ORIGINAL ARTICLE

The role of mesenchymal stem cells in allergic rhinitis and its relationship with IL-10, plasma cells and regulatory T cells

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ABSTRACT

Aim Allergic rhinitis (AR) is an IgE-mediated inflammation of the nose. Regulatory T cells (Tregs), plasma cells and inflammatory cytokines have shown to play a critical role in allergic airway inflammation. The aim of the study was to investigate the role of mesenchymal stem cells (MSCs) in generating Treg cells and plasma cells associated with regulating interlukin-10 (IL-10) in AR model.

Methods Fifteen male Wistar rats (6 to 8 weeks old) were randomly divided into three groups (control group, sham group, and MSCs treatment group). Ovalbumin (OVA) nasal challenge was conducted daily from day 15 to 21, and MSCs ($1x10^6$) were administrated intraperitoneally to OVA-sensitized rats on day 21. Sneezing was observed from day 24 to 27. The rats were sacrificed on day 24 and day 27. The expression of Treg and plasma cells was analysed by flow cytometry assay. The level of IL-10 was analysed under ELISA assay.

Results This study showed that the percentage of sneezing and rubbing times significantly decreased in MSCs treatment associated with the regulation of IL-10 level and plasma cell. This finding was aligned with the significant increase of Treg level.

Conclusion MSCs administration regulates IL-10 and plasma cell-mediated immune and inflammatory responses while increasing Treg cell production. MSCs may be a promising therapeutic target for treating Treg-mediated allergic diseases.

Key words: allergic rhinitis, mesenchymal stem cells, regulatory T cells, IL-10

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INTRODUCTION

Allergic rhinitis (AR) is a an IgE-mediated chronic inflammation of the nose characterized by sneezing, rubbing, and rhinorrhoea (1,2). Allergic rhinitis is a common health problem both in children and adults (3). Nearly 30% of the world population suffers from this disease (4). Cytokine interleukin-10 (IL-10) plays a central role in the pathogenesis of AR (5). IL-10, which is mainly produced by regulatory T cells (Treg) leading to suppressed allergic inflammation through inhibition of many proinflammatory cytokines, and prolongs a lifespan of B cells (6,7). In addition, Treg produced transforming growth factor (TGF-ß) suppresses the production of IgE on allergic (8). Recently, regulatory T cells (Treg) were identified as being essential for immune tolerance (9). In addition, Treg cells play an 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 (10,11). Recently, the first line therapy of AR temporarily inhibit immune cells and inflammatory mediators (12). Therefore, a novel therapeutic strategy of AR is urgently needed.

Mesenchymal stem cells (MSCs) are multilineage stem cells that can be isolated from umbilical cord that has promising therapeutic strategy to many inflammatory diseases including AR (9,13–16). The immunomodulatory properties of MSCs are supported by various specific mechanisms, such as MSCs leading to a shift from Th2 to Th1 responses in AR and being able to regulate the functions of regulatory Tregs as well (17,18). Indeed, the MSCs can target several sub-sets of lymphocytes, including CD4+ Th cells, CD8+ cyto-toxic T-lymphocytes (CTLs), natural killer (NK) cells, NKT cells, and B cells (19). In addition, several anti-inflammatory cytokines released by MSCs, including TGF-β and IL-10 can promote the development of Treg cells (16,20). Controlling inflammation to prevent prolonged inflammation in AR cases has shown to increase the effectiveness of the treatment (21,22). Thus, restoring Treg generation by MSCs could potentially regulate the excessive immune activation in AR (23,24). All these mechanisms suggested that MSCs have anti-inflammatory and immunomodulatory properties to control allergic diseases, including in AR. Therefore, exploring the role of MSCs in regulating cytokine anti-inflammation IL-10, Treg cell, plasma cell, and symptom nasal dan rubbing is crucial to the future management of AR. However, the mechanism of MSCs in AR is still unclear.

The aim of this study was to analyse the role of MSCs in regulating IL-10, Treg cell, plasma cell, and symptom nasal dan rubbing.

MATERIALS AND METHODS

Materials and methods

This study was conducted in Stem Cell and Cancer Research Indonesia in the period between February and July 2022. Fifteen male Wistar rat 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).

MSCs culture and isolation. Rat MSCs were isolated from a 19-day 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, USA) supplemented with 10% PBS, 100 IU/mL penicillin/streptomycin (GIBCO, Invitrogen, USA) then incubated at 37 °C with 5% CO_2. 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 (16,25).

