

ORIGINAL PAPER

doi: 10.5455/medarh.2021.75. 256-261

MED ARCH. 2021 AUG; 75(4): 256-261

RECEIVED: JUN 28, 2021

ACCEPTED: JUL 30, 2021

<sup>1</sup>Departement of Doctoral Degree Program, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia

<sup>2</sup>Departement of Otorhinolaryngology-Head and Neck Surgery, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia

<sup>3</sup>Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Medan, Indonesia

<sup>4</sup>Departement of Doctoral Degree Program, faculty of medicine, universitas sumatera utara, Medan, Indonesia

<sup>5</sup>Departement of Otorhinolaryngology-Head and Neck Surgery, Faculty of Medicine, Universitas Sumatera Utara, Medan, Medan, Indonesia

<sup>6</sup>Pusat Unggulan Inovasi (PUI) Stem Cell, Universitas Sumatera Utara (USU), Medan, Indonesia

<sup>7</sup>Stem Cell and Cancer Research (SCCR), Medical Faculty, Sultan Agung Islamic University (UNISSULA), Semarang, Indonesia

<sup>8</sup>Department of Pathology, Medical Faculty, Sultan Agung Islamic University (UNISSULA), Semarang, Indonesia

<sup>9</sup>Department of Postgraduate Biomedical Science, Medical Faculty, Sultan Agung Islamic University (UNISSULA), Semarang, Indonesia.

<sup>10</sup>Departement of Otorhinolaryngology-Head and Neck Surgery, Faculty of Medicine, Universitas Padjadjaran/ Hasan Sadikin General Hospital, Bandung, Indonesia

<sup>11</sup>Departement of Otorhinolaryngology-Head and Neck Surgery, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia

<sup>12</sup>Departement of Doctoral Degree Program, faculty of medicine, universitas sumatera utara, Medan, Indonesia

<sup>13</sup>Department of Pharmacology and Therapeutics, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia

<sup>14</sup>Pharmacy Study Program, Faculty of Mathematics and Natural Sciences, Universitas Negeri Semarang, Semarang, Indonesia

**Corresponding author:** Dr. dr. Agung Putra, M.Si Med., Stem Cell and Cancer Research (SCCR), Faculty of Medicine, Sultan Agung Islamic University (UNISSULA), Semarang, Central Java, Indonesia. Address: , Jl. Raya Kaligawe Km. 4 Semarang, Jawa Tengah, Indonesia, 50112. Phone: +62 8164251646. E-mail: dr.agungptr@gmail.com. ORCID ID: <https://orcid.org/0000-0003-4261-9437>

© 2021 Lia Restimulia, Syafruddin Ilyas, Delfitri Munir, Agung Putra, Teti Madiadipoera, Farhat Farhat, Rosita Juwita Sembiring, Muhammad Ichwan, Nur Dina Amalina, Iffan Alif

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

# The CD4+CD25+FoxP3+ Regulatory T Cells Regulated by MSCs Suppress Plasma Cells in a Mouse Model of Allergic Rhinitis

Lia Restimulia<sup>1,2</sup>, Syafruddin Ilyas<sup>3</sup>, Delfitri Munir<sup>4,5,6</sup>, Agung Putra<sup>7,8,9\*</sup>, Teti Madiadipoera<sup>10</sup>, Farhat Farhat<sup>11</sup>, Rosita Juwita Sembiring<sup>12</sup>, Muhammad Ichwan<sup>13</sup>, Nur Dina Amalina<sup>7,14</sup>, Iffan Alif<sup>7</sup>

## ABSTRACT

**Background:** Allergic Rhinitis (AR) is the most common immunological disease that has been associated with inflammatory responses and is characterized by sneezing. Previous studies found that AR's allergen exposure significantly induces plasma cells and reduces regulatory T (Treg) cells, a population that contributes to control AR. Therefore, upregulating Treg expression can regulate plasma cells leading to inhibit sneezing in AR. Mesenchymal stem cells (MSCs) are multipotent stem cells that have the immunoregulatory and antiinflammation ability by secreting various cytokines including IL-10 and TGF- $\beta$  which potent as a promising therapeutic modality for allergic airway diseases, including AR. **Objective:** To investigate the role of MSCs in generating CD4+, CD25+, and Foxp3+ Regulatory T cells associated with suppressing plasma cell in AR model. **Methods:** In this study, fifteen male Wistar rats (6 to 8 weeks old) were randomly divided into three groups (control group, sham group, and MSCs treatment group). OVA nasal challenge was conducted daily from day 15 to 21, and MSCs ( $1 \times 10^6$ ) were administrated intraperitoneally to OVA-sensitized rats on day 21. Sneezing was observed from day 22 to 28. The rats were sacrificed on day 22 and day 28. The expression of CD4+ CD25+ Foxp3+ in Treg and plasma cells was analyzed by flow cytometry assay. **Results:** This study showed that the percentage of plasma cell and sneezing times significantly decreased in MSCs treatment. This finding was aligned with the significant increase of CD4+CD25+Foxp3+ Treg level. **Conclusion:** MSCs administration suppress plasma cells population and sneezing times by up regulating Treg to control AR.

