IJBCS
ISSN: 2147-1428

Original Article

International Journal of Basic and Clinical Studies (IJBCS)
2019; 8(1): 46-60 Veranyurt U, Akalin B and Akkoc T

Immunoregulatory Effect of DF-MSCs Derived from Lymphocytes of Children on Patients with Asthma

Ulku Veranyurt[1], Betul Akalin[2], Tunc Akkoc[3]

[1]PhD Student, Department of Health Management, Institute of Health Sciences, Health Sciences University, Istanbul, Turkey
[2]Assistant Professor, Department of Health Management, Faculty of Health Sciences, Health Sciences University, Istanbul, Turkey
[3]Professor, Faculty of Medicine, Pediatric Allergy-Immunology, Marmara University, 12 Istanbul, Turkey

Abstract

Aim: This study aims to develop regenerative medicine and support the formation of new resources in terms of health tourism. We investigated the immunomodulatory effects of human dental follicle mesenchymal stem cells (hDF-MSCs) pre-stimulated with/without IFN-γ; in Derp $p1^+$ asthma patients lymphocytes in-vitro.
Materials and methods: Dental follicles were obtained from University of Marmara Faculty of Dentistry Oral and Maxillofacial Surgery Department. Dental follicle derived MSCs were isolated, characterized and differentiated into osteogenic, adipogenic, chondrogenic linageages. Peripheral blood mononuclear cells (PBMC) were isolated from healty (hPBMC) and asthma patients (aPBMC). They were cultured with/without hDF-MSCs. All cultures were stimulated with Derp1, CDmix pre and post stimulated IFN-γ. Lymphocyte proliferation, Annexin V/PI apoptosis and $CD4^+CD25^+FoxP3^+$ Treg ratio were analyzed after 72 hours of culture period.
Results: hDF-MSCs suppressed $CD4^+$T cell proliferation significantly ($p<0.05$). Previously stimulated hDF-MSCs with IFN-γ suppressed $CD4^+$T lymphocyte proliferation more than un-stimulated MSCs ($p=0.01$). hDF-MSCs increased Treg ratio significantly ($p<0.05$). IFN-γ -stimulation of hDF-MSCs enhanced Treg ratio compared to un-stimulated MSCs ($p=0.01$). Both IFN-γ stimulated and un-stimulated hDF-MSCs suppressed lymphocyte apoptosis significantly ($p<0.05$). In accordance with the results, it would be appropriate to use IFN-γ stimulated hDF-MSC's in the treatment of inflammatory and allergic diseases to modulate immune responses.
Key words: Dental Follicle Mesenchymal Stem Cells, Regenerative Medicine, Health Tourism, Immunomodulation, Allergic Asthma.

Address all correspondence to: Ulku Veranyurt, PhD Student, Department of Health Management, Institute of Health Sciences, Health Sciences University, Istanbul, Turkey
Tel: +90 5457327271, E-mail: ulkuveranyurt@gmail.com

IJBCS
ISSN: 2147-1428

Original Article

International Journal of Basic and Clinical Studies (IJBCS)
2019; 8(1): 46-60 Veranyurt U, Akalin B and Akkoc T

1. Introduction

It has been articulated that augmenting visibility of Asthma and allergies over the past years is related to the environmental issues such as house dust mite. Asthma is a chronic inflammatory disease of the airways characterised not only by cellular infiltration into the airways, but also by an accompanying increase in the sensitivity and response to contractile agents (airway hyper-responsiveness-AHR) and to allergen exposure (early and late asthmatic responses) (1).

House dust mites (HDMs) constitute a major, persistent source of indoor aeroallergens and constitute the leading cause of respiratory allergies such as allergic rhinitis (AR) and allergic asthma. These conditions affect more than 500 million people worldwide (2). There is an association between Asthma and exposure to HDM allergens that facing those allergens in early phases of life increases prevalence risk of Asthma. The airways of atopic individuals with asthma are characterized by infiltration of mast cells, CD4 T cells and eosinophils, which are activated by exposure to allergens. CD4 T cells play a central role in the disease process by producing Th2 effector cytokines (e.g. IL-4, IL-5, IL-9, IL-13) that drive many of the hallmark phenotypic changes observed in asthma; these include airways hyper responsiveness, increased mucus production, mucus cell hyperplasia, and eosinophilic inflammation (16). CD4 T cell responses are themselves heterogeneous, comprising multiple subsets that can either promote (Th1, Th2, Th9, Th17) or negate (Treg, Tr1) the airway inflammatory processes that underpin asthma (3).

Mesenchymal stem cells (MSCs) are spindle shaped cells with the potential for clonogenic proliferation. MSCs were initially reported as fibroblast-like cells that could be isolated from bone marrow via their adherence to plastic in culture and subsequently confirmed as a population (the colony-forming unit-fibroblast) of bone marrow-derived non-hematopoietic cells (4). MSCs are multipotent adult stem cells. They have been isolated from various sources, such as cord blood, Wharton's jelly, the placenta, bone marrow, teeth, and adipose tissue (5). In our research the dental follicle that is a loose connecting tissue surrounding the developing tooth is used. Hence Dental follicle stem cells could be a source of cell for mesenchymal stem cells.

It is well known that MSCs have immunosuppressive and immune modulatory properties in vitro and in vivo. Most in vitro studies showed that MSCs were able to interact with almost all subsets of lymphocytes, including T cells, B cells, natural killer cells, monocyte/ macrophages, dendritic cells, and neutrophils. MSCs can efficiently suppress the proliferation of Th2 by enhancing the IL-4 secretion (6).