Sensitization and challenge with OVA and MSCs administration. For the detailed experimental protocol, we followed the methods of (26) with slight modification. For the induction 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 ($1x10^6$ cells) were administrated via intraperitoneal injection. The rats were terminated on day 3 and day 6 after the MSCs administration.

IL-10 level analysis using ELISA. The plasma IL-10 levels were measured by ELISA (Bioenzy, SG) according to the manufacturer's protocols. The minimal detectable concentration was 1 pg/mL. All samples were detected in triplicate.

T-reg cell population analysis using flow cytometry. After the treatment, all peripheral blood mononuclear cell (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 phycoerythrin (PE)-conjugated anti-human FoxP3 intracellular antibody (BD Biosciences, San Jose, CA, USA) for 30 min at room temperature in the dark. All the data were collected on BD Accuri C6 Plus flow cytometer (BD Biosciences, San Jose, CA, USA) and analysed using BD Accuri C6 Plus software (BD Biosciences, San Jose, CA, USA) (9).

Plasma cell analysis using flow cytometry. Blood was isolated using PBMC technique and counted as much as 1x105 cells/mL in phosphate buffer saline (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 analysed 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 labelled on each cage to determine the mouse code. Furthermore, sneezing behaviour was observed in the rats for 10 minutes of observation time (27).

Rubbing 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 labelled on each cage to determine the mouse code. Furthermore, nasal rubbing behaviour was observed in the rats for 10 minutes of observation time

Statistical analysis

Data were presented as the mean±SD. The statistical significance of differences between the groups was examined using ANOVA with post-hoc Fisher's LSD analysis. The $p<0.05$ was considered significant.

RESULTS

IL-10 cytokine levels in the treatment group were detected as high in the day 3 compared to control group (Figure 1). As a result this condition was still in the inflammatory phase, however on day 6 IL-10 level began to decrease because not being in the inflammatory phase.

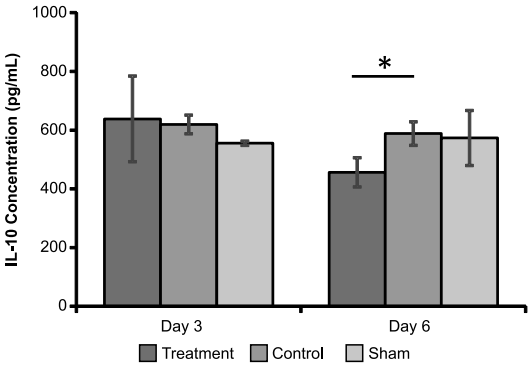

Figure 1. IL-10 levels induced by mesenchymal stem cells (MSCs) on day 3 in allergic rhinitis (AR) models. These experiments were repeated three times.
*p <0.005

To evaluate the effects of administration of MSCs toward Treg expansion on AR rat model, it was determined using flow cytometry from PBMCs

isolation. It was found that on day 3 the Tregs levels were still lower than sham groups, however in day 6 significantly increased the ratio of CD4+CD25+Foxp3+ Tregs in the CD4+ subpopulation (p<0.005) (Figure 2).

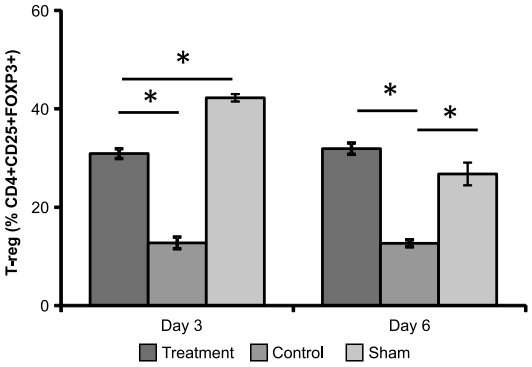

Figure 2. MSCs induce Treg cell in allergic rhinitis (AR) on day 6. Treg cells in peripheral blood mononuclear cell (PMBC) with AR and heathy control (sham). The experiments were repeated three times.
*p<0.005

For the evaluation of the effects of administration of UC-MSC toward plasma cells expansion on AR rat model, flow cytometry from PBMCs isolation was used. It was found that on day 3 the plasma cells tended to be the same, not significantly different. On day 6, a significant decrease the ratio of CD38+CD138+ plasma in the CD45+CD20- subpopulation was found (p<0.005) (Figure 3).

