mL) was significantly decreased in UC-MSCs-treated OVA group compared with the OVA-treated group, along with a significant decrease in the number of macrophages (1.09×10^5/mL), eosinophils (0.97×10^5/mL), and neutrophils (0.16×10^5/mL) (**Figure 16)**. In addition, the eosinophil infiltration around the bronchi and the blood vessels was markedly reduced in the UC-MSCs-treated OVA group (**Figure 17**).

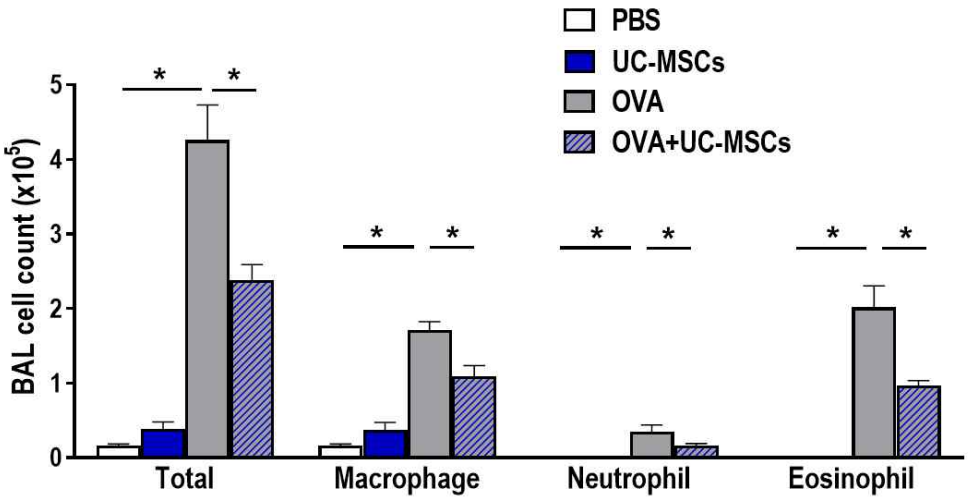


Figure 16. Effect of UC-MSCs on cell counts in bronchoalveolar lavage (BAL) fluid from asthmatic mice. The number of total inflammatory cells, macrophages, neutrophils, and eosinophils was counted in BAL fluid. The number of inflammatory cells was significantly higher in OVA-challenged groups but was significantly reduced upon UC-MSCs treatment. BAL; bronchoalveolar lavage. The data are presented as the mean ± SEM. * $p < 0.05$.

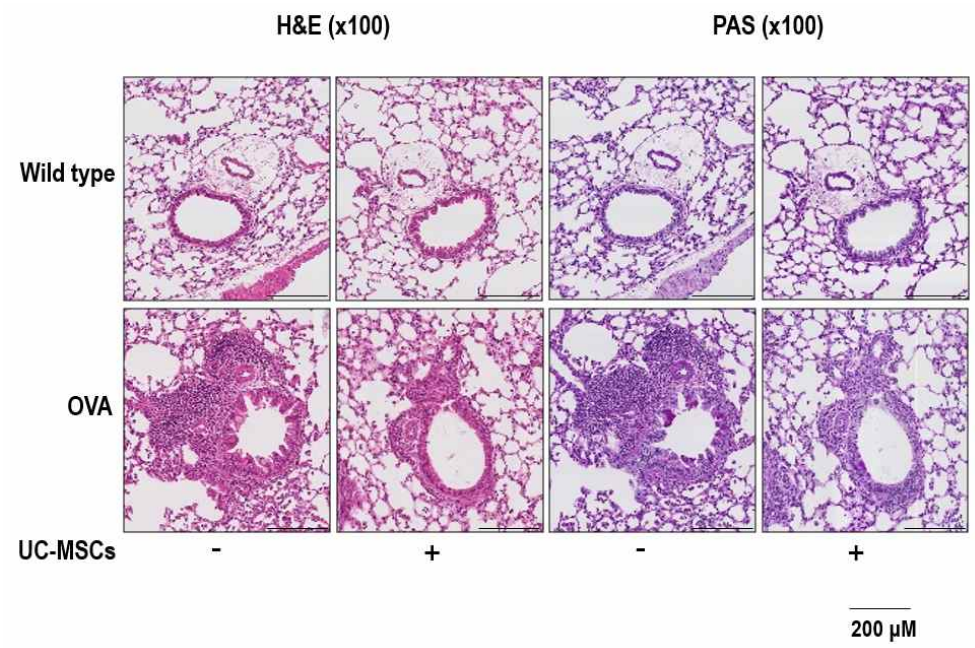


Figure 17. Effect of UC-MSCs on inflammatory cells in lung tissues stained with H&E and PAS. OVA-sensitized mice treated with UC-MSCs exhibited reduced airway inflammation, including goblet cell hyperplasia and the thickness of the epithelium, subepithelial smooth muscle layers, and basement membrane.

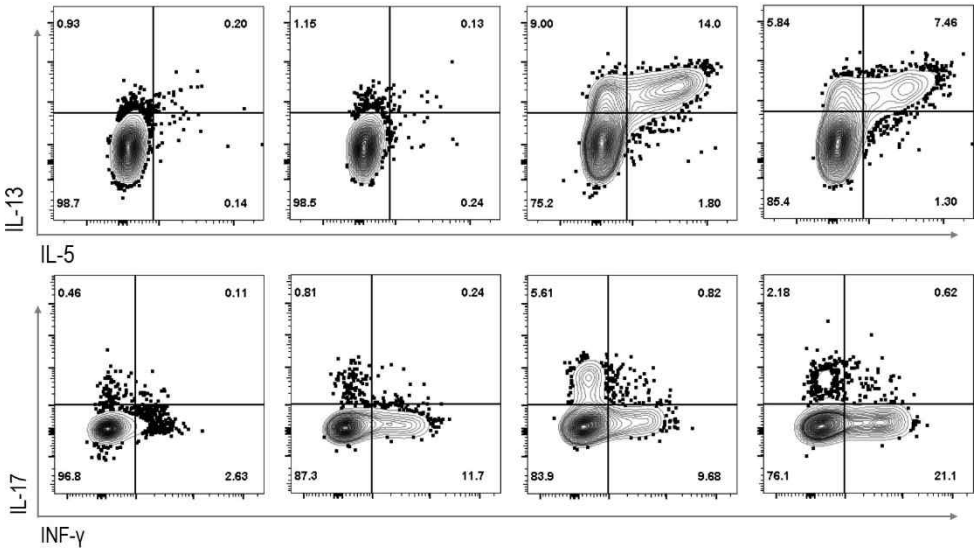
Changes in lymphoid lineages induced by UC-MSCs treatment

The number of IL-13, IL-5/IL-13, and IL-17-secreting CD4 T^+ cells ($2.06 \pm 0.12 \times 10^5$, $2.69 \pm 0.36 \times 10^5$, and $1.04 \pm 0.06 \times 10^5$) was significantly increased in the OVA group. Treatment with UC-MSCs significantly decreased the number of these cells ($1.43 \pm 0.18 \times 10^5$, $1.65 \pm 0.21 \times 10^5$, and $0.54 \pm 0.14 \times 10^5$). On the other hand, UC-MSCs treatment significantly

increased the number of IFN-γ-secreting $CD4^+$ T cells ($3.55 \pm 0.44 \times 10^5$) compared to the OVA group ($2.04 \pm 0.17 \times 10^5$). An enhancement of the Th2/Th1 ratio in OVA-treated group and a reduction of the Th2/Th1 ratio after UC-MSCs treatment were observed (**Figure 18**). In the lung tissues, the proportion of $Foxp3^+$ Treg cells (21.08% ± 1.68% of CD4 T^+ cells) in the UC-MSCs-treated OVA group was significantly higher than that in the non-treated OVA group (13.78% ± 1.61%), while the number of Treg cells from the spleen was reduced following UC-MSCs treatment (**Figure 19**).

In addition to the changes observed regarding T cells, there was a significant increase in IL-13 and IL-5/IL-13 producing ILC2s ($2.16 \pm 0.21 \times 10^5$ and $5.57 \pm 1.02 \times 10^5$) in mice exposed to OVA. UC-MSCs treated mice resulted in a significant decrease in IL-13- and IL-5/IL-13-producing ILC2s ($1.00 \pm 0.25 \times 10^5$ and $2.26 \pm 0.60 \times 10^5$) compared to OVA-treated mice. The impact of ILC3s following UC-MSCs treatment was the same as that observed for ILC2s. IFN-γ-secreting ILC1s were increased in the OVA-induced group but were not changed due to UC-MSCs treatment (**Figure 20**).