In recent years, MSCs have been important in the fields of regenerative medicine and immunotherapy depending on their biological characteristics. MSCs have an important place for use in many chronic or acute diseases in medicine

IJBCS ISSN: 2147-1428

Original Article

International Journal of Basic and Clinical Studies (IJBCS)
2019; 8(1): 46-60 Veranyurt U, Akalin B and Akkoc T

with their immune-regulating and immunosuppressive properties, which can emit factors that accelerate tissue regeneration by migrating to the region of inflammation after tissue damage in the body, they can be transformed into different cell types (15). It can be envisaged that

regenerative medicine will play an important role in the development of new treatment approaches in our country and in the formation of new resources in terms of health tourism.

In this study, our purpose is to analyse the immune modulatory effects of human dental follicle mesenchymal stem cells (hDF-MSCs) pre-stimulated with/without IFN - γ; in Derp p 1 (+) asthma patients' lymphocytes in vitro.

2. Materials and Methods

This study consists of examination peripheral blood mononuclear cells (PBMC) from 7 healty (hPBMC) and 7 Derp 1+ asthma patients (aPBMC). The study is carried out at Marmara University, Faculty of Medicine and Paediatric Allergy-Immunology Department. Dental follicles were obtained from 6 healthy adults between the age of 19-25 at University of Marmara Faculty of Dentistry Oral and Maxillofacial Surgery Department.

The study protocol had the approval of the Ethics from Committee of Clinical Research at the Marmara University, Faculty of Medicine.

Patients diagnosed with Asthma and Derp 1 + who applied to University of Marmara, Department of Child Health and Diseases were included in this study based on the approval of their families. Patients between age 1 and 15 and showing significant sympthoms of Astma with cronological diseases (Diabetis, Cystic fibrosis etc) were excluded from this research.

2.1 Isolation of Stem Cells

Dental follicles (DF) were collected from the Marmara University Faculty of Dentistry Oral and Maxillofacial Surgery. The legitimate delegate of all patients provided informed consent according to the guidelines of the Ethics Committee of the Marmara University Medical Faculty in Istanbul, Turkey (09.2014.0226/70737436-050.06.04).

Teeth were obtained from 6 adult donors aged 19–25 years, healthy third molar was collected.

The follicles were carried in Dulbecco's phosphate-buffered saline (DPBS, Gibco, Grand Island, NY 14072, USA) including 1% penicillin/streptomycin (Gibco, USA). All laboratory work was executed in a laboratory in the Department of Pediatric Allergy-Immunology, Marmara University Research Hospital. The follicles were isolated under sterile conditions. The follicles were enzymatically treated with 3.5 mg/mL collagenase type I (Gibco, USA) for 40 minutes at 37∘C to completely digest follicles tissue. Then, 3.5 mL of Dulbecco's adjusted Eagle's medium (DMEM,Gibco,USA) supplemented with 10% fetal bovine serum (FBS, Gibco, USA) and 1% penicillin/streptomycin was added to digest the pulp and follicle tissue followed by centrifugation at 1500 rpm

IJBCS ISSN: 2147-1428

Original Article

International Journal of Basic and Clinical Studies (IJBCS)
2019; 8(1): 46-60 Veranyurt U, Akalin B and Akkoc T

for 5 minutes. Cells pellets were obtained, and the supernatant was aspirated. DFSCs were cultivated in T-25 flasks in a 5% CO2 atmosphere under 37◦C in culture medium composed of DMEM, 10% FBS, and 1% penicillin/streptomycin. The stem cells were washed with DPBS and

provided with fresh culture medium. The culture medium was changed every 2 to 3 days until the cells reached confluence. The cells were detached with 0.25% trypsin-EDTA (Gibco, USA) when they reached 70–80% confluence. Adherent cells cultured for 3 passages were characterized and analyzed for specific surface markers. The cellular analyses and differentiation were performed using flow cytometry.

2.2 Flow Cytometry Analysis

To analyse the cell surface antigen expressions, the cells from the third passage were used DFSCs were incubated with antibodies for human CD73 phycoerythrin (PE), CD90 PE, CD146 fluorescein isothiocyanate (FITC), CD105 PE, CD45 FITC, CD14 FITC, CD20 APC (BD Biosciences, San Diego, CA, USA) at room temperature in the dark. Control antibodies were phycoerythrin-conjugated or fluorescein isothiocyanate-conjugated and allophycocyanin-conjugated mouse IgG1 and mouse IgG2 (BD Biosciences, San Diego, CA, USA). The flow cytometry results were analysed using BD FACS Calibur.

2.3 Differentiation of Stem Cells

To induce osteogenic (MesenCult, Stemcell Technologies, North America), adipogenic, and chondrogenic differentiation, a human MSC functional identification kit (Gibco, Grand Island, USA) was used. For differentiation, the cells were plated in 6-well plates (5 × 104 cell/well), and the differentiation medium was prepared according to the manufacturer's instructions and changed 3 times per week. After 14 days, the adipocytes and chondrocytes were stained with Oil Red O and Alcian blue, respectively, and after 28 days, the osteocytes were stained with Alizarin red.

