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Human Adipose Tissue-Derived Mesenchymal Stem Cells Alleviate Atopic Dermatitis via Decreased Serum Level of IgE and Number of Mast Cells

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Abstract

Atopic dermatitis (AD) is a major inflammatory skin disease. Most patients with atopic dermatitis have elevated serum levels of mast cells and IgE. Mesenchymal stem cell (MSC) has been applied for the therapy of allergic disorders due to its beneficial immunomodulatory abilities. We sought to investigate the safety and the efficacy of human adipose tissue-derived MSCs (hAT-MSCs) in mouse atopic dermatitis (AD) model and to determine the serum levels of mast cells (MC) and IgE after subcutaneous administration.

Keywords: Mesenchymal stem cells • Atopic dermatitis • Subcutaneous • IgE • Mast cells

Introduction

Mesenchymal stem cells (MSCs) possess immunomodulatory activities and have been known to interact with cell types of both innate and adaptive immune systems, which results in the immunosuppressive effects on T-cell proliferation, inhibition of dendritic cell function, suppression of B-cell proliferation, and immunomodulation of other immune cells such as natural killer (NK) cells and macrophages [1-4]. MSC commonly have anti-inflammatory properties, because of their ability to modulate immune responses [5-8]. MSC includes some stem cells with an inherent ability for self-renewal and differentiation potential for types of cell lineages, including adipocytes, osteocytes, chondrocytes, hepatocytes, neurons, epithelial cells, and muscle cells [9-11]. It appears that micro-environment parameters have a major impact on differentiation properties of MSC and the immunomodulatory activity that makes it attractive for the therapy of autoimmune and inflammatory diseases by subcutaneous infusion of these cells. Atopic dermatitis (AD) is a chronic and very common inflammatory skin disorder accompanied by xerosis, eczematous lesions and affects approximately 5-20% of children worldwide [12]. The pathogenesis of acute AD is associated with type 2 helper T cell (Th2)-dominant inflammatory responses, characterized by dermal infiltration of CD4+ T cells and eosinophils and increased levels of immunoglobulin E (IgE) and number mast cells (MC) also Th2 cytokines [13]. There are several treatment approaches for AD such as topical glucocorticosteroids, emollients, calcineurin inhibitors, and systemic immunosuppressants [14,15]. However, these drugs and therapies reduce inflammation, but they have been reported to carry the risk of side-effects [16-18]. Therefore, the development of new therapeutic approaches is necessary for AD treatment. Mesenchymal stem cells (MSCs) can be a promising candidate to replace current therapeutics because of their ability to modulate immune responses, MSCs can regulate multiple factors simultaneously in response to the treatment of patients with inflammation-related diseases such as multiple sclerosis (MS) [19]. Several recent studies have demonstrated that MSCs could suppress allergic responses in disease development AD [20,21]. A few recent studies revealed that the administration of MSCs could ameliorate symptoms of allergic disease AD in a mouse model through the regulation of MC de-granulation [22]. In this study, we hypothesized that MSCs exert their immunomodulatory effects on allergic inflammation in skin disorders. In the present study, we attempted to investigate the therapeutic efficacy of subcutaneously administered hAT-MSCs into 1-Fluoro-2,4-dinitrobenzene (DNFB)-induced AD mouse model to provide the evidence and the reference in the field of stem cell therapeutics for allergic diseases. We used adipose tissue in this study because adipose tissue is easily obtainable in sufficient quantities using a minimally invasive procedure and an attractive source of MSCs for stem cell therapy [23,24]. Furthermore, adipose tissues contain more MSCs than marrow stromal cells from bone marrow (BMMSC) (about 100,000 MSCs per gram of fat) [25]. The safety and efficacy of AdMSCs in the treatment of various diseases have been reported *in vivo* [26].