(a)


0.93
0.20
98.7
0.14
1.15
0.13
98.5
0.24
9.00
14.0
75.2
1.80
5.84
7.46
85.4
1.30
IL-13
IL-5
0.46
0.11
96.8
2.63
0.81
0.24
87.3
11.7
5.61
0.82
83.9
9.68
2.18
0.62
76.1
21.1
IL-17
INF-γ

(b)

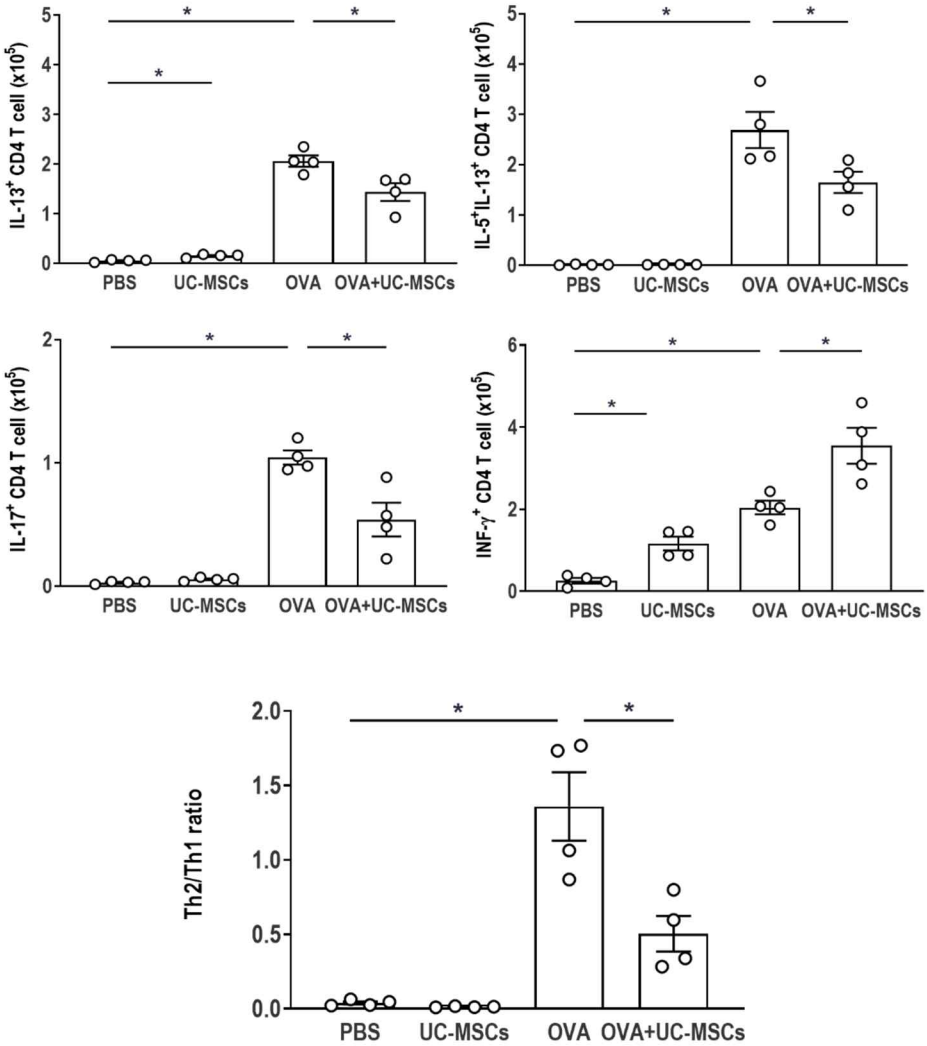


Figure 18. Population of $CD4^+$ T cells in lung tissues following UC-MSCs treatment. $IL13^+$, $IL\text{-}5^+/IL\text{-}13^+$, $IL17^+$, and $IFN\text{-}\gamma^+$ T cells were gated from $CD4^+$ T cells using flow cytometry (a). The number of $IL13^+$ and $IL\text{-}5^+/IL\text{-}13^+$ $CD4^+$ T cells was significantly lower in UC-MSCs-treated OVA cells compared with non-treated OVA cells. $IL17^+$ CD4 T cells were decreased in

UC-MSCs-treated OVA cells, but IFN-γ^+ CD4 T cells were increased after UC-MSCs treatment (b). The data are presented as the mean ± SEM. * $p < 0.05$.

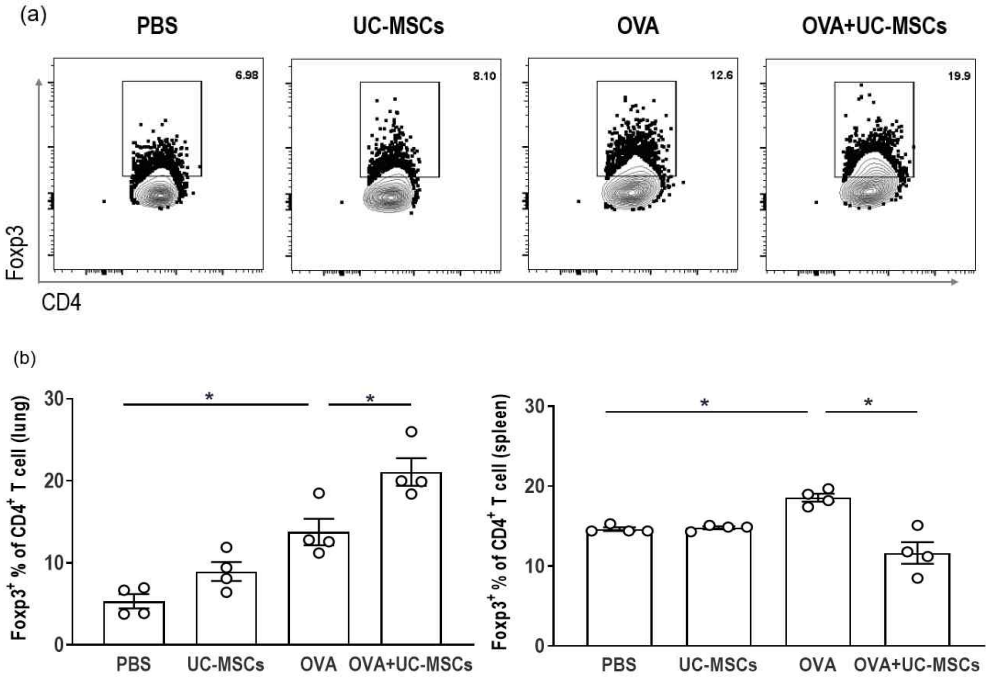
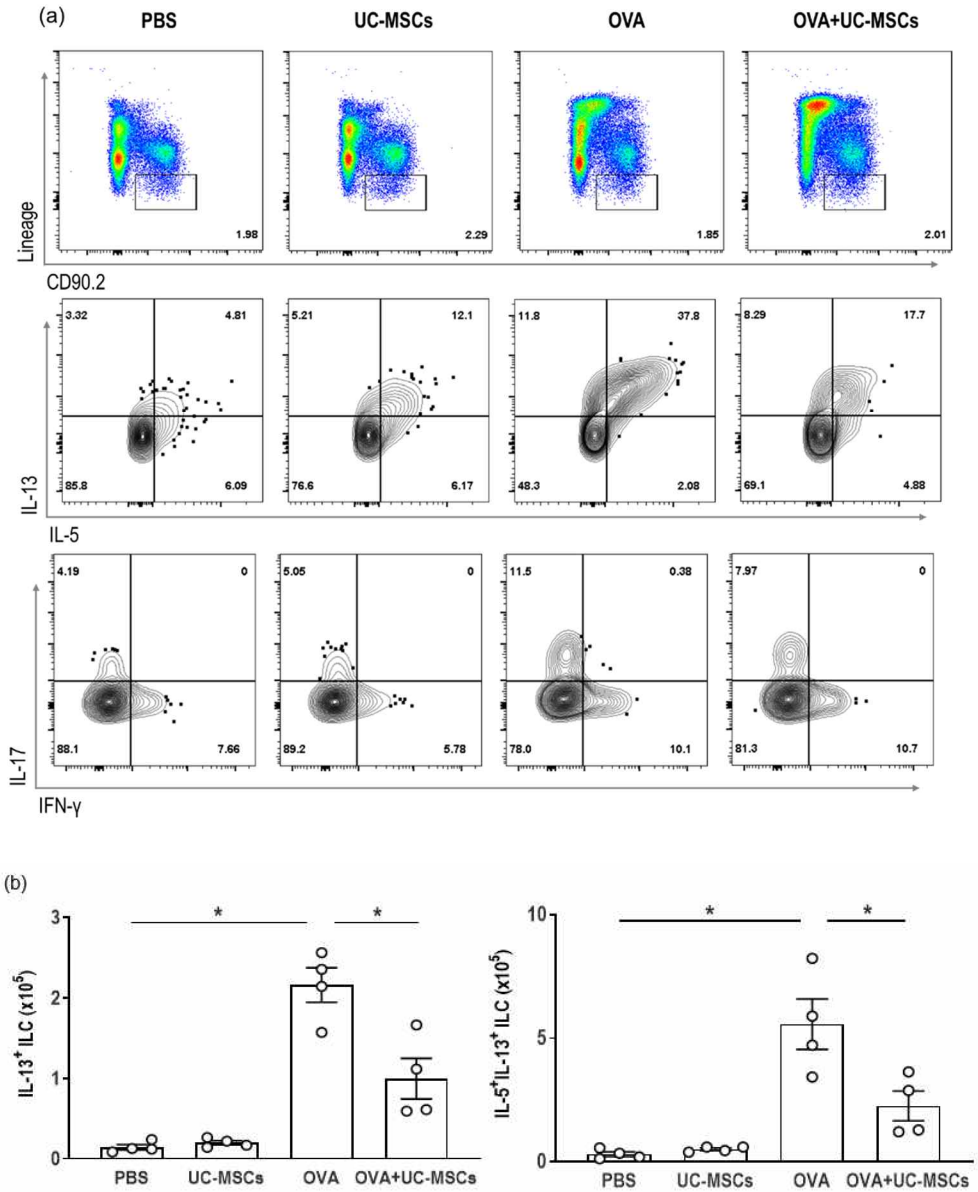