2.4 Lymphocyte Isolation

Peripheral blood was obtained from 7 healty (hPBMC) and 7 Derp 1+ asthma patients (aPBMC) aged between 1 to 15 years and was added to heparin tubes. The legitimate delegate of all patients provided informed consent according to guidelines of the Ethical Committee of the Marmara University Medical Faculty in Istanbul, Turkey. PBMCs were obtained via Ficoll-Paque (GE Healthcare Bio-Sciences) density gradient from heparinized peripheral blood samples, as previously described. The cellswere cultured in RPMI (Gibco, USA) supplemented with 10% FBS and 1% penicillin/streptomycin. PBMCs were stimulated with 5μL of anti-CD2 (0.5 μg/mL, eBioscience, San Diego, CA)/anti-CD3 (0.5 μg/mL, Life Span Biosciences, USA)/anti-CD28 (0.5 μg/mL,Millipore, California) (CDmix), 2,5 µl Derp 1 and 2,5 µl IFN- γ (Millipore, California; 2309018)

IJBCS ISSN: 2147-1428

Original Article

International Journal of Basic and Clinical Studies (IJBCS)
2019; 8(1): 46-60 Veranyurt U, Akalin B and Akkoc T

(applied at the commencement and 48th hours after) at 37°C in a humidified atmosphere containing 5% CO_2 for 72 hours.

2.5 Coculture of Human PBMCs and DFSCs

PBMCs and DFSCs (5 × 104/well in a 48-well plate) were plated 48 h prior to the addition of ten times number of lymphocytes in the culture medium. DFSCs, and lymphocytes (1:10) were co-cultured for 3 days. The cultures were stimulated using 5 μL of the CDmix , 2.5μL of IFN-Γ γ (5 μg/mL, Millipore, CA, USA), 2,5 µl Derp 1 .Then, lymphocyte proliferation (carboxyfluorescein succinimidyl ester, CFSE), apoptosis (Annexin V), $CD4^+CD25^+FoxP3^+$ cell expression and cytokine expression were analyzed via flow cytometry.

2.6 CFSE Assays for Lymphocyte Proliferation

After 3 days of co-culturing, the cell proliferation behaviour of the lymphocytes was quantified using carboxy fluorescein succinimidyl ester (CFSE) (Invitrogen, Grand Island, USA). The cells were labelled with CFSE, and 10 μm CFSE dye was used to stain the lymphocytes after co-culturing. The lymphocytes were stimulated in vitro with and without DFSCs and were tested for CFSE dilution via flow cytometry.

2.7 Detection of Apoptosis of the Lymphocytes by Annexin V

After 3 days of consecutive culturing, the apoptotic rate of the lymphocytes was quantified using an Annexin V kit (BD Biosciences, USA), based on the manufacturer's instructions. The kit included PI- Per CP, Annexin FITC.

2.8 CD4+FoxP3+ Treg Cell Assessment

After co-culturing for 3 days, the Treg lymphocyte cells were quantified using a Human FoxP3 Buffer Set (BD Biosciences, USA). We clarified the percentage of Treg (CD4+FoxP3+) markers that had developed from the lymphocytes. The cultures were examined by flow cytometry using Human FoxP3 Buffer Kit by following the manufacturer's instructions. The kit included Buffer A, and anti-human CD4 and anti-human FoxP3 PE (BD Biosciences, USA).

2.9 Statistical Analyses

The differences between groups were analyzed via a one-way ANOVA test using SPSS v20 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 6 software. Graphs were generated using GraphPad Prism. *P* values less than 0.05 were considered significant.

IJBCS
ISSN: 2147-1428

Original Article

3. Results

3.1. Isolation, Characterization, Differentiation

DFSCs attached separately to the culture flasks and exhibited a fibroblast-like and spindle-shaped morphology during the early days of incubation. The DFSCs reached 70% confluency in the primary culture 5-6 days after being plated in their first passages (P1). Most of the DFSCs exhibited fibroblast like morphology in the later passages (P1, P2, and P3; Figure 1. Then, immunophenotyping and differentiation of the three cell passages were observed. DFSCs were analyzed via flow cytometry. These cells exhibited positive staining for CD90, CD146, CD105 and CD73 (Figure 2B) but were negative for CD14, CD45 and CD20 (Figure 2A).

Figure 1. Morphological appearance and isolation of DFSCs. P0 (Passage 0)3rd Day Day, (b) P1(Passage 1): 3rd Day, (c) P3(Passage 3): 3rd Day, Original magnifications: ×10.

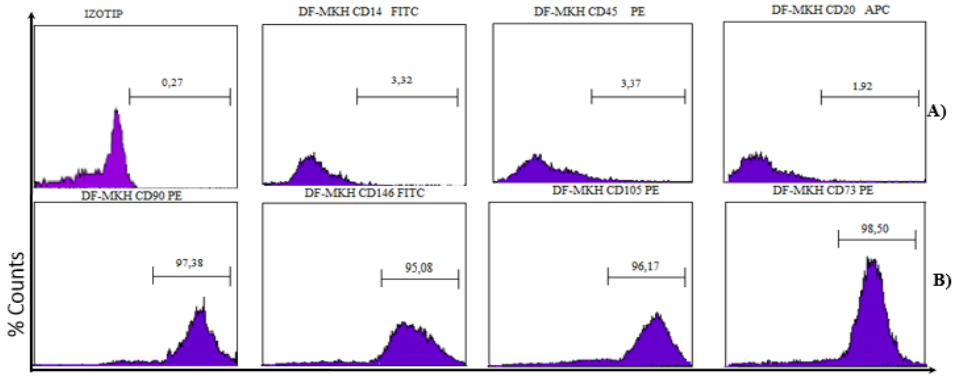

Figure 2. Cell surface stem cell antigens analysed by flowcytometry:
A) lsotype and negative expression for CD14, CD45 and CD20.,
B) Positive expression of CD90, CD146, CD105 and CD73 indicate a mesenchymal stem cell phenotype.