**Keywords:** Allergic Rhinitis, Mesenchymal Stem Cells, Plasma Cell, T regulator, Sneezing.

## 1. BACKGROUND

Allergic rhinitis (AR) is a non-infectious chronic inflammatory disease of the nasal mucosa characterized by nasal obstruction, paroxysmal sneezing and rhinorrhea (1, 2). AR is a global health problem in both children and adults, affecting nearly 30% population of the world, with symptoms regularly increasing, its severity varies constantly, which makes it difficult to gauge (3). In the first line therapy of AR, corticosteroids,  $\beta_2$ -adrenergic receptor agonist, and antihistamines temporarily inhibit immune cells and inflammatory mediators (4). However, long-term therapy causes several negative side effects including drug resistance and intolerance. Therefore, a novel therapeutic strategy of AR is urgently needed. Recently, mesenchymal stem cells (MSCs) have been receiving increased attention for their immunomodulatory and anti-inflammatory properties in controlling allergic diseases (5), indicating MSCs as a promising approach to control AR.

MSCs are ubiquitous fibroblast like-multipotent cells which possess immunomodulatory capability, as well as capable to differentiate into specific somatic cell lineages, such as osteocytes, adipocytes, chondrocytes and neural cells (6). These cells could be characterized by high level of surface antigens, such as CD90, CD105, CD73, CD29 and low level of CD31, CD34, CD45, CD14, CD19 and HLA-DR (7, 8). The immunomodulatory properties of MSCs are supported by various specific mechanisms, such as the activation and generation of Treg cells followed by the release of several anti-inflammatory cytokines, including TGF- $\beta$  and IL-10 (9, 10). Recent studies

revealed growing evidence regarding immunodeficiency of Treg cells as the main factor of the Th2 cells superiority in AR, characterized by the overactivation plasma cells (11). The Th2-released cytokines can maintain the inflammatory milieu to further irritate the nasal mucosa leading to a series of allergy symptoms (12). In contrast, several anti-inflammatory cytokines released by MSCs, including TGF- $\beta$  and IL-10 can promote the development of Treg cells (9, 13). Thus, restoring Treg generation by MSCs could potentially regulate the excessive immune activation in AR.

AR is caused by release of interleukin (IL)-4, IL-5, and IL-3 by CD4+ Th2 effector cell in response to harmless environmental antigens (14, 15). The T cells-released cytokine that promoted immunoglobulin E (IgE) production by plasma cells which could activate mast cells leading to a degranulation response and the secretion of allergic mediators, followed by acute or chronic inflammation of the nasal mucosa during AR (16). Recently, regulatory T cells (Treg) were identified as being essential for immune tolerance (17). In addition, Treg cells play important role as immunotherapy targets in AR sensitization phase by suppressing the inflammatory response and controlling acquired immunity by suppressing the response of effector T cells, B cells, eosinophils and mast cells (18, 19). A previous study reported that MSCs could potentially induce Treg cell phenotype through suppressing proinflammatory T cell subset differentiation. The suppressive mechanism of MSCs may occur through the release of IL-10 and TGF- $\beta$  that regulated Treg cell associated with Th1 and Th2 cells immune response (20-22). On the other hand, plasma cell is one of the important targets for immunomodulation in immunotherapy (23). Several studies reported that after immunotherapy administration significantly increase level of memory B cells (24). Another study also reported that changes in plasma-cell subpopulations after immunotherapy treatment, mainly in circulating inflammatory plasma-cells that affect the response to the allergen in AR (25). All of this mechanism suggested that MSCs have anti-inflammatory and immunomodulatory properties to control allergic diseases, including in AR. Therefore, exploring the role of MSCs in regulating Treg cell associated with suppressing plasma cell is crucial to the future management of AR. However, the mechanism of MSCs in AR especially regulate Treg and plasma cell still unclear.

## 2. OBJECTIVE

The aim of this study was to analyzed the role of MSCs in regulating Treg and plasma cells in AR mouse model.

## 3. MATERIAL AND METHODS

### MSCs culture and isolation

Rat MSCs were isolated from a 19-days pregnant female rat. Briefly, donor rats were anesthetized, and the abdomens were dissected out. Under sterile conditions, the umbilical cord (UC) was collected and washed in phosphate-buffered saline (PBS). The UC artery and vein were removed, then the UC was cut into lengths of

2–5 mm using a sterile scalpel. The sections were then distributed evenly in T25 flasks using Dulbecco's modified Eagle's medium (DMEM) (Sigma-Aldrich, St. Louis, MO) supplemented with 10% PBS, 100 IU/ml penicillin/streptomycin (GIBCO, Invitrogen), then incubated at 37 °C with 5% CO<sub>2</sub>. The medium was renewed every 3 days, and the cells were passaged after reaching 80% confluence. UC-MSCs at passages 4-6 were employed for the following experiments (9, 26).