Figure 19. Population of Foxp3$^+$ Treg cells in lung tissues following UC-MSCs treatment. Foxp3$^+$ Treg cells were gated from CD4+ T cells using flow cytometry (a). In the lung tissues, the number of Foxp3$^+$ Treg cells was significantly increased after UC-MSCs treatment compared to OVA-treated Foxp3$^+$ Treg cells. A decrease in Foxp3$^+$ Treg cells from the spleen was observed after UC-MSCs treatment (b). The data are presented as the mean ± SEM. * $p < 0.05$.


(a)
PBS
UC-MSCs
OVA
OVA+UC-MSCs
Lineage
CD90.2
1.98
2.29
1.85
2.01
IL-13
IL-5
3.32
4.81
85.8
6.09
5.21
12.1
76.6
6.17
11.8
37.8
48.3
2.08
8.29
17.7
69.1
4.88
IL-17
IFN-γ
4.19
0
88.1
7.66
5.05
0
89.2
5.78
11.5
0.38
78.0
10.1
7.97
0
81.3
10.7
(b)
IL-13+ ILC (x10^5)
IL-5+IL-13+ ILC (x10^5)
PBS
UC-MSCs
OVA
OVA+UC-MSCs
*

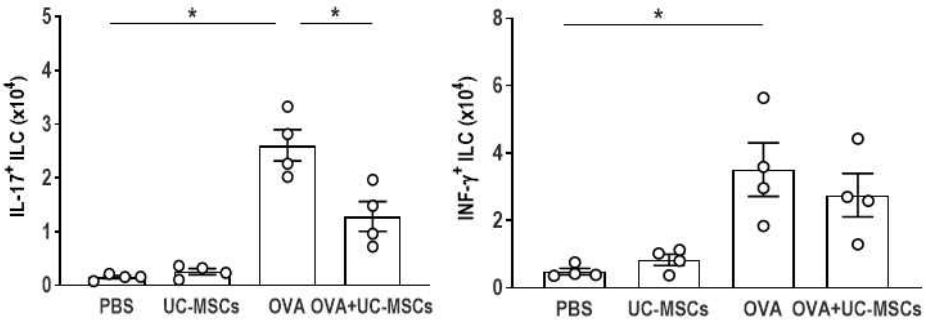


Figure 20. Population of ILCs in lung tissues following UC-MSCs treatment. ILCs were gated from $Lin^{-}CD90.2^{+}$ cells using flow cytometry (a). IL-5^{+}/IL-13^{+}, IL-13^{+}, and IL-$17A^{+}$ ILCs were significantly decreased after UC-MSCs treatment compared to OVA-treated ILCs (b). The data are presented as the mean ± SEM. * $p < 0.05$.

Changes in dendritic cells and macrophages induced by UC-MSCs treatment

OVA challenge increased the number of DCs ($2.62 \pm 0.49 \times 10^{5}$) in the lungs, especially those with an enhanced expression of MHCII ($4.35 \pm 0.67 \times 10^{5}$). UC-MSCs treatment reduced the total number of DCs and mature DCs ($1.23 \pm 0.16 \times 10^{5}$ and $2.05 \pm 0.27 \times 10^{5}$) with antigen-presenting properties ($MHCII^{+}CD11c^{+}$ DCs) (**Figure 21**).

Similarly, the increase in the number of total macrophages ($F4/80^{+}$ cells) in the OVA group ($1.54 \pm 0.19 \times 10^{6}$) was also reduced ($0.72 \pm 0.07 \times 10^{6}$) by the treatment with UC-MSCs. The proportion of $CD11c^{+}CD11b^{-}$ macrophages, defined as AMs, was significantly decreased in the

OVA group (3.64% ± 0.48% of total macrophages), but was significantly increased by UC-MSCs treatments (7.30% ± 1.15%). Moreover, MHCII$^-$CD11c$^+$CD11b$^-$ macrophages were decreased by OVA challenge and MHCII$^+$CD11c$^+$CD11b$^-$ macrophages showed the opposite trend. UC-MSCs treatment reduced the population of MHCII$^+$ AMs and increased the population of MHCII$^-$AMs, although there was no significant difference between the OVA group and the UC-MSCs-treated OVA group. The number of AMs expressing high levels of Siglec-F, considered as resident AMs, was significantly reduced in OVA-treated groups (3.51% ± 0.49% of total macrophages), and they were restored by UC-MSCs treatment (7.18% ± 1.17%) (**Figure 22**).

The number of CD11b$^+$ macrophages, defined as IMs, and their subgroups expressing Ly6C and MHCII (13.88 ± 1.59 × 10^5, 3.38 ± 0.39 × 10^5, and 3.87 ± 0.52 × 10^5) was increased in the OVA group compared to the PBS group. These increases were attenuated by UC-MSCs transplantation (6.03 ± 0.54 × 10^5, 1.09 ± 0.12 × 10^5, and 1.81 ± 0.21 × 10^5). Based on CD206 and CD86 expression, IMs were divided into M1 (CD206$^-$CD86$^+$), M2 (CD206$^+$CD86$^-$), and double positive (CD206$^+$CD86$^+$) macrophages. The cell number of the three populations (0.72 ± 0.11 × 10^4, 2.45 ± 0.26 × 10^5, and 2.85 ± 0.49 × 10^5) was significantly higher in the OVA group than in the PBS group. After UC-MSCs treatment, lower numbers of M1, M2, and double positive macrophages (0.45 ± 0.04 × 10^4, 0.89 ± 0.10 × 10^5, and 1.51 ± 0.17 × 10^5) were found. Particularly, we found that the change in M2 was higher than that observed in M1. There was an enhancement of the M2/M1 ratio in the OVA-treated group and a reduction in the M2/M1 ratio after UC-MSCs treatment.

Of the M2 subtypes based on CD206, CD86, and MHCII expression, the number of M2a and

M2c macrophages (2.70 ± 0.35 × 10^5 and 1.78 ± 0.33 × 10^5) was shown to be markedly increased in the OVA challenge group than in the control group. Treatment with UC-MSCs decreased the number of M2a and M2c macrophages (1.18 ± 0.12 × 10^5 and 0.74 ± 0.08 × 10^5) compared to those in the OVA challenged groups. The number of M2b macrophages was decreased, although no significant difference was observed following UC-MSCs treatment (**Figure 23**).