IJBCS ISSN: 2147-1428

Original Article

International Journal of Basic and Clinical Studies (IJBCS)
2019; 8(1): 46-60 Veranyurt U, Akalin B and Akkoc T

DFSCs differentiated into chondrocytes, adipocytes and osteocytes. First, chondrogenic differentiation capability was investigated in vitro during a fourteen-day culture period in chondrogenic induction medium, and cell differentiation into chondrocytes was confirmed with Alcian blue staining. Next, the in vitro adipogenic differentiation capability was assessed by culturing the cells in adipogenic induction medium and staining with Oil Red. Intracellular lipid droplets were observed in these cells. Finally Intracellular proteoglycans were observed in these cells the osteogenic differentiation capability was investigated in vitro during a twenty-eight-day culture period in osteogenic induction medium. DFSCs were stained with Alizarin red, and the cells formed calcified bone nodule structures (Figure 3).

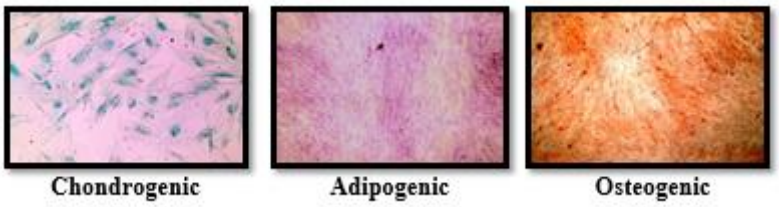

Figure 3. Alcian blue staining of chondrogenic induced DFSCs. Oil Red staining of adipogenic induced DFSCs. Alizarin red staining of osteogenic induced DFSCs.

3.2. The suppression of Lymphocyte Proliferation with DFSC's Derp 1+ and CDmix Stimulated Co-cultures

Lymphocyte proliferation was quantified via flow cytometry. In the CFSE labeling assay, lymphocyte proliferation was suppressed at day 3.The proliferation of lymphocytes was made from a group of healthy and Derp1+ Asthma Patients. The Lymphocytes were stimulated with IFN-γ, CDmix and Derp1 with DFMC's and without their presence. In group of healthy and Derp1+ Asthma Patients stimulation with CDMix in the presence of DFMC's were found significant ($P < 0.01$). In the presence of IFN-γ and DFMC's, the proliferation was suppressed significantly for the group of Derp1+ Asthma Patients ($P < 0.01$). The groups with/without DFMC's stimulated with Derp1 has shown no significant result ($P > 0.05$) (Figure 4). For the healthy group of patients presence of DFMC's stimulated with Derp1 and IFN-γ has shown no significant result ($P > 0.05$) (Figure 5).

IJBCS
ISSN: 2147-1428

Original Article

International Journal of Basic and Clinical Studies (IJBCS)
2019; 8(1): 46-60 Veranyurt U, Akalin B and Akkoc T

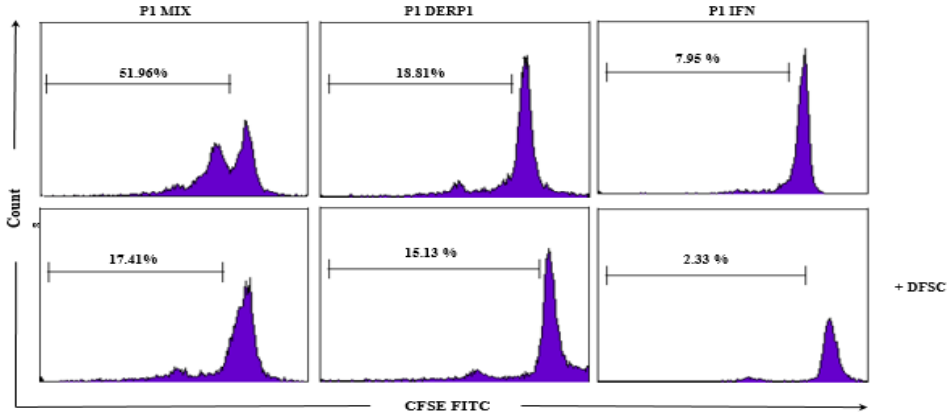

Figure 4. Proliferation of Derp1+ Asthma Patient's results.