### MSCs In-vitro osteogenic and adipogenic differentiation assay

The MSCs were grown in a 24 well plate (1.5 x 10<sup>4</sup> cells/well) with a standard medium containing DMEM (Sigma-Aldrich, Louis St, MO), enriched with 10% FBS (Gibco™ Invitrogen, NY, USA) and 1% penicillin (100 U/mL)/streptomycin (100  $\mu$ g/mL) (Gibco™ Invitrogen, NY, USA) at 37 °C, 5% CO<sub>2</sub> and  $\geq$  95% humidity. After 80% confluent, the osteogenic and adipogenic protocol was initiated. For osteogenic differentiation, the standard medium was aspirated and replaced with an osteogenic differentiation medium containing Human MesenCult™ Osteogenic Differentiation Basal Medium (Stem Cell Technologies, Singapore), augmented with 20% Human MesenCult™ Osteogenic Differentiation 5X Supplement (Stem Cell Technologies, Singapore) and 1% L-Glutamine (Gibco™ Invitrogen, NY, USA). The differentiation medium was renewed every 3 days. After bone matrix formation occurred, the osteogenic differentiation was visualized by staining with 1 ml of 2% alizarin red solution. For adipogenic differentiation, the growth medium was switched to Human MesenCult™ Adipogenic Differentiation Basal Medium (Stem Cell Technologies, Singapore). The medium was changed every other day and at day 35 the cultures were stained with Oil Red O and observed under microscope.

### Characterization of MSCs

MSCs were analyzed for specific surface markers expression by flow cytometry. Briefly, the cultured cells were incubated in the dark with primary antibodies mouse anti-human CD29, mouse anti-human CD90, and mouse anti-human Lin negative (CD45/CD31) followed by secondary conjugated antibody. MSCs were stained with a specific antibody for 30 minutes at 4 °C, examined with a BD Accuri C6 Plus flow cytometer (BD Biosciences, San Jose, CA, USA) and analyzed with BD Accuri C6 Plus software (BD Biosciences, San Jose, CA, USA) (26).

### Animals

Fifteen male Wistar rat, which were 6 to 8 weeks old were purchased from local breeders (Semarang, Indonesia). They were raised in a controlled environment, with a regular 12-hour light-dark cycle and unrestricted access to OVA-free food and water. All the mice that were used in this study were handled according to a protocol, which was approved by Ethical Committee Universitas Sumatera Utara (142/KEP/USU/2020).

### Sensitization and challenge with OVA and MSCs administration

For the detailed experimental protocol, we followed the methods of (27) with slight modification. For the in-

duction of allergic asthma, the rats were first sensitized with an intraperitoneal (i.p.) injection of 1 mg of OVA (Sigma-Aldrich, St. Louis, MO, USA) and 2.25 mg aluminum hydroxide gel (alum adjuvant; Thermo Fisher Scientific, Waltham, MA, USA) in 100 µl of sterile saline on days 0, 5 and 10. After systemic sensitization, the mice were locally challenged by intranasal (i.n.) instillation of 50µg/10µL of OVA into their nostrils from days 15 to 21. Furthermore, after sensitization, on day 21 MSCs (1x10<sup>6</sup> cells) were administrated via intra peritoneal injection. The rats were terminated on day 1 and day 7 after MSCs administration (Figure 1).

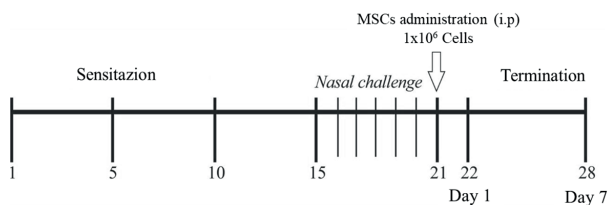


Figure 1. Implementation process of animal experimentation

**T-reg cell population analysis using flow cytometry**

After treatment, all of PBMCs were immunolabelled using antibodies against surface protein CD4, CD25 and intracellular protein FoxP3 according to the manufacturer’s instructions. Briefly, the cells were incubated with FITC- and PE-conjugated anti-human CD4 and CD25 respectively, for 30 min at room temperature in the dark. Then, the cells were washed with 1 ml staining buffer (BD Biosciences, San Jose, CA, USA) and fixed with fixation buffer (BD Biosciences, San Jose, CA, USA) for 10 min at room temperature. The cells were permeabilized using permeabilization buffer (BD Biosciences, San Jose, CA, USA) for 30 min at room. The PBMCs were rinsed again and stained with PE-conjugated an-

ti-human FoxP3 intracellular antibody (BD Biosciences, San Jose, CA, USA) for 30 min at room temperature in the dark. All data were collected on BD Accuri C6 Plus flow cytometer (BD Biosciences, San Jose, CA, USA) and analyzed using BD Accuri C6 Plus software (BD Biosciences, San Jose, CA, USA) (17).