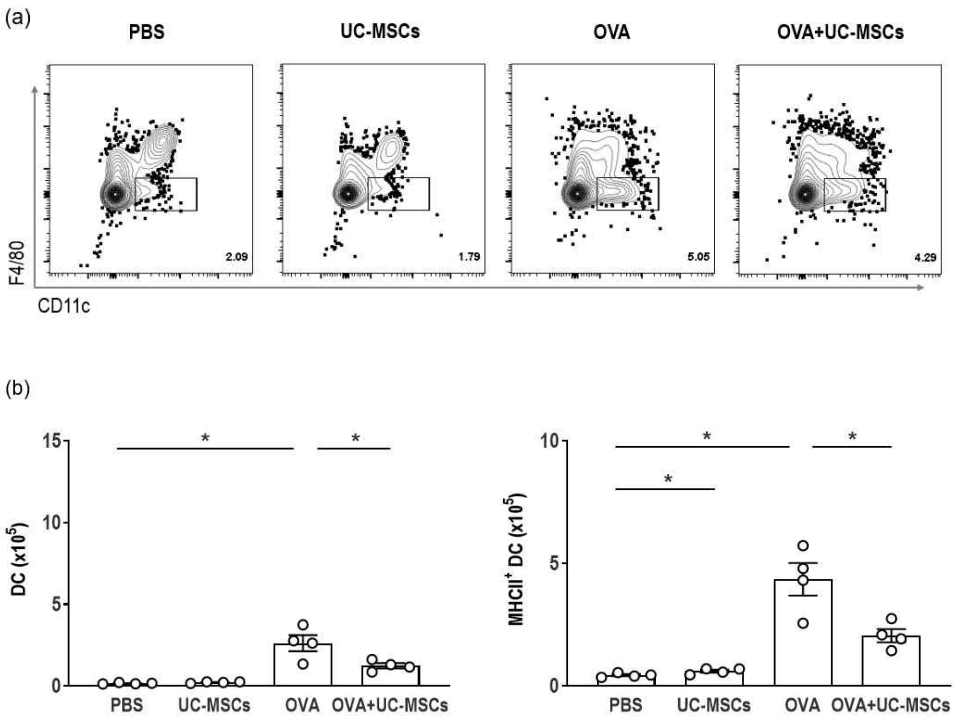
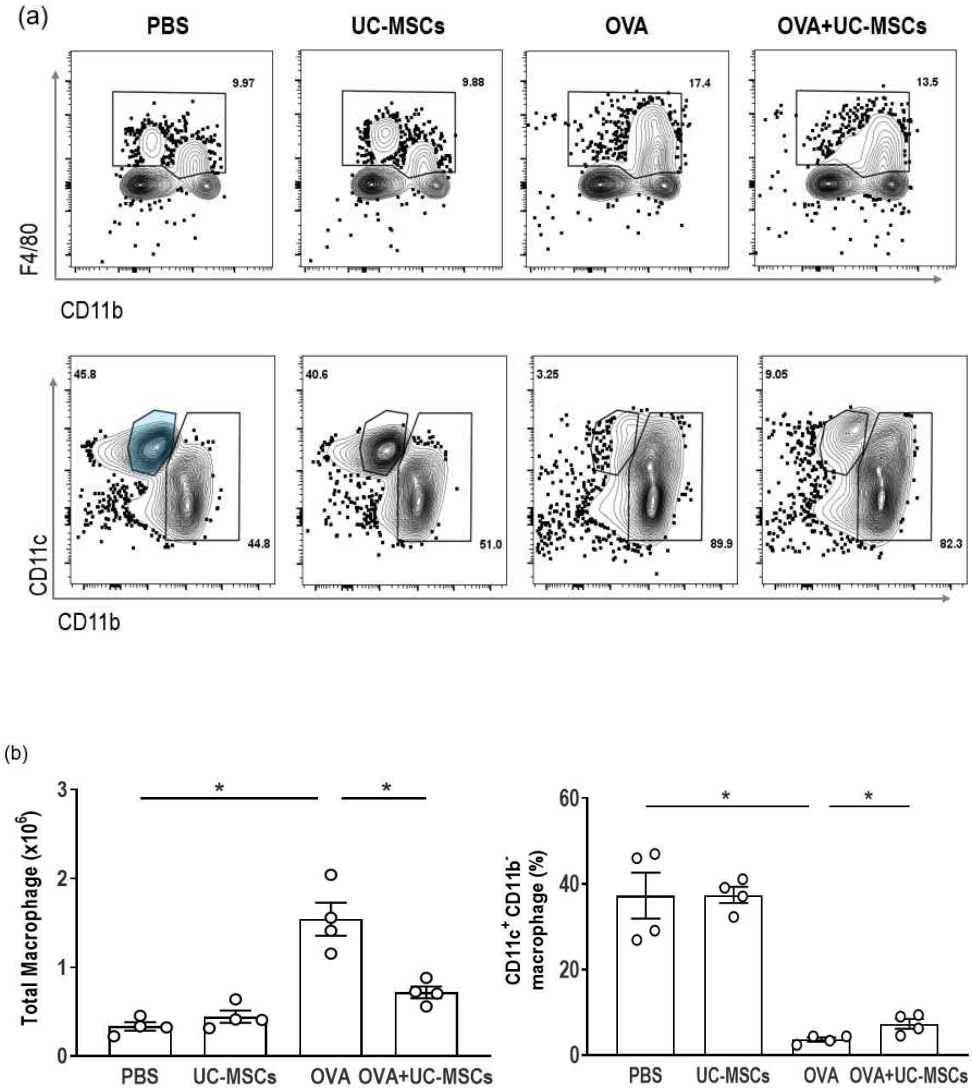


Figure 21. Population of DCs in lung tissues following UC-MSCs treatment. DCs were gated from F4/80$^-$CD11c$^+$ cells using flow cytometry (a). Total DCs and MHCII$^+$ DCs were

significantly decreased after UC-MSCs treatment compared to OVA-treated DCs (b). The data are presented as the mean ± SEM. * $p < 0.05$.



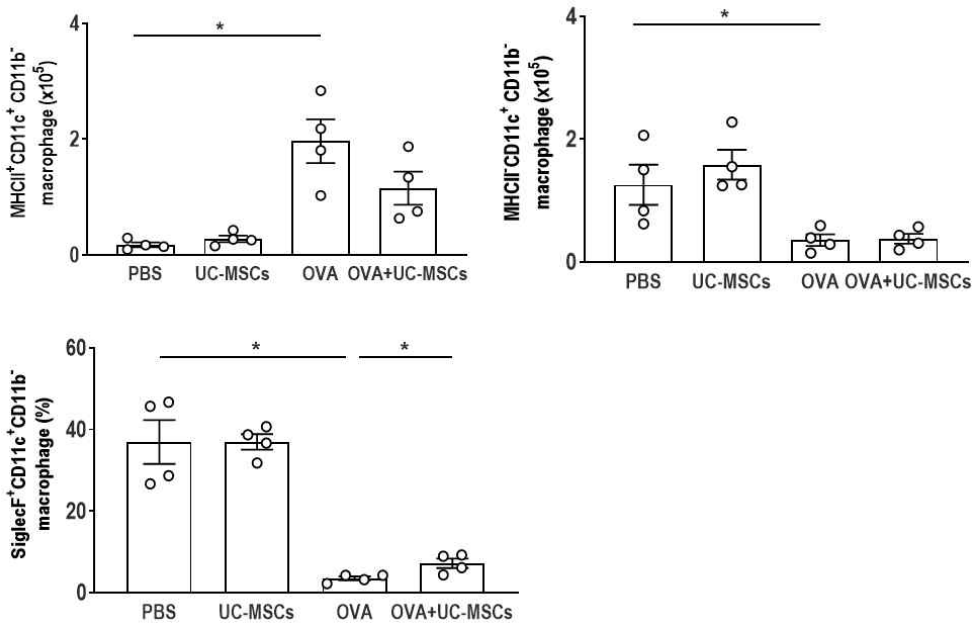
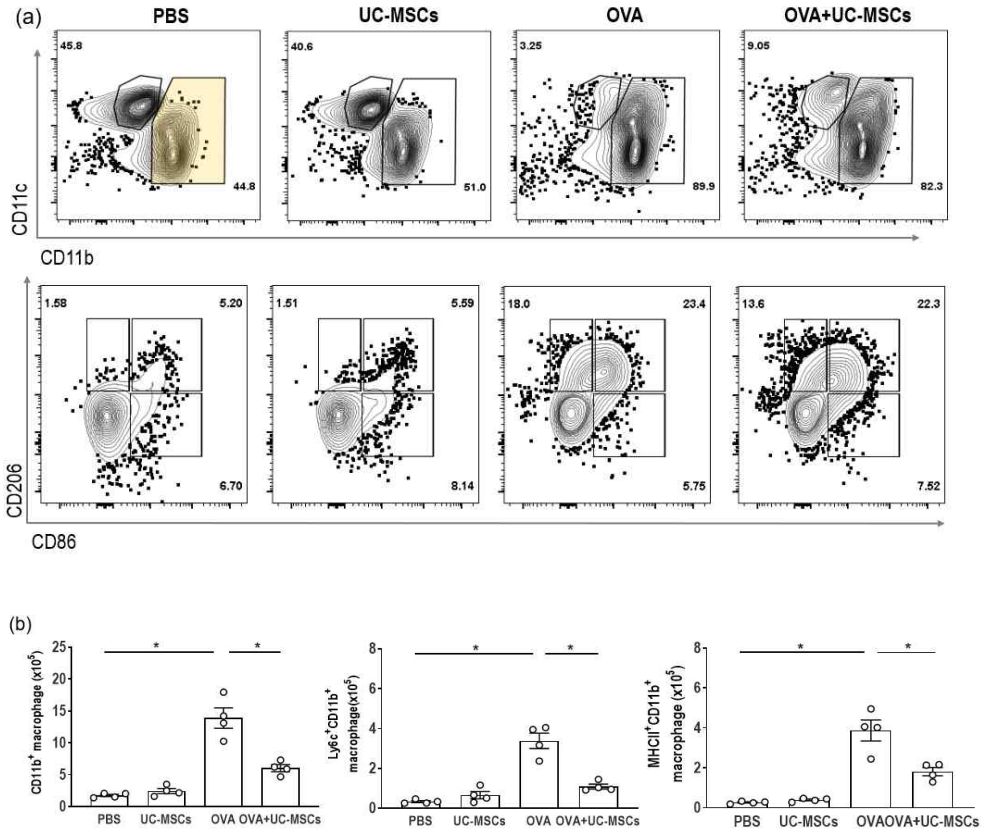