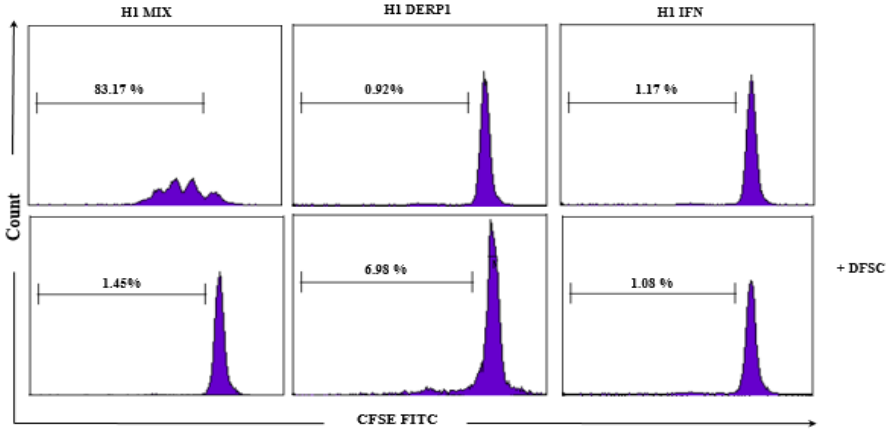

Figure 5. Proliferation of Healthy groups's results

3.3. Effects DFSCs on CD4+FoxP3+ Treg Cell Expansion of Lymphocytes

We studied the effects of DFSCs on the Treg frequency for both healthy group and patients with Derp1+ Asthma. CD4+FoxP3+ Treg cells were significantly induced by the stimulated lymphocytes with the CDmix compared with unstimulated lymphocytes for both groups. (P < 0.0001). CD4+FoxP3+ Treg cells were significantly induced when
lymphocytes stimulated with the CDmix were co-cultured with DFSCs (P < 0.05) within the same manner. In addition, the CD4+FoxP3+ Treg cells were significantly induced when stimulated with the CDmix in the presence of IFN-

IJBCS
ISSN: 2147-1428

Original Article

γ lymphocytes when co-cultured with DFSCs ($P < 0.05$) (Figure 6).

IFN-Γ γ pre-stimulated hDF-MSCs suppressed lymphocyte proliferation, apoptosis and increased the number of CD4+CD25+FoxP3+ Treg cells of Derp 1+ asthma patients' lymphocytes (Figure 7). IFN-γ stimulation of hDF-MSCs has more suppressive effect on CD4+ T cell response by enhanced CD4+CD25+FoxP3+ Treg cells. Same stimulation demonstrated no significant result for healthy patients (Figure 8).

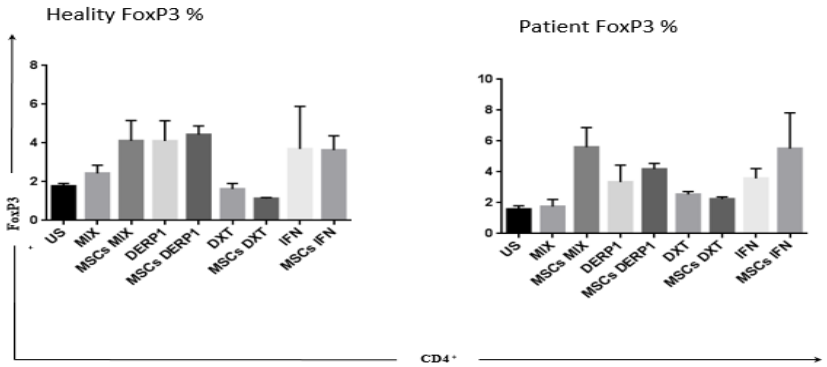

Figure 6. CD4+FoxP3+ Treg cells were displayed statistically. Induced effects of DFSCs on CD4+FoxP3+ Treg cells were displayed both for healthy and Derp1+ asthma patients by flow cytometry.

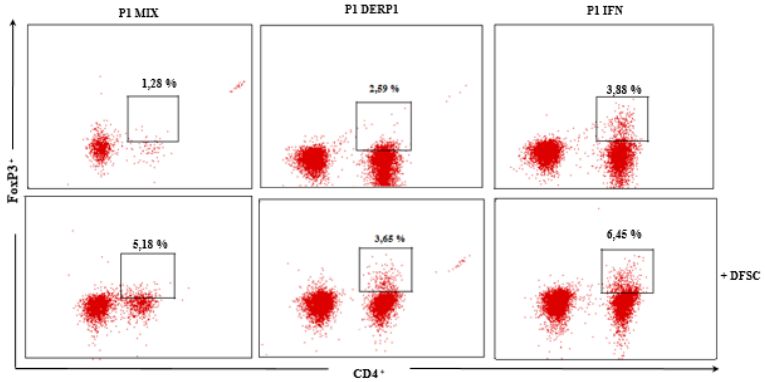

Figure 7. Derp1+ Asthma Patients of result CD4+CD25+FoxP3+ T regulatory lymphocyte (Treg) by flow cytometry.

IJBCS
ISSN: 2147-1428

Original Article

International Journal of Basic and Clinical Studies (IJBCS)
2019; 8(1): 46-60 Veranyurt U, Akalin B and Akkoc T

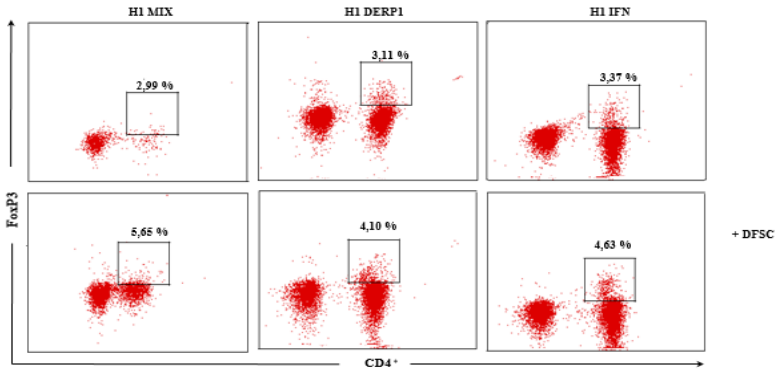

Figure 8. Healthy's of result CD4+CD25+FoxP3+ T regulatory lymphocyte (Treg).