**Plasma cell analysis using flow cytometry**

Blood was isolated using PBMC technique and counted as much as 1x10<sup>5</sup> cells / mL in PBS, then incubated using antibody Fluorescein isothiocyanate (FITC)-and Phycoerythrin (PE)-anti-human mouse CD45 5µl, CD20 5µl, CD38 5µl and CD138 5µl for 30 minutes on a dark condition. After incubation, cells were washed with 1000 µl PBS and centrifuged 800g for 5 minutes. Cells were resuspended in 300 µl PBS and analyses using flow cytometry (BD) Acurri C6 plus.

**Sneezing analysis**

Observation of clinical symptoms was carried out by 2 observers on the same subject for 10 minutes. Each mouse was put into a transparent cage, then labeled on each cage to determine the mouse code. Furthermore, the rats were observed sneezing behavior for 10 minutes of observation time (28).

**Statistical analysis**

Data were presented as the mean ± SD. The statistical significance of differences between the groups was examined on SPSS 23.0 (IBM Corp., Armonk, NY, USA) using ANOVA with post-hoc Fisher’s LSD analysis. p < 0.05 were considered significant.

**4. RESULTS**

**Characteristics of MSCs based on cell morphology, differentiation capacity and immunophenotypic profile**

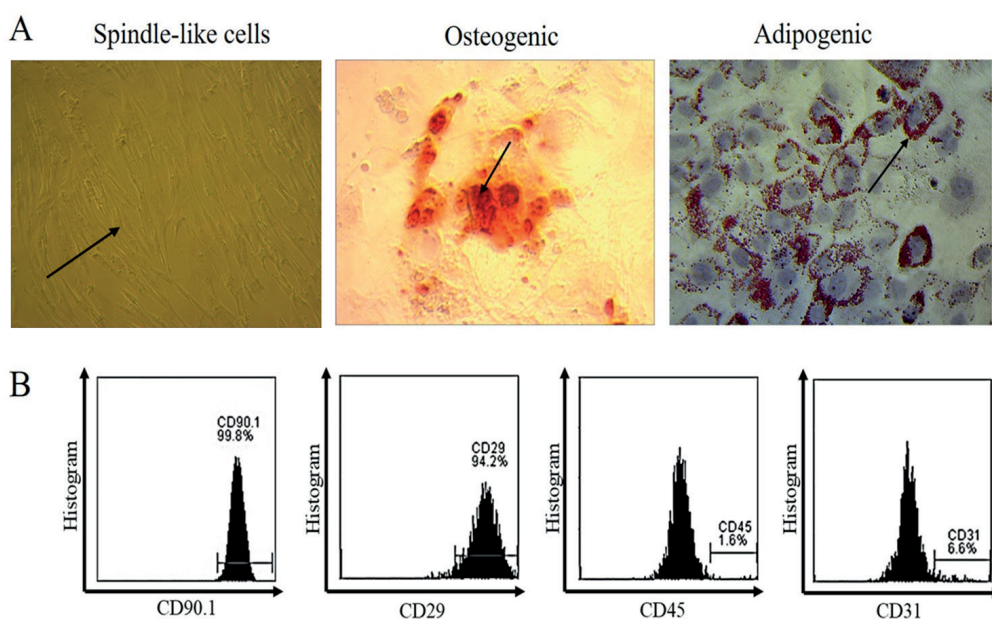
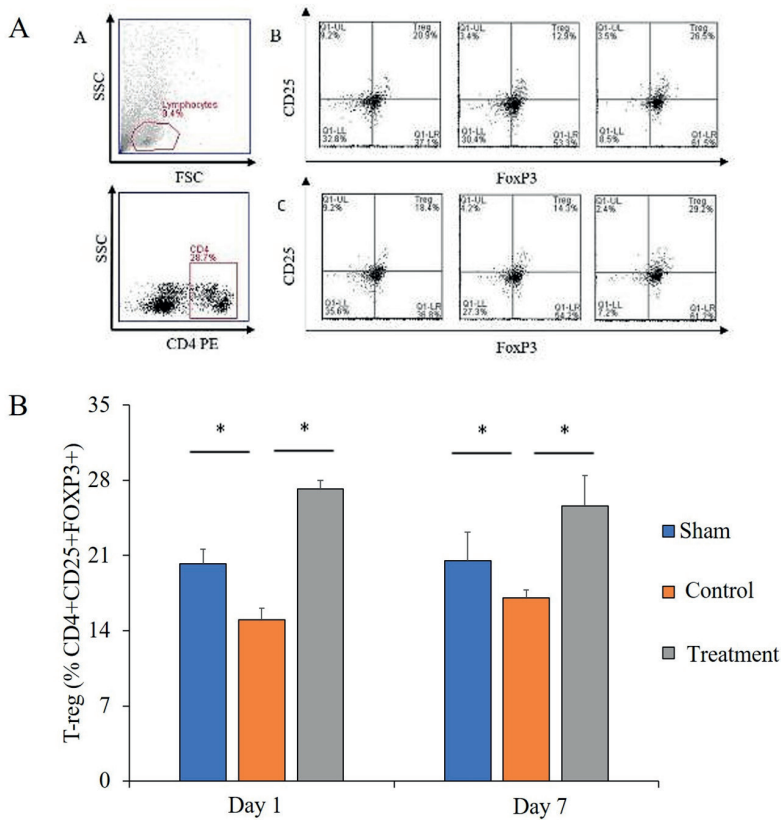