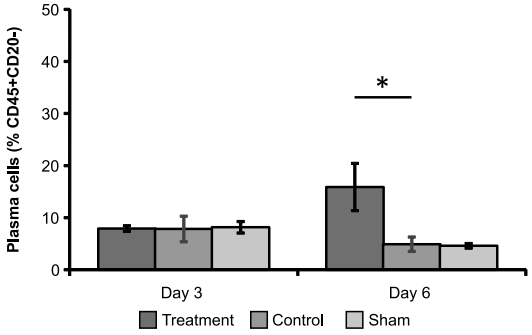

Figure 3. MSCs increase plasma cell in peripheral blood mononuclear cell (PMBC) with allergic rhinitis (AR) and heathy control (sham). The experiments were repeated three times.
*p<0.005

To investigate the role of UC-MSCs in AR, we generated an AR rat model using administered OVA with aluminum hydroxide in order to evaluate the intensity of sneezing and rubbing after the last nasal challenge for 10 minutes (Figure 4A). The results showed that the rats in the control group sneezed (9.31±0.13) more frequently than the treatment group (3.14±0.72) on day 3 (p<0.05). On day 6 the rats in the control group sneezed (7.56±1.67) more frequently than the treatment group (4.98±1.13) (p<0.05). Along with the rubbing, administering MSCs on days 3 and 6 was able to significantly reduce the number of nose rubs, 2.16±0.02 and 4.98±1.56 n/10 minutes, compared to the control group, 9.42±0.30 and 7.81± 0.16 n/10 minutes, respectively (Figure 4B). All experiments in these data were repeated three times.

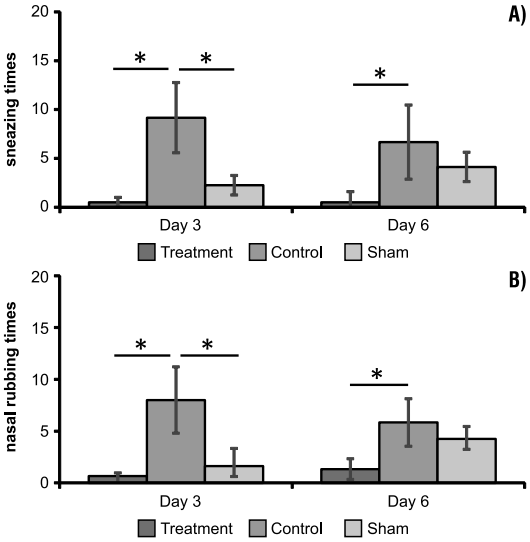

Figure 4. Intraperitoneal administration of mesenchymal stem cells (MSCs) reduced (A) sneezing and (B) rubbing in the treatment group.
*p<00.05

DISCUSSION

The capacity of MSCs that alter phenotype and function of immune cells largely attributes to the production of soluble factors (28,29). MSCs produce and release various soluble factors that are accountable for the immunosuppression function, including IL-10. In this study, IL-10 were used to determine Treg function (25, 30–32). Tregs responsible for enabling immune homoeostasis with immune regulatory mechanisms suppress cytokines having a role in Th1- and Th2-mediated responses and allergic immune responses (6,33). The IL-10 levels on day 6 were detected as lower than the control group. This finding is in line with a previous study which reported that serum IL-10 levels of AR models had been detected as lower than those of controls (34). Interestingly, our stu-

dy found the synergy of the level of IL-10 with Treg level, especially on day 6. In addition, the regulatory B cell-derived IL-10 has been described to promote IgG4 expression by B cells of allergic patients, and hints to a possible AR mechanism involving IL-10 apart from IgG4 upregulation (35). The previous study also reported that plasma cells mediate suppression of Th1 and Th17 differentiation by IL-10 secretion as shown in autoimmune disorders, including AR (36,37). Our findings suggest that the MSCs administration increased plasma cell population by upregulating Treg. It is obvious that Treg cell and cytokines have an important role in AR immunopathogenesis.

In this study, MSCs had significantly suppressed sneezing and rubbing frequencies in the rhinitis rat model. The lower frequency of sneezing and rubbing might be attributed to the increased Treg population (38,39). Treg was crucial in allergy mechanisms, including AR (40). Treg was reported to have immunomodulation properties that are crucial in controlling allergies (41). The 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 and rubbing (20,42). This finding suggests that MSCs may suppress histamine production which resulted in reduced sneezing and rubbing frequencies. Taken together, MSCs had immunosuppressive effect on activated Treg, which suggested different suppressor functions of MSCs according to the phases of diseases.

In conclusion, our findings suggest that MSCs regulate plasma cell-mediated immune and inflammatory responses while increasing Treg cell production. Furthermore, MSCs may be a promising therapeutic target for treating Treg-mediated allergic diseases.

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TRANSPARENCY DECLARATION

Conflict of interest: None to declare.

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