3.4. Apoptosis of Lymphocytes using Annexin V

In order to determine the effect of DFMC cells on apoptosis of lymphocyte cells, the rate of Annexin-V live cells was examined. For this purpose, lymphocytes, both in combination with both DFMC and in non-hDF-MSC culture, are used as anti-CD2, anti-CD3, anti-CD28 (CDmix), Derp1, dexamethasone stimulated with IFN-γ for 24 and 48 hours were cultured for 72 hours. Annexin-V analysis was performed on flow cytometry.

It was proven that hDF-MSC cells had an enhancing effect on the rate of Annexin-V live cell in healthy (control) individuals in lymphocyte cells (Figure 9).

Similarly for the group with Derp1 Asthma patients stimulated with IFN-γ in presence of DFMC and in its absence an increase in the Annexin-V cells were observed. (Figure 10).

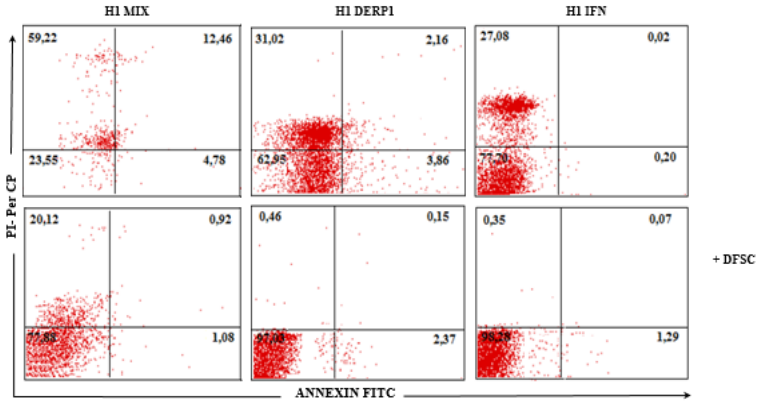

Figure 9. Healthy Group's results with Annexin V.

IJBCS
ISSN: 2147-1428

Original Article

International Journal of Basic and Clinical Studies (IJBCS)
2019; 8(1): 46-60 Veranyurt U, Akalin B and Akkoc T

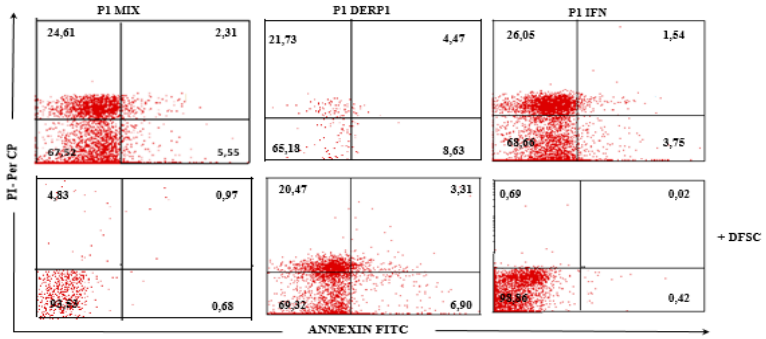

Figure 10. Derp1+ Asthma Patient's results with Annexin V.

4. Discussion

In this study, we investigated the effect of hDF-MSC cells on immune system cells. For this purpose, mesenchymal stem cell isolation was performed from the dental follicle. Isolated stem cells were cultured with lymphocyte cells isolated from the venous blood of healthy individuals and Derp1 + asthmatic individuals. Immunological analyzes were performed after 72 hours of culture. As immunological analyzes; after culture, flow cytometry device showed lymphocyte proliferation, apoptosis in lymphocyte cells, CD4 + FoxP3 + lymphocyte cells.

Asthma, Global Initiative for Asthma (GINA) according to the definition of chronic inflammatory disease and is emerging as a result of this inflammation, airway hyper -reactivity, common variable, spontaneously or with treatment follows a smooth course. In addition to this, it manifests itself as attacks of cough, shortness of breath and wheezing. In asthma, these complaints occur especially at night or in the morning, and these symptoms may occur in different ages at different ages in childhood. In general, asthma symptoms are reversible, spontaneously or with treatment.

In a study of Miura et al. Conducted in 2003, mesenchymal stem cell isolation was performed from milk pulp. Approximately 15, 20 cells were isolated from each poured tooth. It was shown that these cells adhere to plastic surfaces and are characteristic of other stromal cells (7). In a study by Suchanek et al., Dental stem cells were isolated and were shown to express CD44, CD73, CD90, CD117, CD166, HLA I medium CD29, CD105 and low CD45, CD63, CD71. In addition, these cells have been shown not to express CD18, CD31, CD34, CD49d, CD49e, CD106, CD133, CD184, CD197, CD146 and HLA II (8). In this study, dental follicle stem cell isolation was performed from a 20-year-old burial tooth and was isolated in a manner similar to that of Suchanek et al. These cells were shown to adhere to plastic surfaces and have high proliferation capabilities (Figure 1). The isolated characterization of these cells was made similar to that of Suchanek et al. As in the study of Suchanek et al. These cells were shown to express CD90, CD146, CD105 and CD73 on their surface and not to express CD14, CD45, and CD20 (Figure 2).