Figure 2. Characterization and validation of MSCs. (A) Morphological MSCs. The cells appeared as homogeneous spindle-shaped. Calcium deposition under osteogenic differentiation assay following Alizarin Red staining. accumulation of neutral lipid vacuoles that stained with Oil Red O (B) Graphs displayed the phenotype of MSCs: CD90 (99.8%), CD29 (94.2%), CD45 (1.6%), and CD31 (6.6.0%).





**Figure 3. MSCs induce Treg cell in AR (A) Flow cytometry gating strategy used to identify Treg cells, which were defined as CD4 CD25 FoxP3 and flow cytometry analysis of Treg cells in PMBC with AR and healthy control (sham). (B) These experiments were repeated three times (\*p < .005).**

MSCs were analyzed based on their plastic adherent capability under standard culture. After passage four, MSCs showed adhere cells with typical monolayers of spindle-shaped fibroblast-like cells (Figure 1A). To confirm the in vitro differentiation potential of MSCs, we used osteogenic and adipogenic differentiation media to evaluate that these MSCs can differentiate into osteogenic and adipogenic cells. We found a red color at osteogenic differentiation assay as calcium deposition indicating the MSCs differentiate to osteogenic (Figure 1A) and adipogenic differentiation was indicated by accumulation of neutral lipid vacuoles that stained with Oil Red O (Figure 2A). Immunophenotyping UC-MSCs using Flow cytometric analysis indicated that MSCs have positive for CD29 and CD 29, negative for CD45 and 31 (Figure 2B).

**MSCs administration enhance CD4+C-D25+Exop3+ Treg cell in AR rat model**

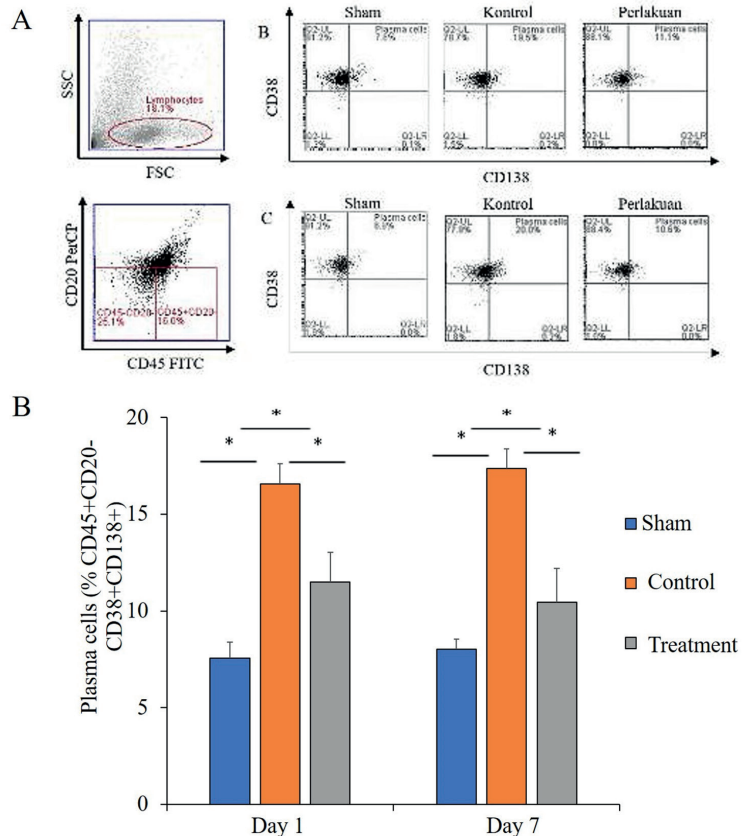
To evaluate the effects of administration of MSCs toward Treg expansion on AR rat model, we were determined using flow cytometry from PBMCs isolation. We found that both day 1 and day 7 significantly increased the ratio of CD4+CD25+Foxp3+ Tregs in the CD4+ subpopulation (p < .005) (Figure 3).