Figure 22. Population of macrophages in lung tissues following UC-MSCs treatment. Macrophages were gated from F4/80$^+$ cells using flow cytometry. Based on CD11b and CD11c expression, F4/80$^+$ cells were divided into CD11c$^+$CD11b$^-$, CD11c$^+$CD11b$^+$, CD11c$^-$CD11b$^+$, and CD11c$^-$CD11b$^-$cells. Of these, CD11c$^+$CD11b$^-$ cells were defined as alveolar macrophages (AMs) (a). The total macrophage number was significantly decreased after UC-MSCs treatment compared to that of OVA-treated macrophages. OVA challenge decreased the number of alveolar macrophages (CD11c$^+$CD11b$^-$ cells) and of their subtype with the following expression: MHCII$^-$ and SiglecF$^+$. The increased number of total AMs and resident AMs was observed after UC-MSCs treatment (b). The data are presented as the mean ± SEM. * $p < 0.05$.


(a)
PBS
UC-MSCs
OVA
OVA+UC-MSCs
45.8
44.8
40.6
51.0
3.25
89.9
9.05
82.3
CD11c
CD11b
1.58
5.20
6.70
1.51
5.59
8.14
18.0
23.4
5.75
13.6
22.3
7.52
CD206
CD86
(b)
CD11b+ macrophage (x10^5)
Ly6c+CD11b+ macrophage(x10^5)
MHCII+CD11b+ macrophage (x10^5)
PBS
UC-MSCs
OVA
OVA+UC-MSCs

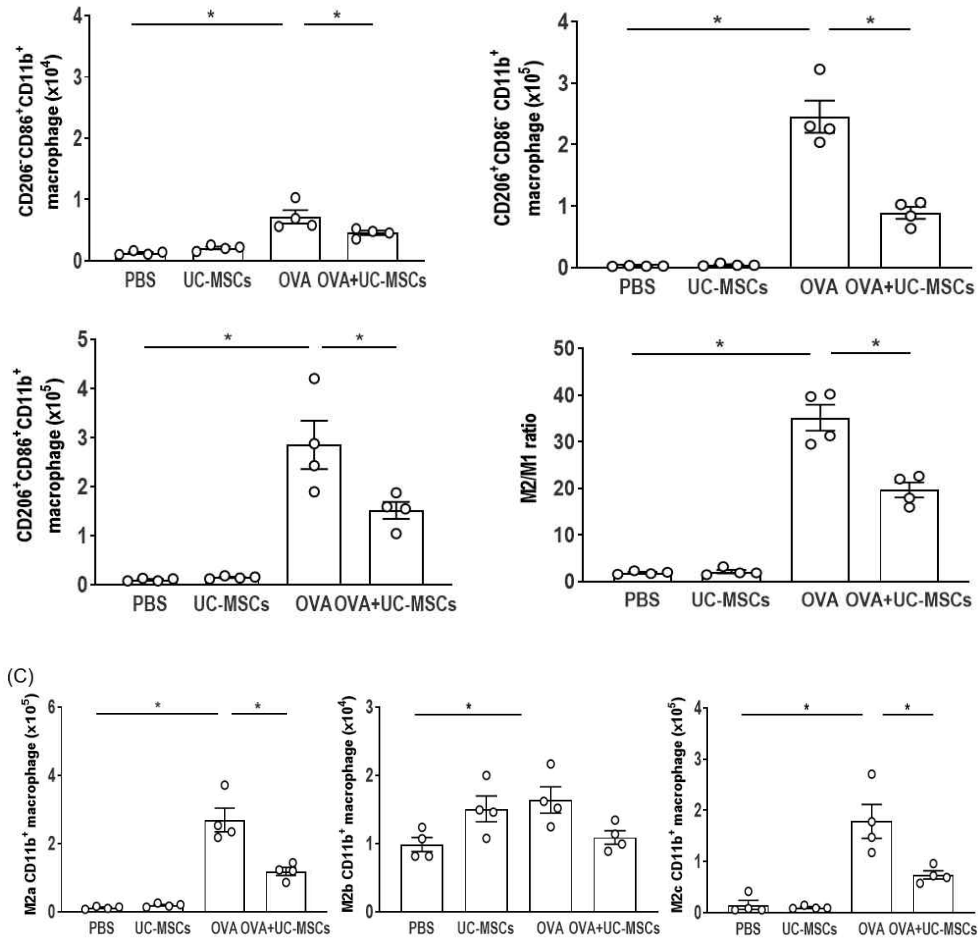


Figure 23. Population of interstitial macrophages in lung tissues following UC-MSCs treatment. Interstitial macrophages (IMs) were gated from $CD11b^+$ cells using flow cytometry. Based on CD206 and CD86 expression, $CD11b^+$ cells were divided into M1 ($CD206^-CD86^+$), M2 ($CD206^+CD86^-$), and double positive ($CD206^+CD86^+$) macrophages. Of the M2 subtypes based on CD206, MHCII, and CD86, cells were classified as M2a ($CD206^+CD86^-$ $MHCII^+$), M2b ($CD206^-$ $CD86^+$ $MHCII^+$), and M2c ($CD206^+CD86^-MHCII^-$) cells (a). The number of

total IMs, Ly6c^{+} IMs, and HMCII^{+} IMs was increased by OVA challenge, whereas the number of these cells was decreased in the UC-MSCs-treated OVA group. The number of M1, M2, and double positive macrophages was increased in the OVA group, and UC-MSCs treatment significantly reduced the number of M1, M2, and double positive macrophages (b). M2a and M2c macrophages were significantly decreased in the UC-MSCs treatment group compared to the OVA-challenged group (c). The data are presented as the mean ± SEM. * $p < 0.05$.

Impact of UC-MSCs treatment on macrophage expression

Analysis of mRNA expression levels in mouse lung revealed that UC-MSCs treatment significantly downregulated the expression levels of M2 markers (CD206 and Arg-1), but not that of IL-13. Murine macrophages were collected from BAL fluid and stimulated with IL-4 and IL-13 to induce M2 macrophage polarization in the presence and absence of UC-MSCs treatment. CD206 expression by macrophage cell line was enhanced by IL-4 and IL-13 treatment (16.63 ± 7.14- fold and 3.20 ± 0.39-fold), and the expression of IL-13-stimulated macrophages was only significantly reduced by UC-MSCs treatment (1.05 ± 0.10-fold). In addition, indirect treatment of UC-MSCs significantly suppressed CD206 expression of IL-13-stimualted macrophages (1.56 ± 0.32-fold) (**Figure 24**).