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Materials and Methods

Isolation and culture of hAT-MSCs

Human adipose tissues were obtained by simple liposuction from freshly excised human fat tissue with informed consent. Subcutaneous adipose tissues were digested with 0/1% collagenase (type I, Gibco) under gentle agitation for 30 minutes at 37°C. The digested tissues were filtered through a 100 µm nylon mesh to remove cellular debris and were centrifuged at 2,000 rpm for 10 min to obtain a pellet. The pellet was resuspended cells in DMEM (Invitrogen)-based media containing 0.2 mM ascorbic acid and 15% Fetal bovine serum (FBS). The cell suspension was re-centrifuged at 2,000 rpm for 10 min. The supernatant was discarded and the cell pellet was collected. The cell fraction was cultured overnight at 37°C 5% CO_2 in DMEM- based media containing 0,2mM ascorbic acid and 15% FBS. After 24h, the cell adhesion was checked under an inverted microscope, and non-adherent cells were removed by washing with phosphate-buffered saline (PBS). The cells were maintained for 2-3 days until confluent (passage 0). When the cells reached 90% confluency, they were used in this study. Given that hAT-MSCs isolated from 8 different donors were used after verification of characteristics for MSCs by observing the surface markers and differentiation capabilities.

Reagents

1- Fluoro-2, 4-dinitrobenzene (DNFB) from merckInc (Germany). N-terminal amino acids of proteins and peptides. PBS, PS, Fungizone, DMEM, FBS, L_Glutamine, Dispase, Trypsine, Collagenase, DMSO, ELISA kit, MTT assay kit, CD44, CD90, CD34, CD45, Hypotonic ammonium chloride, Sodium dodecyl sulfate, Osteoblast differentiation medium, Chondroblast differentiation medium, toluidine blue, H&E, Alizarin red, Ketamine, were purchased from sigma Aldrich.

Mice

NC/Nga mice (male, 8wk old) were used for the experiments. Animal care and all experimental procedures were approved by and followed the regulations of the Institute of Laboratory Animal Resources. They were obtained from Pasteur Institute and group housed in wire mesh cages under specific pathogen free conditions in the animal care facility.

AD model induction in NC/Nga mice

Atopic dermatitis (AD) was induced as previously method described with some modifications [27-29]. We used 8 mice per group. Briefly, the upper backs of the mice were shaved with a clipper. Sodium dodecyl sulfate (4%, 150 µl/head) was treated on the shaved dorsal skin including surfaces of ears to achieve skin barrier disruption. After 3-4 hours, in a volume of 150 µl DNFB (Merck, Inc.) was topically applied. 1-Fluoro-2,4-dinitrobenzene (DNFB) was treated 3 times a week for 3-week intervals (i.e., days 1, 3, 6, 9, 12, 15, 18, 21 and 24). Thereafter, hAT-MSCs ($0/75 \times 10^5$ or $0/375 \times 10^5$ cells/200 µl normal saline) were subcutaneously injected into mice on days 32, 40, 48, and 56 (Figure 1A) after sacrifice on day 64, sera and skin biopsy specimens were obtained to detect the concentration of total IgE using a commercial ELISA kit (Zell Bio) or to evaluate histopathological lesions, respectively. Mast cells in skins were determined by toluidine blue.

Image J analysis to assess lesions area

To investigate changes in lesions surface area, and measure the percentage of lesions reduction, on different days (8, 16, after treatment) during the treatment period, the surface of the lesions using Image J software with cm^2 unit. The surface of the lesions was first photographed with a digital camera under the same conditions, and then using image j software, the size of the lesions was calculated with high accuracy.

Histopathological evaluation

Skin samples were collected, fixed in 10% formalin followed by consecutive tissue processing steps including alcohol-xylene changes, and embedding in paraffin. Sections of 5 µm thicknesses were prepared and stained with H&E or toluidine blue. Mast cell infiltration was determined by toluidine blue staining.

Statistical analysis

Statistical analysis was performed using Prism5 and excels software (Microsoft Office 2013). For quantitative data analysis, One Way ANOVA in case of cluster comparison was applied. $P < 0.05$ was considered statistically significant.

Results

Sub-cutaneous administration of hAT-MSCs reduces the symptoms of DNFB- induced atopic dermatitis in mice

We first investigated whether the xenogeneic administration of hAT-MSCs could exert a therapeutic effect against DNFB-induced murine AD. To assess the therapeutic effects, two different doses (low dose: $0/375 \times 10^5$; high dose: $0/75 \times 10^5$) of hAT-MSCs were injected sub-cutaneously at day 32 when AD was fully induced (Figure 1A). Phosphate buffer saline (PBS) was infused as a cell control group. None of the mice that received hAT-MSCs showed any adverse events or lethality. Interestingly, subcutaneous administration of high dose hAT-MSCs significantly reduced the clinical severity of AD mice, whereas the low dose group showed their therapeutic effects over a longer period of time (Figure 1B).