**MSCs administration reduce CD45+CD20-CD38 CD138 Plasma cell in AR rat model**

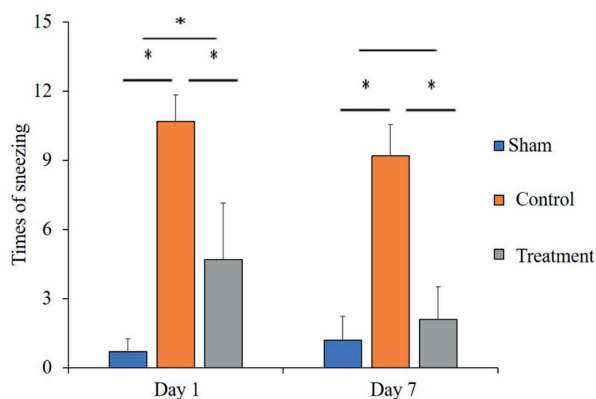
To evaluate the effects of administration of UC-MSC toward Plasma cells expansion on AR rat model, we were determined using flow cytometry from PBMCs isolation. We found that both day 1 and day seven significantly decrease the ratio of CD38+CD138+ Plasma in the CD45+CD20- subpopulation (p < .005) (Figure 4).

**MSC reduce sneezing in AR rat model**

To investigate the role of UC-MSCs in AR, we generate an AR rat model using administered OVA with aluminum hydroxide. We evaluated the intensity of sneezing after the last nasal challenge for 10 minutes (Figure 5). The results showed that the rats in the control group sneezed (10.7 ± 1.13) more frequently than treatment group (4.7 ± 1.13) in day one. there was a significant difference (p < 0.05). In the seven days showed that the rats in the control group sneezed (9.2 ± 1.13) more frequently than treatment group (2.1 ± 1.13). there was a significant difference (p < 0.05).



**Figure 4. MSCs decrease plasma cell in AR (A) Flow cytometry gating strategy used to identify plasma cells, which were defined as CD45+ CD20- CD38+ CD138+ and flow cytometry analysis of plasma cells in PMBC with AR and healthy control (sham). (B) These experiments were repeated three times (\*p < .005).**



**Figure 5. Intraperitoneal administration of MSCs reduced sneezing in treatment groups. \* indicate significant differences among the experimental groups at  $p < 0.05$ .**

## 5. DISCUSSION

Allergic rhinitis is a common inflammatory disease worldwide characterized by a well-defined phenotype, such as frequent sneezing, breathing difficulty, and increased airway resistance (29-31). Furthermore, a previous study reported that AR is correlated with the increased of B cells and plasma cells (32). Current therapies towards AR are limited to chemical drugs that alleviate allergic symptoms but fail to regulate the allergic reaction and occasionally have several side effects including drug resistance and intolerance (33, 34). Thus, finding alternative therapies to regulate allergic reactions without resulting side effects is needed. A previous study reported that mesenchymal stem cells (MSCs) possess immunomodulatory and anti-inflammatory capacities by regulating inflammatory cells including Treg and Plasma B Cell (35, 36). Furthermore, MSCs are considered good options for clinical therapies due to low immune rejection and are legally regulated (37). However, the role of MSC to control AR was unclear.

In this study, MSCs had significantly suppressed sneezing frequencies in the rhinitis rat model. The lower frequency of sneezing might be attributed to the reduced B cell plasma population. B cell plasma was crucial in allergy mechanisms, including AR (38). IgE-produced B cell plasma activates the production of histamine by activating cell mast (16, 38). This study revealed that MSCs administration suppressed B cell plasma population leading to the reduced histamine via inhibition of cell mast activation (36, 39). This finding suggests that MSCs may suppress histamine production which resulted in reduced sneezing frequencies.

Our study also reported the increased Treg cell population post MSCs administration. Treg was reported to have immunomodulation properties that are crucial in controlling allergies (40). Previous study revealed that the depletion of Tregs during the early phase of AR development resulted in a remarkable exacerbation of inflammation that was marked by frequent sneezing (13, 41). Treg as an inflammatory cell mainly releases regulatory cytokines including TGF- $\beta$ , IL-10, and IL-35 (17). Previous studies report that both TGF- $\beta$  and IL-10 played a critical role in immunosuppression through in-

hibiting B-cell proliferation (42, 43). Our findings suggest that the MSCs administration reduced B cell plasma population by up regulating Treg. However, in this study, the pathway regarding Treg reduced B cell plasma was not investigated and became this study limitation.

## 6. CONCLUSION

Our findings suggest that MSCs alleviate plasma cell-involved immune and inflammation response and augment production of Treg cells. Thus, MSCs become a therapeutic target for AR. Further, MSCs might be an attractive therapeutic target to treat Treg mediated allergic diseases.

- **Author's Contribution:** A.P and D.M.: conception, design, and manuscript writing; L.R: provision of study material; L.R and M.I: administrative support; N.D.A, L.R, S.I and T.M: provision of study material, data analysis and interpretation; F.F and R.J.S: conception and design; provision of study material and data analysis. All authors read and approved the final manuscript.
- **Conflicts of interest:** There are no conflicts of interest.
- **Financial support and sponsorship:** This work was carried out with the support of the "Stem Cell and Cancer Research (SCCR) Laboratory". The funding bodies have played role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.
- **Acknowledgements:** We thank the SCCR Laboratory of Sultan Agung Islamic University for finishing this study.