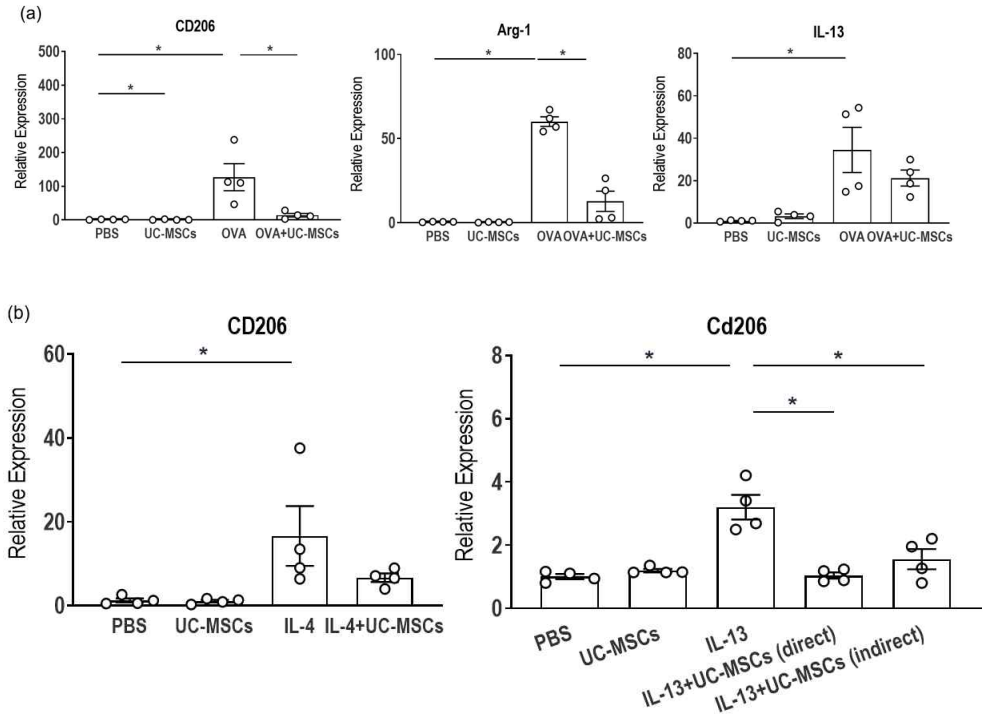


Figure 24. mRNA expression in murine macrophages induced by UC-MSCs treatment. The elevated expression of M2 markers (CD206 and Arg1) in the OVA group was significantly reduced by UC-MSCs treatment (a). mRNA expression of M2 marker (CD206) was enhanced by IL-4 or IL-13 treatment and was recovered by UC-MSCs treatment. In addition, UC-MSCs treatment indirectly affected IL-13-stimulated macrophages (b). The data are presented as the mean ± SEM. * $p < 0.05$.

Discussion

MSCs can be found in tissues all over the body, such as bone marrow, amniotic fluid, adipose tissue and umbilical cord.[62] Unlike hematopoietic stem cells, MSCs readily differentiate into any cell type, having great potential for regenerative medicine.[14] Moreover, the hypo-immunogenic properties of MSCs enable them to survive and differentiate in MHC-mismatched allogeneic or even xenogeneic transplant recipients.[63] BM-MSCs have been the major source of hMSCs and the most widely investigated in preclinical or clinical settings.[64] As suggested in earlier studies, BM-MSCs exert immunomodulatory effects of Th2 response in allergic airway inflammation.[16-22] However, BM-MSCs are difficult to obtain sufficient quantities to use in therapies. Their harvesting procedure is painful and invasive. Moreover, the cell number and proliferative/differentiation capacity of BM-MSCs are significantly decreased with age.[65] In contrast, human UCB and UC is a promising source of MSCs that does not require such an invasive procedure. Human UCB and UC can be easily collected without causing pain and having ethical or technical issues associated.[26] There are various culture methods and protocols used for the isolation of MSCs from the various compartments of UC, including Wharton's jelly, umbilical vessel, UC lining membrane, and sub-amnion and perivascular regions.[26] MSCs harvested from UCB and UC have shown multipotency and self-renewal abilities after accumulating at the damaged or inflamed regions, promoting tissue repair, and modulating immune responses.[66-70] Compared with BM-MSCs and embryonic stem cells (ESCs), UCB-MSCs and UC-MSCs show a gene expression profile that is more similar to that of ESCs and have a faster self-renewal than BM-MSCs.[13] Another advantage of UCB-MSCs and UC-MSCs is that they grow faster (shorter doubling time) than BM-MSCs and are viable at higher passage numbers.[13] Moreover, they secrete more immunomodulatory substances,

such as IL-10, IL-8, TGF-β2, and hepatocyte growth factor (HGF).[71,72] The upregulation of costimulatory and human leukocyte antigen (HLA) molecules by activated MSCs is a concern for administering allogeneic MSCs. Interestingly, UC-MSCs showed a minimal expression of HLA-A, -B, -C, and -DR, in response to a high dose of IFN-γ.[73] In contrast with peripheral blood mononuclear cells injected group of humanized mice, the serum allo-responsive IgG titer, the number of pro-inflammatory cytokines, such as IFN-γ and tumor necrosis factor (TNF)-α, and splenic T-cell activation were lower in mice injected with UC-MSCs.[74] Furthermore, UCB-MSCs are known to be hypo-immunogenic or non-immunogenic because they have low expression levels of MHCI and lack the MHCII molecules that induce an immune rejection in allogeneic transplantation.[75] Similar to BM-MSCs, UCB-MSCs and UC-MSCs may provide a source of autologous and allogeneic cell therapies. Autologous use is useful in gene therapies, regenerative or anti-inflammatory therapies for neonatal injuries, such as cerebral palsy or hypoxic brain damage. Allogeneic use of cells could be expanded and cryopreserved in a cell bank for patients in need.[76] The only consideration of autologous and allogeneic use is that the baby's health as a donor is needed to confirm because it cannot be determined in advance whether the donor will continue to grow normally without underlying health problems. Hence, genetic tests and chromosomal tests are required.[26] Compared with AD-MSCs, UCB-MSCs and UC-MSCs show comparable inhibitory effects on activated T-cell proliferation via Indoleamine 2,3-dioxygenase (IDO) and cyclooxygenase (COX)-2 pathways.[73,77] UC-MSCs have the shortest doubling time and proliferation rate, which is three- to four-fold higher than that of AD-MSCs.[73] In addition, human leukocyte antigen-G (HLA-G)-induced T cell inhibitory effects are more remarkable in UC-MSCs.[73] In AD-MSCs, there are considerable

differences in the cell yield and the performance of adipose tissue, which are dependent on various factors, including anatomic site, harvesting technique, body mass index, donor age, and gender.[78] Therefore, UCB and UC, which are the alternative sources of MSCs, may provide significant clinical benefits regarding allogeneic use, ease of accessibility, and regenerative capacity. Specifically, UCB-MSCs are already being utilized for the treatment of patients in several clinical trials for neurodegenerative diseases, autoimmune diseases, severe acute graft-versus-host disease, and liver diseases (www.clinicaltrials.gov).[79-82]

In experiment I, UCB-MSCs treatment was effective in an eosinophil-predominant asthmatic mouse model established via OVA challenge.[53] Intravenous administration of UCB-MSCs significantly attenuated airway hyperresponsiveness, eosinophilic inflammation, and histopathological changes in BAL fluid. UCB-MSCs treatment significantly suppressed the production of IgE and IgG1. It resulted in a down-regulation of Th2 cytokines, such as IL-4, IL-5, and IL-13, at local (lung tissue) and systemic (spleen) levels. Our findings are in line with those of previous studies.[25] In AHR, most studies reported a significant reduction due to MSCs treatment, regardless of the timing of administration. Total cell and eosinophil count of BAL fluid were also reduced by MSCs treatment in most studies, but some studies did not report the effects of the treatment.[18,40,49] The BAL levels of cytokines, such as IL-4, IL-5, and IL-13, were generally found to be significantly lower by MSCs treatment. However, no significant effect in IL-4 was observed in several studies.[35,38,40,47] IL-5 and IL-13 did not result in significant effects in some studies, respectively.[18,35,36,38,40] UCB-MSCs treatment decreased allergen-specific IgE and IgG1 production, indicating sensitization in Th2 response. The effect on serum allergen-IgE, IgG1, and G2 levels from previous studies had variable results. MSCs treatment resulted

in reduced levels of serum IgE.[18,19,36,47,49] However, there were no significant changes reported in another study.[40] The same trend has also been reported regarding the changes in allergen-specific IgG1 and G2a.[18,36,47,49]

We conducted *in vivo* bioluminescent imaging as described previously to determine whether injected MSCs migrated into the lungs.[21] We found that UCB-MSCs are deposited in the lungs whereas virtually no cells were seen in other organs within 48 hours after tail vein injection of UCB-MSCs (data not shown). Recent findings are verified by *in vivo* cell tracking studies, which showed that administered MSCs first accumulated in the lung vasculature, and then moved to other major organs, such as the kidney and liver. About 10% of the injected cells remained in the spleen, whereas there are no cells in the lymph nodes.[83] The survival or ability to detect the cells, regardless of the donor and different homing organs, were relatively short and varied at a low level among different studies.[83-86] Less than 0.01% of injected MSCs were detectable in the lungs of mice after 4 weeks. Most of the cells are not detectable 7 – 14 days post transplantation.[87]

Several mechanisms by which MSCs may function at cellular and molecular levels include differentiation into multiple cell lineages and regulation of different stages of immune responses.[12,88] Recent studies have shown the ability of MSCs to induce Treg cells, and MSCs-induced Treg cells have been associated with protective effects of allergic airway diseases.[19,21] In this study, UCB-MSCs increased the percentage of $FoxP3^+$ Treg cells in the lung lymph nodes, together with an increasing trend in the spleen. Previous reports have demonstrated that Treg cells migrated and accumulated in the site of inflammation and draining lymph nodes.[89,90] The expansion of Treg cells in this study was accompanied by the upregulation of IL-10 and