In another study by Suchanek et al., Cells were differentiated osteogenic, adipogenic and chondrogenic, and then stained with alizarin red, oil redo and von kossa respectively (8). In our study, after the isolation of hDF-MSC cells, osteogenic, adipogenic and chondrogenic differentiated, and then these cells were stained with alizarin red, oil redo and alcian blue respectively. These cells were shown to be different in three soy. Osteogenicly differentiated osteoblast nodules, showed adipogenicly differentiated fat droplets and proteoglycans were seen in chondrogenically differentiated (Figure 3.). In another study by Suchanek et al., Cells were differentiated osteogenic, adipogenic and chondrogenic, and then stained with alizarin red, oil redo and von kossa respectively (8). In our study, after the isolation of hDF-MSC cells, osteogenic, adipogenic and chondrogenic differentiated, and then these cells were stained with alizarin red, oil red and alcian blue respectively. These cells were shown to be different in three soy. Osteogenicly differentiated osteoblast nodules (Figure 3) showed adipogenicly differentiated fat droplets and proteoglycans were seen in chondrogenically differentiated (Figure 3).

Dental follicle is the mesenchymal tissue surrounding the developing tooth structure. Dental follicle, cement, periodontal ligament, alveolar bone marrow cells together with the female form (9). These cells should be taken after the withdrawal of the twenty-year-old female who is in the process of development for orthodontic reasons. The isolation of dental follicle-derived stem cells was first performed by Handa et al. Since the ability to be easily available and proliferating is high, we used stem cell origin stem cells in this study.

Recently, different effects of MSCs have been studied. The regulatory effect of MSCs in the immune system has been discovered for the first time once it has been able to avoid immune system control after transplantation. MSCs can suppress different T-Lymphocyte activities in vitro and in vivo (16). Naive and memory cells T-cells are subjected to suppression by MSC, and the inhibitory effect of stem cells does not require the presence of antigen presenting cells and does not require the mediation of CD4 + / CD25 regulator T cells (11). In the light of this information, co-cultures were performed with lymphocyte cells and hDF-MSC cells of individuals with asthma susceptible to household dust (Derp 1). In the in-vitro environment, hDF-MSCs were shown to suppress lymphocyte proliferation (Figure 4).

Recent research and reports have highlighted the importance of immunosuppressive factors and the importance of immunosuppression via MSCs in cell-cell contact. MSCs need direct and soluble mediators to provide a platelet-enhanced immunomodulatory effect that responds to stimuli such as cytokine, chemokine, T-cell, NK-cell. (11). Similar studies have shown that IFN-γ bask exposed MSCs do not have a reducing effect on T cell proliferation, but the expression of the Hepatocyte Growth Factor has been shown to increase and suppress multiple response

characteristics at the concentration of TGF-β1 (11).

Studies have shown that the survival of T cells suppressed by MSCs and less apoptosis is observed in the analyzes resulting from cultures of lymphocyte cells with MSCs (12). In our study, apoptosis was shown to be suppressed in the presence of stem cells. In the presence of hDF-MSC cells, it was shown to have an enhancing effect on the rate of Annexin V live cells in lymphocyte cells. At the same time, it was shown that the suppression of IFN-γ results in culture was even greater (Figure 10).

In our study, it was seen that the increase in CD4 + FoxP3 + cell amount was higher than non-stem cell groups and this increase was higher in the presence of IFN-γ in the flow cytometry analysis of stem cells and lymphocytes co-cultures. In addition, IFN-γ was added to the environment and its effect on MSCs was investigated (Figure 7). The main functions of MSC have different therapeutic properties. Examples of these properties include anti-inflammatory and immunomodulatory effects, and the production of different mediators that trigger or support tissue repair. Recently, these features have been used in preclinical and clinical therapies of different fetuses (13).

Another area in which MSCs have been used, limited success and difficulty in directed tissue regeneration technique in tissue engineering has revealed the need for a new regeneration therapy using MSCs. Due to its characteristics (proliferation rate, multiple differentiation, stem cell markers), mesenchymal stem cells have become an important target study area in tissue engineering. In order to get a more concrete output on this issue, it is necessary to try new approaches in tissue engineering based on the creation of scaffolding suitable for periodontem regeneration and dental applications (14).

In recent years, MSCs have gained a significant place in the fields of regenerative medicine and immunotherapy depending on their biological characteristics. These stem cells migrate to the site of inflammation after tissue damage in the body, secrete factors that accelerate tissue regeneration, transform into different cell types, immunoregulatory and immunosuppressive properties, and provide significant advantages for use in many chronic or acute diseases in the clinic (15). Today, more than five out-of-date MSC-based clinical trials, each of which are in different clinical stages, are announced on the ClinicalTrials.gov website. These studies investigating the efficacy of MSCs have been carried out for the treatment of various diseases such as autoimmune diseases,
cardiovascular diseases, liver failure, osteoarthritis, respiratory failure, spinal cord injury, and renal failure (15).