The hAT-MSCs suppress AD in the mouse model

We aimed to exclude the xenogeneic rejection response in this AD mouse model. Therefore, we used human adipose tissue-derived mesenchymal stem cells. It is now generally accepted that MSCs are hypo immunogenic as they do not express MHC class II activity [30]. We used the DNFB-induced AD mice models that were subcutaneously sensitized with 150 µl of DNFB on days 1, 3, 6, 9, 12, 15, 18, 21 and 24. The hAT-MSCs ($0/375 \times 10^5$ or $0/75 \times 10^5$) were then subcutaneously injected 4 times for 4 weeks (Figure 1A). Decreased cell infiltration in the skin was observed in mice treated with both high dose and low dose hAT-MSCs compared with that in the PBS control group (Figure 1B). The severity score of skin lesions was significantly decreased by hAT-MSCs (Figure 1C). Mast cells were identified by toluidine blue. These cells were also significantly decreased by hAT-MSCs (Figures 2A and 2B). The total and DNFB- specific IgE levels were significantly decreased in sera of mice treated with both high dose and low dose hAT-MSCs. These results indicate therapeutic effects on AD by xenogeneic hAT-MSCs in two doses, high and low.

The hAT-MSCs decrease serum IgE concentration

We investigated whether hAT-MSCs directly decrease IgE concentration. To determine the serum immunoglobulin level after hAT-MSCs administration, serum IgE concentration was measured. The serum level of IgE was increased by AD induction and its level was significantly decreased by hAT-MSCs administration. This suppression could be due to the cell-cell contact (Figure 3A). However, PBS injection did not suppress the serum IgE level (Figure 3B). These results suggested that hAT-MSCs decrease IgE concentration.

Histopathological analysis of hAT-MSCs efficacy in AD mice

Histological evaluation using H&E staining revealed that the epidermal hyperplasia exerted by AD induction was attenuated by hAT-MSCs treatment in a dose-dependent manner. After completion of a 20-day observation period, lesions of each group were subjected to biopsy, fixed, and stained with hematoxylin and eosin. A decrease in the epidermal thickness and in the number of infiltrating cells in the dermis was observed only in the hAT-MSCs-treated skin areas (Figure 4). We next performed toluidine blue staining to determine the degranulation of MCs infiltrated in lesions. hAT-MSCs administration significantly reduced the number of mast cells (Figures 2A and 2B). These results indicate that histological as well as macroscopic improvement of AD was achieved by the subcutaneous administration of hAT-MSCs.

Taken together, our results indicate that the subcutaneously delivered hAT-MSCs exhibit a dose- dependent efficacy against DNFB- induced AD in both criteria of gross and histopathological evaluation, and that mechanisms regulation IgE production might be involved in this effect. For example, we can refer to cell- cell contact in this field.

Discussion

In the present study, we demonstrate for the first time that subcutaneous administration of xenogeneic hAT-MSCs can alleviate DNFB-induced AD in mice, probably by decreasing the number of mast cells (MCs) and the concentration of IgE. Several recent studies have shown that MSCs can prevent allergic airway inflammation [31-33]. Frequently, immunologic mechanisms of AD are characterized by dominant Th2-mediated abnormal inflammatory responses and elevated serum immunoglobulin E (IgE) and eosinophils [34,35]. MCs, as well as DCs, express FcεRI and specific IgE- bearing MCs. Called sensitized MCs, are abundant in AD lesion. Upon exposure to the specific allergen. IgE-mediated MC degranulation results in the release of performed inflammatory mediators, such as histamine, serotonin, PG and leukotrienes, which contribute to disease exacerbation through the itch-scratch cycle and inflammatory processes through the recruitment of eosinophils and lymphocytes into the dermis [36]. We showed in this study that xenogeneic subcutaneous administration of hAT-SCs into mouse models to be well-tolerated and sufficiently effective, we state precisely in this regard that hAT-