TGF-β expression. These findings are in line with previous reports using a murine model of allergic asthma.[15] Cho *et al.* demonstrated that murine MSCs derived from adipose tissue can induce the generation of Treg cells via secretion of soluble factors (IDO, TGF-β, and prostaglandin E2 (PGE2)), and that MSCs activated Treg cells might secrete anti-inflammatory cytokines, such as IL-10 and TGF-β, which lead to a decrease in lung eosinophilic inflammation, Th2 cytokines, and IgE antibody production.[15] Although we did not address the function of Treg cells after administration of UCB-MSCs experimentally, we assume that UCB-MSCs display therapeutic potential in asthma by the regulation of Th2 cells and Treg cells. Along with many soluble factors, cell-to-cell contact, reprogramming of antigen-presenting cells to tolerogenic phenotypes, and induction of extracellular vesicles, such as exosomes, have emerged as possible mechanisms via which MSCs induce immune-modulatory Treg cell proliferation.[91]

In experiment II, UC-MSCs attenuated allergy-specific pathological changes after the challenge phase via the regulation of innate immune cells. UC-MSCs treatment decreased ILC2s, ILC3s, total DCs, and DC subsets with antigen-presenting capacity. In response to various innate signals and several innate immune cells, ILCs have been identified to link innate and adaptive immunity.[92] ILCs are classified into three subpopulations, such as ILC1, ILC2, and ILC3, that are thought to play a crucial role in the pathogenesis of asthma. Previous reports have shown that ILC2s, which secrete IL-4, IL-5, and IL-13, contribute to eosinophilic inflammation in allergic asthma and coordinate adaptive immune responses. By releasing IL-17 and/or IL-22 that promote neutrophilic inflammation, ILC3s are mainly found in some endotypes of asthma, especially non-allergic and severe asthma.[93] In this study, the numbers of $MHCII^+$ DCs and $MHCII^+$ AMs were much higher in the OVA group, but UC-MSCs treatment suppressed these

numbers. These findings suggest that UC-MSCs play roles in the alleviation of asthma by regulation of the antigen-presenting DCs and AMs. Similarly, Zeng *et al.* reported that murine BM-MSCs suppressed the antigen-presenting ability of DCs in an OVA-induced asthmatic mouse model.[50] Recent several studies have shown that the role of macrophages is extended to developmental processes and maintenance of tissue homeostasis in many ways.[94] Macrophages are the most numerous cells presented in the respiratory tract and can be broadly divided into two populations depending on their localization: AMs, that line the surface of alveoli, and IMs, that reside in the space between the alveolar epithelium and vascular endothelium.[95] SiglecF, a marker related to eosinophil apoptosis, is expressed in eosinophils and AMs, but is not found in IMs or inflammatory macrophages.[96-98] Hence, SiglecF^{+}CD11c^{+}CD11b^{-} macrophages can represent typical resident AMs with homeostatic function in a steady state.[99] In this study, UC-MSCs increased resident AMs, and these results suggest that UC-MSCs may reduce type 2 inflammation in asthma with recovery of the depleted resident AM population. In line with this study, CD11c^{+}CD11b^{low} AMs were significantly reduced upon OVA challenge but were also restored upon treatment with hMSCs, and *in vivo* depletion of AMs abrogated the therapeutic effects of hMSCs on allergic airway inflammation and AHR.[41] Similar to the Th1/Th2 dichotomy, macrophages can differentiate into distinct effectors, including classically (M1) and alternatively (M2) activated macrophages.[100] M1 macrophages are known as classically activated macrophages induced by IFNγ, lipopolysaccharide (LPS), and TNFα. M2 macrophages are known as alternatively activated macrophages induced by IL-4 and IL-13. M2 macrophages had already been sub-categorized into M2a, M2b, and M2c based on their distinct gene expression profiles. The M2a subtype is driven by IL-4, IL-13, or fungal and helminth

infections. M2b is induced by IL-1 receptor ligands, immune complexes, and LPS, whereas M2c is elicited by IL-10, TGF-β, and glucocorticoids.[101-103] Macrophage activation has been considered a dynamic process. Early macrophages respond to environmental signals, such as cytokine signaling, and they develop and act into functional macrophages when exposed to foreign substances.[104] Changes in the tissue microenvironment also affect macrophage polarization.[105] Braza *et al.* revealed that murine BM-MSCs were phagocytosed by murine macrophages, which altered their proinflammatory signature to a suppressive phenotype, following IL-10 and TGF-β expression.[31] Song *et al.* found that BM-MSCs provoked a pronounced polarization in alveolar macrophages to the M2 subtype, which is mediated by TGF-β-signaling-dependent alveolar macrophage polarization.[106] In IMs, Nie *et al.* observed that OVA-induced asthmatic mice expressed phenotypic markers associated with the M2 subtype, such as Arg-1, and reduced levels of IL-10.[107] These data suggest that IMs undergo a phenotypic switch from a regulatory macrophage under normal conditions to alternatively activated macrophage in OVA-induced asthma. This study revealed that UC-MSCs reduced the number of IMs and their subtypes (M1, M2, and $CD206^{+}CD86^{+}$ cells, which represent a transition state from M1 to M2). Moreover, M2-polarized macrophages such as M2a and M2c macrophages, showed a similar pattern following UC-MSCs treatment. These findings suggest that potential therapeutic effects of UC-MSCs in asthma may be mediated by the regulation of these M2 macrophages. Altogether, UC-MSCs can sequentially change the phenotype of macrophages depending on their anatomic location. Additional investigations are warranted to better understand this contradictory phenomenon.

Recent studies have shown that the immunomodulatory effects of MSCs are mainly mediated

by soluble factors, which may be the primary mechanism for their therapeutic effects.[18,77,108-111] MSCs-secreted water-soluble agents, such as IL-6, IL-10, PGE2, and nitric oxide have been reported to play a role in the modulation of the allergic airway response.[77,108-111] From the *in vitro* experiments, we confirmed that the expression of M2-related markers was decreased with UC-MSCs treatment. These findings support that UC-MSCs affected macrophage polarization, even without direct cell-to-cell contact, suggesting that the paracrine action induced by transplanted MSCs may be a critical event in the UC-MSCs effect observed.

This study has some limitations that should be acknowledged. First, two experiments were carried out with MSCs derived from different sources, at different dose, and with a different timing of administration. The pre-challenge administration was done to understand the effects of disease development and the treatment during the challenge was done to identify the therapeutic effect of the actual disease. This heterogeneity bias limits the consistency of the interpretation of the results, but the overall results are similar with the data obtained from current preclinical studies. Second, the cytokine protein expression levels in experiment I were not evaluated in lung tissues, and the protein levels of IL-13 from spleen had some degree of uncertainty when comparing between groups. The cytokine levels of IL-10 and TGF-β in BAL fluid determined by ELISA were increased in the UCB-MSCs-treated OVA groups, whereas the concentration of IL-4 and IL-13 was decreased. However, the differences in BAL fluid between groups were low when converted into protein levels. Third, the therapeutic effects of MSCs were evaluated using murine asthma models, which were randomly divided into three or four groups (negative control, OVA treated group, MSCs treated group, and group for MSCs treated OVA-challenged mice). We did not perform direct comparisons of MSCs and

corticosteroid as standard of care for asthma. Currently, inhaled corticosteroids (ICSs) are the mainstay of asthma management because ICSs are the most potent and effective anti-inflammatory agents available so far. However, ICSs do not facilitate tissue repair or treat asthma in a radical way. About 10% of patients with asthma remain uncontrolled despite ICSs use.[112] In previous studies, intranasally administered mesenchymoangioblast-derived MSCs (MCA-MSCs) from human iPSCs predominantly demonstrated protection against airway remodeling and AHR than dexamethasone from OVA induced chronic allergic airways diseases. Combining MCA-MSCs with dexamethasone provided an equivalent protection to that offered by either of the therapies alone.[113] An *in vitro* study demonstrated that the treatment of hMSCs with budesonide-loaded poly microparticles enhanced IDO expression following IFN- γ stimulation.[114] These two studies revealed that MSCs could be used to augment therapeutic potency of conventional therapies by suppressing active inflammation and inducing tolerance. Moreover, striking antifibrotic and wound-healing effects could serve as a suitable adjunct therapy to corticosteroids as anti-inflammatory therapy. Lastly, we could not rigorously demonstrate the mechanistic link between MSCs and Treg cells, and macrophage function. Therefore, our findings must be interpreted with caution.