## REFERENCES

1. Prescott S, Allen KJ. Food allergy: Riding the second wave of the allergy epidemic. *Pediatr Allergy Immunol.* 2011; 22(2): 155-160.
2. Wesemann DR, Nagler CR. The Microbiome, Timing, and Barrier Function in the Context of Allergic Disease. *Immunity* [Internet]. 2016; 44(4): 728-738. Available from: <http://dx.doi.org/10.1016/j.immuni.2016.02.002>
3. Chong SN, Chew FT. Epidemiology of allergic rhinitis and associated risk factors in Asia. *World Allergy Organ J.* 2018; 11(1).
4. Akdis M, Akdis CA. Therapeutic manipulation of immune tolerance in allergic disease. *Nat Rev Drug Discov* [Internet]. 2009; 8(8): 645-660. Available from: <http://dx.doi.org/10.1038/nrd2653>
5. Li H, Tian Y, Xie L, Liu X, Huang Z, Su W. Mesenchymal stem cells in allergic diseases: Current status. *Allergol Int.* 2020; 69(1): 35-45.
6. Hocking AM, Gibran NS, Box C. Mesenchymal stem cells a paracrine signaling and differentiation during cutaneous wound repair. *Cell.* 2011; 316(14): 2213-2219.
7. Munir D, Lubis RR, Darlan DM, Putra A, Alif I. The role of mesenchymal stem cells in decreasing interleukin-12 human systemic lupus erythematosus. *Open Access Maced J Med Sci.* 2020; 8(A): 787-792.
8. Lv FJ, Tuan RS, Cheung KMC, Leung VYL. Concise review: The surface markers and identity of human mesenchymal stem cells. *Stem Cells.* 2014.
9. Darlan DM, Munir D, Putra A, Jusuf NK. MSCs-released TGF $\beta$ 1 generate CD4+CD25+Foxp3+ in T-reg cells of hu-