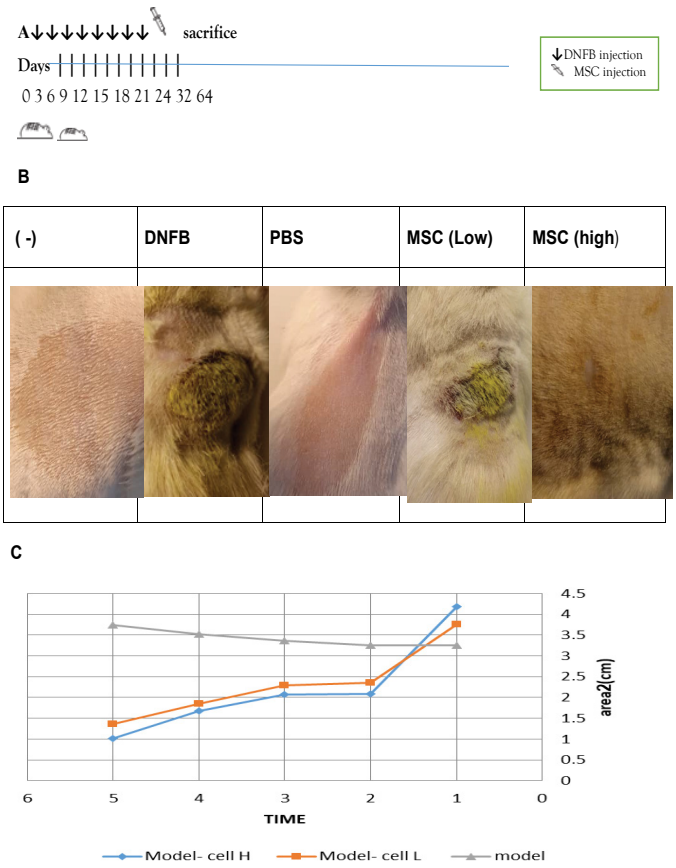

Figure 1. Therapeutic effect of S.C. injected hAT-MSCs in AD mice. (AD) Atopic dermatitis was induced by the repetitive application of 1-Fluoro-2,4-dinitrobenzene (DNFB) on day 24, after the onset of disease. Two different doses of hAT-MSCs or phosphate buffer saline (PBS) were injected subcutaneously (s.c). **(A)** Scheme of AD induction and cell injection. **(B)** Photographs of skin gross lesions were taken for pathological evaluation**. (C)** Lesions surface area on different days (8, 16, after treatment) during the recovery period on control and treatment groups.

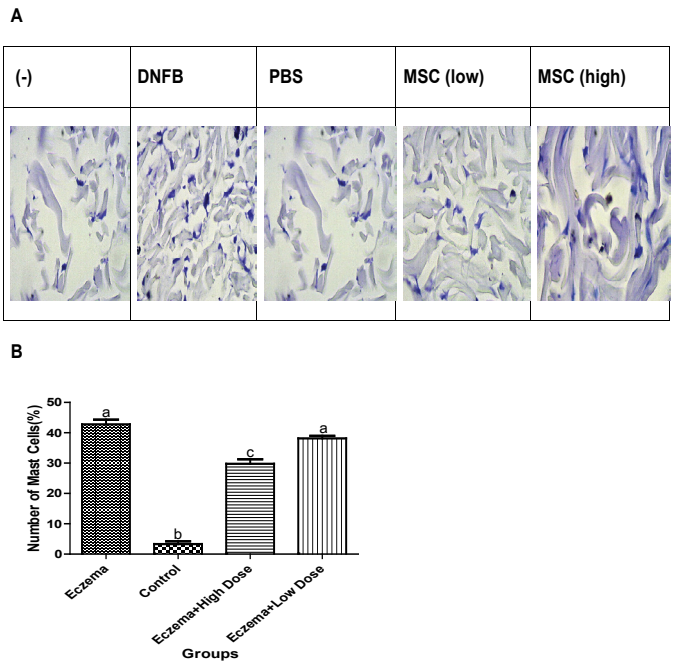

Figure 2. Histopathological analysis of hAT-MSC efficacy in AD mice. **(A)** Skin sections were stained with toluidine blue, scale bar = 200 μm and **(B)** The number of decreased mast cells was counted. The decrease in the number of infiltrating cells was observed only in the dermis of the hAT-MSCs-treated skin areas. Eight mice per group were used. *P<0.05, **P<0.01, ***P<0.001. Results are shown as mean ± SD.