In terms of classical cellular (bone marrow / hematopoietic stem cell) treatments, there are specialized centers and experienced physicians who meet the international standards in our country, but their numbers remain limited in terms of the dimensions of our country. In our country, there is an Institute and five R & D centers operating in the field of stem cell and regenerative medicine. In addition to

these, there are five publicly licensed centers with a production license for cellular treatment products, five centers for private treatment, and seven centers licensed for banking and new ones are added (15). Presence of these centers and regenerative medicine studies can be predicted to play an important role in the development of new treatment approaches in our country and in the formation of new resources in terms of health tourism.

In our study, hDF-MSC significantly suppressed CD4+ T lymphocyte proliferation in parallel with the literature results. In the presence of IFN-γ it was observed that hDF-MSC suppressed CD4+ T lymphocyte proliferation. hDF-MSC T significantly increased the regulator cell ratio. It was observed that the rate of IFN-γ stimulated hDF-MSC T regulator cells increased significantly. IFN-γ stimulated and non-stimulated hDF-MSC significantly suppressed apoptosis in lymphocytes. According to the results obtained in this study, it has been shown that hDF-MSC cells suppress proliferation of lymphocytes obtained from patients with Derp1 + asthma.

In conclusion, according to the data obtained in our study, it was observed that IFN-γ stimulated hDF-MSC immune system could be used as the cells in the immunomodulatory functions in the treatment approaches of autoimmune, inflammatory and allergic diseases. This sheds light on the therapeutic use of hDF-MSC in the literature.

Ethical approval

The project "A regenerative medicine study: Immunoregulatory effect of DF-MSCs on lymphocytes of children with house dust mites sensitive asthma patients" was approved by Marmara University (Ethical code: 09.2014.0226/ 70737436-050.06.04-)

References

1. Raemdonck et al, CD4+ and CD8+ T cells play a central role in a HDM driven model of allergic asthma, Respiratory Research, s12931-016-0359-y.

2. Demoly et al, A year-long, fortnightly, observational survey in three European countries of patients with respiratory allergies induced by house dust mites: Methodology, demographics and clinical characteristics, BMC Pulmonary Medicine, s12890-016-0246-9.

3. Troy et al, Differential gene network analysis for the identification of asthma-associated therapeutic targets in allergen-specific T-helper memory responses, BMC Medical Genomics, s12920-016-0171-z.

4. Liu et al, Concise Reviews: Characteristics and Potential Applications of Human Dental Tissue-Derived Mesenchymal Stem Cells, Stem Cells, 2015; 33:627–638P

5. Ulusoy et al, Dental follicle mesenchymal stem cell administration amelio rates muscle weakness in MuSK-immunized mice, Journal of Neuro-inflammation , s12974-015-0451-0.

6.Yi et al, The Role of Recipient T Cells in Mesenchymal Stem Cell-Based Tissue Regeneration, Int J Biochem Cell Biol.,2012, biocel.2012.08.003.

7-.Miura M, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG, et al. SHED: stem cells from human exfoliated deciduous teeth. Proc Natl Acad Sci U S A. 2003; 100:5807-12.

8. Suchánek J, Víšek B, Soukup T, Mohamed SK, Ivančaková R, Mokrý J, Aboul-Ezz EHA, Omran A. Stem cells from human exfoliated deciduous teeth-isolation, long term cultivation and phenotypical analysis. Acta Medica (Hradec Králové) 2010, 53(2):93-99.

9. Yokoi T, Saito M, Kiyono T, Iseki S, Kosaka K, Nishida E. Establishment of immortalized dental follicle cells for generat ing periodontal ligament in vivo. Cell Tissue Res. 2007; 327(2): 301–311.

10. Handa K, Saıto M, Tsunoda A, Yamauchı M, Hattorı S, Sato S, Toyoda M, Teranaka T, Narayanan As. Progenitor cells from dental follicle are able to form cementum matrix in vivo. Connect Tissue Res.2002; 43:406-408.

11. Hiroshi Yagi, Alejandro Soto-Gutierrez, Biju Parekkadan, Yuko Kitagawa, Ronald G. Thompkins, Naoya Kobayashi, Martin L. Yarmush, MSC: Mechanisms of Immunomodulation and Homing, Cell Transplant. 2010; 19(6): 667-679.

12. Di Nicola M, Carlo-Stella C, Magni M, Milanesi M, Longoni PD, Matteucci P, Grisanti S, Gianni AM. Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. Blood 2002; 99: 3838-3843 [PMID: 11986244 DOI: 10.1182/blood.V99.10.3838].

13. G.Z. Racz1, K. Kadar1, A. Foldes1, K. Kallo1, K. Perczel-Kovach1,2, B. Keremı1, A. Nagy3, G. Varga1*, Immunomodulatory and Potential Therapeutic Role Of Mesenchymal Stem Cells In Periodontitis, Journal Of Physıology And Pharmacology 2014, 65, 3, 327-339.

14. Maxim MA, Soritau O, Baciut M, Bran S, Baciut G. The role of dental stem cells in regeneration. Clujul Med. 2015;88(4):479-82. doi: 10.15386/cjmed-475. Epub 2015 Nov 15. Review. PMID: 26733745.

15.Y. Murat ELÇİN, World Stem Cells and Regenerative Medicine Field and Developments in Turkey, April 2016 Issue: 328, Anahtar Journal, ISSN: 1300-2414, 22-26.

16. ARIG U. The Investigation of Immunological Effects Of Dental Follicule Mesenchymal Stem Cells On House Dust Mıtes Sensitive Asthma Patients' Lymphocytes In Vitro. MSc, Marmara University, Istanbul, Turkey, 2016.