- man SLE PBMC. *J Formos Med Assoc* [Internet]. 2021; 120(1): 602-608. Available from: <https://doi.org/10.1016/j.jfma.2020.06.028>
10. Sungkar T, Putra A, Lindarto D, Sembiring RJ. Intravenous Umbilical Cord-derived Mesenchymal Stem Cells Transplantation Regulates Hyaluronic Acid and Interleukin-10 Secretion Producing Low-grade Liver Fibrosis in Experimental Rat. *Med Arch*. 2020; 74(3): 177-182.
  11. Zaretsky AG, Konradt C, Dépis F, Wing JB, Atria DG, Silver JS, et al. T Regulatory Cells Support Plasma Cell Populations in the Bone Marrow. *Cell Rep*. 2017; 18(8): 1906-1916.
  12. Ryu G, Kim DW. Th2 inflammatory responses in the development of nasal polyps and chronic rhinosinusitis. *Curr Opin Allergy Clin Immunol*. 2020; 20(1): 1-8.
  13. Palomares O, Martín-Fontecha M, Lauener R, Traidl-Hoffmann C, Cavkaytar O, Akdis M, et al. Regulatory T cells and immune regulation of allergic diseases: Roles of IL-10 and TGF- $\beta$ . *Genes Immun*. 2014; 15(8): 511-520.
  14. Saggini A, Maccauro G, Tripodi D, De Lutiis MA, Conti F, Felaco P, et al. Allergic inflammation: Role of cytokines with special emphasis on IL-4. *Int J Immunopathol Pharmacol*. 2011; 24(2): 305-311.
  15. Georas SN, Guo J, De Fanis U, Casolaro V. T-helper cell type-2 regulation in allergic disease. *Eur Respir J*. 2005; 26(6): 1119-1137.
  16. Stephen j MT. IgE and mast cells in allergic disease. *Natl Institutes Heal*. 2016; 18(5): 693-704.
  17. Darlan DM, Munir D, Putra A, Jusuf NK. MSCs-released TGF $\beta$ 1 generate CD4+CD25+Foxp3+ in T-reg cells of human SLE PBMC. *J Formos Med Assoc* [Internet]. 2020; (xxxx): 1-7. Available from: <https://doi.org/10.1016/j.jfma.2020.06.028>
  18. Calzada D, Baos S, Cremades-Jimeno L, Cárdbaba B. Immunological mechanisms in allergic diseases and allergen tolerance: The role of Treg cells. *J Immunol Res*. 2018; 2018.
  19. Boonpiyathad T, Sözener ZC, Akdis M, Akdis CA. The role of treg cell subsets in allergic disease. *Asian Pacific J Allergy Immunol*. 2020; 38(3): 139-149.
  20. Kustiyah AR, Putra A, Nasihun T, Ramasamy R. The Normal Ratio of Th17 and Th1 Post-mesenchymal Stem Cells Coculture with PBMCs of Systemic Lupus Erythematosus Patients. 2021; 9: 169-176.
  21. Hirahara K, Nakayama T. CD4+ T-cell subsets in inflammatory diseases: Beyond the Th1/Th2 paradigm. *Int Immunol*. 2016; 28(4): 163-171.
  22. Kim Y, Lee S, Kim YS, Lawler S, Gho YS, Kim YK, et al. Regulation of Th1/Th2 cells in asthma development: A mathematical model. *Math Biosci Eng*. 2013; 10(4): 1095-1133.
  23. Hawker K. B cells as a target of immune modulation. *Ann Indian Acad Neurol*. 2009; 12(4): 221-225.
  24. Jiménez-Saiz R, Patil SU. The Multifaceted B Cell Response in Allergen Immunotherapy. *Curr Allergy Asthma Rep*. 2018; 18(12): 1-15.
  25. Fernández TD, Gómez E, Doña I, Campo P, Rondon C, Gonzalez M, et al. Differential Plasma-cell evolution is linked with Dermatophagoides pteronyssinus immunotherapy response. *Sci Rep*. 2015; 5(September): 1-10.
  26. Hamra NF, Putra A, Tjipta A, Amalina ND, Nasihun T. Hypoxia mesenchymal stem cells accelerate wound closure improvement by controlling  $\alpha$ -smooth muscle actin expression in the full-thickness animal model. *Open Access Maced J Med Sci*. 2021; 9: 35-41.
  27. Jang TY, Jung AY, Kim YH. Hormetic Effect of Chronic Hypergravity in a Mouse Model of Allergic Asthma and Rhinitis. *Sci Rep* [Internet]. 2016;6(May):1-9. Available from: <http://dx.doi.org/10.1038/srep27260>
  28. Ryu G, Bae JS, Kim JH, Kim EH, Chung YJ, Mo JH. Sneezing and rubbing counts in allergic rhinitis mouse models are a reliable indicator of type 2 immune response. *Clin Exp Otorhinolaryngol*. 2020; 13(3): 308-311.
  29. Savi E, Peveri S, Capelli O. Allergic Sensitization in Rhinitis and Asthma. *Prim Care Pract-Integr is Needed*. 2016;
  30. Varshney J, Varshney H. Allergic rhinitis: An overview. *Indian J Otolaryngol Head Neck Surg*. 2015; 67(2): 143-149.
  31. Sin B, Togias A. Pathophysiology of allergic and nonallergic rhinitis. *Proc Am Thorac Soc*. 2011; 8(1): 106-114.
  32. Luo J, Guo H, Liu Z, Peng T, Hu X, Han M, et al. Analysis of peripheral B cell subsets in patients with allergic rhinitis. *Allergy, Asthma Immunol Res*. 2018; 10(3): 236-243.
  33. Aaronson DW. Side effects of rhinitis medications. *J Allergy Clin Immunol*. 1998; 101(2 Suppl.): 379-382.
  34. May JR, Dolen WK. Management of Allergic Rhinitis: A Review for the Community Pharmacist. *Clin Ther* [Internet]. 2017; 39(12): 2410.2419. Available from: <http://dx.doi.org/10.1016/j.clinthera.2017.10.006>
  35. Cho KA, Lee JK, Kim YH, Park M, Woo SY, Ryu KH. Mesenchymal stem cells ameliorate B-cell-mediated immune responses and increase IL-10-expressing regulatory B cells in an EB13-dependent manner. *Cell Mol Immunol* [Internet]. 2017; 14(11): 895-908. Available from: <http://dx.doi.org/10.1038/cmi.2016.59>
  36. Fan L, Hu C, Chen J, Cen P, Wang J, Li L. Interaction between mesenchymal stem cells and B-cells. *Int J Mol Sci*. 2016; 17(5).
  37. James A. Ankrum, Joon Faii Ong JMK. Mesenchymal stem cells: immune evasive, not immune privileged. 2015; 32(3): 252-260.
  38. Saunders SP, Ma EGM, Aranda CJ, Curotto de Lafaille MA. Non-classical B Cell Memory of Allergic IgE Responses. *Front Immunol*. 2019; 10(April): 715.
  39. Glenn JD. Mesenchymal stem cells: Emerging mechanisms of immunomodulation and therapy. *World J Stem Cells*. 2014; 6(5): 526.
  40. Zhang H, Kong H, Zeng X, Guo L, Sun X, He S. Subsets of regulatory T cells and their roles in allergy. *J Transl Med*. 2014; 12(1): 1-11.
  41. Robinson DS, Larché M, Durham SR. Tregs and allergic disease. *J Clin Invest*. 2004; 114(10): 1389-1397.
  42. Komai T, Inoue M, Okamura T, Morita K, Iwasaki Y, Sumitomo S, et al. Transforming growth factor- $\beta$  and interleukin-10 synergistically regulate humoral immunity via modulating metabolic signals. *Front Immunol*. 2018; 9(Jun):1-15.
  43. Molnarfi N, Bjarnadóttir K, Benkhoucha M, Juillard C, Lalive PH. Activation of human B cells negatively regulates TGF- $\beta$ 1 production. *J Neuroinflammation* [Internet]. 2017; 14(1): 1-12. Available from: <http://dx.doi.org/10.1186/s12974-017-0798-5>