Despite the above limitations, our study had several strengths compared to previous studies. This is the first study in which UC-MSCs could favorably change the outcome of asthmatic inflammation targeting of innate immune cells, such as ILCs and macrophages. Additionally, this is the first evaluation detailing the effects of xenogenic UCB-MSCs on allergic inflammation in an acute asthma model. MSCs, including UCB-MSCs and UC-MSCs, could provide immunomodulatory effects in asthma, and it is speculated that their effects could be

related to the regulation of both innate and adaptive immune cells. Based on the promising results using animal studies, we agree that the therapeutic potential of MSCs may be attributed to cellular and molecular mechanisms of action, and still needs in-depth exploration for clinical application.

In summary, we demonstrated that UCB-MSCs and UC-MSCs successfully attenuated allergic airway inflammation and these MSCs are effective in the early or acute phase of the allergic response. Long-term studies with large cohorts are warranted to determine the beneficial effects of UCB-MSCs and UC-MSCs in asthma. Mechanisms underlying the therapeutic effects during the rescue and repair of asthmatic inflammation require further elucidation. Nevertheless, there is no doubt that the potential and safety of UCB-MSCs and UC-MSCs make them a promising tool for developing novel treatment strategies for asthma.

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Table 1. Study design reporting stem cell treatment in rodent models

Study (year)	Animals (numbers per group)	Sensitization (days)	Challenge (days)	MSCs source	MSCs dose per site (route, injection days)
Abreu[27] (2013)	A (6)	0, 2, 4, 6, 8, 10, 12	40, 43, 46,	M	1×10^5 (IT, 47)
Abreu[28] (2014)	A (7)	0, 2, 4, 6, 8, 10, 12	40, 43, 46	M	2×10^6 (IT, 47)
Ahmad[29] (2014)	B (> 5)	0, 7, 14	27-30	M, H	1×10^6 (IT, 30)
	B (> 5)	0, 7, 14	21-27	M, H	1×10^6 (IT, 27)
	B (> 5)	0, 7, 14	25-29	M, H	1×10^6 (IT, 29)
Bonfield[16] (2010)	B (4-6)	0	Every 2 days for 4 weeks starting on day 14	H	1×10^6 (IV, During 6th week)
Bonfield[30] (2010)	B (4-6)	0	14-18	H	1×10^6 (IV, 14 or 16)
Braza[31] (2016)	B (5-15)	0, 7, 14, 21	27, 34	M	5×10^5 (IV, 27)

Chan[32] (2016)	B (10)	1-3	14, 17, 21, 24, 28	H	1×10^6(IV, 21)
Cruz[33] (2015)	A (6)	0, 7	14-16	M, H	1×10^6(IV, 14)
Cruz[34] (2015)	A (3-5)	0, 7	14-16	M, H	1×10^6 (IV, 14)
Firinci[17] (2011)	B (10)	0, 14	×3 weekly for 8 weeks starting on day 21	M	1×10^6(IV, 75)
Ge[35] (2013)	B (10)	1, 14	24-26	M	5×10^5(IT, 23)
Ge[36] (2013)	B (12)	1, 7, 14	×3 weekly for 8 weeks starting on day 21	M	5×10^5(IT, 20)
Martinez-Gonzalez[37] (2014)	B (5-7)	0, 7	14	H	1×10^6(IV, 15)
Goodwin[18] (2011)	A , B (4-10)	0, 7	14-16	M	2×10^6(IV, 0, 7)

Kavanagh[19] (2010)	B (>15)	0, 7, 14	14, 25-27	M	0.5×10^6 (IV, 7, 14)
Lathrop[38] (2014)	A (16-24)	0, 7	14-16 ± 76-78	M	1×10^6 (IV, 14 ± 76)
Lee[20] (2011)	B (6)	1-5	9-11	M	1×10^5 (IV, 8)
Lin[39] (2015)	C (6)	1, 8	15, 22, 29, 36, 43, 50, 56	M	1×10^6 (IV, 22, 29)
Mariñas-Pardo[40] (2014)	B (5-8)	none	×3 weekly for 4 or 6 weeks	M	3×10^5 (IV, 28)
Mathias[41] (2013)	B (9-15)	0	8-10	H	1×10^6 (IV, 5-7)
Mohammadian[42] (2016)	B (6)	0, 14	×3 weekly from day 21 to 74	M	1×10^6 (IV, 67)
Mohammadian[43] (2016)	B (4)	0, 14	×3 weekly from day 21 to 74	M	1×10^6 (IV, 67)
Nemeth[21] (2010)	A (4-9)	0, 5	14 (IT), 15 (IN)	M	0.75×10^6 (IV, 14)

Ogulur[44] (2014)	B (4)	0, 14, 21	×2 weekly for 12 weeks starting on day 26	M	2.5×10^5 (IV, 104)
Ogulur[45] (2014)	B (4)	0, 7	21-28	M	5×10^5 (IN, 28)
Ou-Yang[46] (2011)	A (10)	0, 14	Every day for 4 weeks starting on day 21	M	4×10^6 (IV, 4 weeks before sensitization)
Park[23] (2010)	B (25)	1, 14	21-23	M	2×10^6 (IV, 18-20)
Sun[47] (2012)	B (4-6)	1, 3, 5, 7, 9, 11, 13	21-27	H	1×10^6 (IV, 0, 20)
Tang[48] (2016)	B (6)	1, 7, 14	21-25	H	1×10^6 (IV, 20)
Wang[49] (2013)	B (6)	0, 14	28	M	1×10^6 (IV, 35)
Zeng[50] (2015)	B (6-8)	0, 7	17-19	M	1×10^6 (IV, 16)

A, C57BL/6 mice; B, BALB/c mice; C, Sprague-Dawley (SD) rats; M, murine MSCs; H,

human MSCs; IV, Intravenous; IN, intra-nasal; IT, intra-tracheal administration.

Abstract

Effects of human mesenchymal stem cells in asthmatic airway inflammation

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Background: Asthma is a chronic inflammatory disorder of the airways characterized by variable airflow limitation and airway hyperresponsiveness. The therapeutic effects of human mesenchymal stem cells derived from umbilical cord blood (UCB-MSCs) or umbilical cord (UC-MSCs) on asthma are not clearly understood. This study aims to evaluate the effect of UCB-MSCs and UC-MSCs on allergic airway inflammation in experimental animal models.

Methods: Six-week-old female BALB/c mice were used in the following two groups according to the timing of MSC administration: the preventive MSCs group (UCB-MSCs

intravenous injection before the ovalbumin (OVA) challenge) and the inhibitory MSCs group (UC-MSCs intravenous injection during the OVA challenge). Each group was evaluated for airway hyperresponsiveness, histological changes, cytokines, allergen-specific antibodies, and population of innate and adaptive immune cells. Furthermore, *in vitro* experiments revealed the effect of UC-MSCs treatment on macrophages from bronchoalveolar lavage (BAL) fluid, which were stimulated with interleukin (IL)-4/IL-13. Transwell assays were performed to determine the paracrine effect of UC-MSCs promoting macrophage polarization.

Results: Intravenous administration of UCB-MSCs significantly reduced airway hyperresponsiveness to methacholine and eosinophil counts in BAL cells. Treatment with UCB-MSCs resulted in a significant reduction in airway inflammation, mucus production, and goblet cell hyperplasia. There was a significant decrease in serum OVA-specific immunoglobulin E (IgE) and IgG1 levels, along with T helper 2 (Th2) cytokine production (IL-4, IL-5, and IL-13) in the lung and spleen tissues, whereas an increased percentage of regulatory T (Treg) cells was observed after intravenous administration of UCB-MSCs. Intravenous UC-MSCs treatment also significantly decreased the numbers of type 2 and type 3 innate lymphoid cells, dendritic cells, and macrophages in the lungs of asthmatic mice. Alveolar macrophages ($CD11c^{+}CD11b^{-}$) and resident alveolar macrophages ($SiglecF^{+}CD11c^{+}CD11b^{-}$) were prominently increased after UC-MSCs treatment. However, the number of interstitial macrophages ($CD11b^{+}$) and their subtypes, including M1 ($CD206^{-}CD86^{+}$) and M2 ($CD206^{+}CD86^{-}$) macrophages, was significantly decreased after intravenous UC-MSCs treatment. Of the two subsets, the number of M2 macrophages was more effectively reduced.

IL-4- and IL-13-treated M2 expression (CD206) was reduced after UC-MSCs treatment *in vitro*. Similar results were observed through paracrine action.

Conclusion: The results of this study suggest that UCB-MSCs and UC-MSCs exert cross-species anti-inflammatory activity, which might be mediated by a Th1/Th2 shift, Treg cell induction, and macrophage switching. UCB-MSCs and UC-MSCs not only affect adaptive immunity but also innate immunity. Preclinical findings may provide important clues to support further UCB-MSCs and UC-MSCs research for the development of a new asthma treatment strategy.

Keywords: asthma, mesenchymal stem cells, umbilical cord, umbilical cord blood, T cells, macrophages.

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