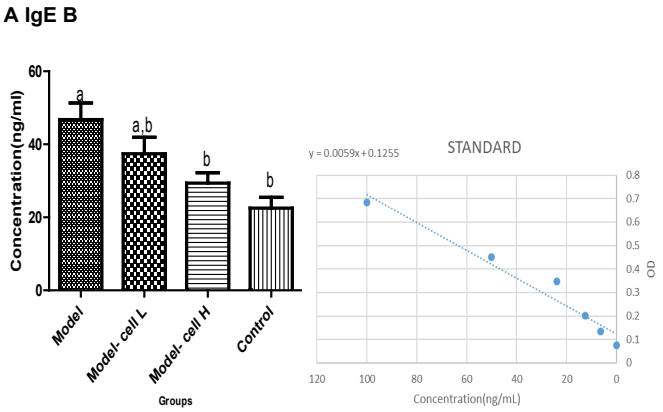

Figure 3. Decrease of IgE concentration by hAT-MSCs injection. To measure IgE concentration, on day 56, all mice were sacrificed for further analysis and serum level of IgE was measured by ELISA. **(A)** IgE concentration were significantly decreased by subcutaneously (s.c) administered with hAT-MSCs**. (B)** The total level of IgE was significantly decreased by both low dose and high dose of hAT-MSCs, but DNFB- specific IgE was not affected. Eight mice per group were used. *P<0.05, **P<0.01, ***P<0.001. Results are shown as mean ±SD.

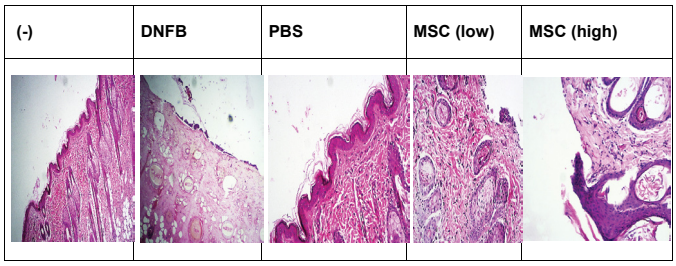

Figure 4. Hematoxylin and eosin (H&E) staining of mice treated with PBS or hAT-MSCs**.** Paraffin-embedded sections of skin tissue from AD mice were stained with hematoxylin and eosin, scale bar = 200 μm. Decreased cell infiltration in the skin was observed in mice treated with both low dose and high dose of hAT-MSCs compared with that in the PBS control group. H&E staining showed an improvement of acanthosis and a decrease in the number of invasive lymphocytes in the dermis only in the hAT-MSCs- treated group.

MSCs can favorably exert cross-species immunosuppressive effects. Studies in this field have also been conducted by other researchers [37]. Furthermore, we revealed that the subcutaneous administration of hAT-MSCs not only decreased the serum level of IgE but significantly reduced the number of mast cells (MCs). Scuderi et al. showed that subcutaneous injection of autologous AT-derived MSCs (AT-MSCs) with hyaluronic acid (HA) scaffold resulted in the significant improvement of skin symptoms in patients with SSc, and the number of passages for injected MSCs, was between 2 and 3 [38]. Previous studies showed that subcutaneously (SC) administered human UCB-derived MSCs (hUCB-MSCs) can effectively ameliorate the experimental mouse model of AD as well as psoriasis [39]. In addition, subcutaneous injection of allogeneic hUCB-MSCs represented promising clinical efficacy and safety in patients with moderate - to- severe AD [40]. When both high dose ($0/75 \times 10^5$) and low dose ($0/375 \times 10^5$) of hAT-MSCs were subcutaneously administered in DNFB-induced AD, cell infiltration into the skin lesions and the level of IgE production in sera were significantly decreased as compared with those observed in the PBS- treated control mice. Administration of the high dose of hAT-MSCs appeared to be better for the therapeutic effects of the AD than the low dose of hAT-MSCs. However, both doses of hAT-MSCs significantly decreased total IgE production in sera and number of mast cells (MCs). We demonstrate for the first time that subcutaneous administration of xenogeneic hAT-MSCsdid not exhibit any embolism-related, clumped cells-related symptoms, supporting the safety of hAT-MSCs. Our findings along with the human clinical trial data could open the door for the development of a new therapeutic strategy to treat AD patients.

Conclusion

MSC-based cell therapy has been spotlighted as a promising approach

for the treatment of inflammatory skin disorders, and relevant clinical trials are ongoing. Human AdMSCs can be isolated from small amounts of adipose tissue, efficiently expanded to achieve more than 10^9 cells after 3 to 4 passages independent on donor age and disease status. In this study, we proved that a subcutaneous administration of hAT-MSCs can be successfully used for the treatment of AD and is well tolerated without any safety issues.

Conflict of Interest

The authors declare that there is no conflict of interest.

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