The Egyptian Journal of Hospital Medicine (October 2014) Vol. 57, Page 403-417

Effect of Human Adipose-derived Mesenchymal Stem C on The Differentiation of Monocytes

Sobh, M. A. [*]; Habbak, L. Z.[**]; Mansy S. E.[**]; El-Chennawi, F.A. [***]; Zeed, S.M.[**]
* Stem cell, Urology and Nephrology Center, Mansoura University, Egypt.
** Zoology Department, Faculty of Science,Damietta University, Egypt.
*** Clinical Pathology Department, Faculty of Medicine, Mansoura University, Egypt.

Abstract

Background: The ideal stem cell for use in functional tissue engineering needs to be abundantly available, harvested with minimal morbidity, differentiated reliably down various pathways and able to be transplanted safely and efficaciously. Adult human adipose tissue contains a population of mesenchymal stem cells; adipose-derived stem cells, which seem to fulfil most, if not all, of these criteria. In this work, we investigated the immunogenicity properties of human adipose-derived mesenchymal stem cells (HAMSCs) and their effect on monocytes differentiation.

Materials and methods: The HAMSCs have been isolated and specified. Human peripheral blood mononuclear cells (PBMCs) were isolated and passed through a column with magnetic beads coated with anti-CD14 antibody. CD14+ ve cells were isolated and cultured independently or co-cultured with HAMSCs in the presence of cytokines (IL-4, Granulocyte-macrophage colony-stimulating factor (GM-CSF)) to induce their differentiation into dendritic cells (DCs). Their further maturation was induced by LPS added on the 6^{th} day of culture.

Results: The major part of the independently cultured cells (CD14+ ve) was found to express the markers which are considered to be specific for the mature dendritic cells such as Human leukocyte antigen-DR (HLA-DR) (40.44 %) and low percentage of cells (6.9 %). Nevertheless dendritic cells of monocyte origin (mDCs) co-cultured with HAMSCs showed significant shifts in the pattern of surface markers. The percentage of HLA-DR cells was much lower (6.44%) compared to control cultures ($p < 0.001$). Similarly, the secretion of IL-10 by DCs was up-regulated in co-cultures of HAMSCs and DCs.

Conclusion: The results show that human adipose-tissue mesenchymal stem cells (HAMSCs) could inhibit the differentiation of the blood monocytes into dendritic cells.

Keywords: Human adipose tissue; mesenchymal stem cells; Monocyte differentiation; Allogeneic transplantation.

Introduction

Mesenchymal stem cells (MSCs) are pluripotent cells that can be found in several adult and fetal tissues. Human mesenchymal stem cells (MSCs) have the capacity to differentiate into a variety of tissues including bone, cartilage, stroma, fat, muscle, and tendon. The major properties of MSCs are based on their plasticity, self-renewal and differentiation capacity. These characteristic features define MSCs as a very promising means for the purposes of regenerative medicine.[1] Mesenchymal stem cells are morphologically symmetrical fibroblastoid type cells. They express CD44, CD29, CD105, CD73, and CD166 and lack markers that are consistent with hematopoietic cells, in particular CD45 and CD34.[2]

Adult mesenchymal stem cells have been isolated from various adult tissues or peripheral blood. It has been reported that significant differences can be detected when studying MSCs isolated from different sources.

Although embryonic stem cells seem to exhibit unlimited differentiation potential both *in vitro* and *in vivo*, they are subject to significant ethical, legal and political concerns and are not generally available in current medical practice or research. Stem cells from adult tissue, on the other hand, suffer from few such restrictions.

Multipotent stem cells can be isolated from various mesenchymal tissue sources in adults, most commonly bone marrow. The harvest of bone marrow stem cells (BMSCs) has practical constraints. These include pain at the harvest site, and harvest of only a small volume of bone marrow (and therefore a small number of stem cells),[3] Umbilical cord blood (UCB) from the placenta of infants also contains mesenchymal stem cells. The use of UCB has been limited by the practical difficulties in obtaining and isolating the mesenchymal stem

DOI: 10.12816/0008474

cells at the time of birth and ensuring adequate long-term storage for autologous use, and the fact that their differentiation potential seems to be lower than that of BMSC. [4]

Adipose tissue represents an alternative source of stem cells. Subcutaneous adipose depots are accessible, abundant, and replenishable, thereby providing a potential adult stem cell reservoir for each individual. Many groups working independently have shown that adult stem cells derived from white adipose tissues can differentiate along multiple pathways invitro including the adipocyte, chondrocyte, endothelial, epithelial, hematopoietic support, hepatocyte, neuronal, myogenic, and osteoblast lineages.[5]

Recently extensive research efforts have been concentrated on the ability of the MSCs to modulate the immune response. It has been reported that MSCs can suppress the proliferation of T cells in vitro. The mechanisms underlying the suppressive effects seem to be quite various including induction of cell division arrest causing anergy. [6]

One of the mechanisms of the immunosuppression exerted by the MSCs has been shown to be the influence on the differentiation and maturation of the dendritic cells (mDCs) from monocyte origin. [7,8]

The dendritic cells are the most potent antigen-presenting cells and parallel to that possess quite powerful immunoregulatory activities. These mDCs originate from the blood monocytes then differentiate passing through the stage of immature dendritic cells and their later maturation to functional maturation stage. Every differentiation and/or maturation stage can be strictly identified by the expression of surface markers (CD14, CD80, CD86, CD83, HLA-DR) analyzed through immunophenotyping. [9,10,11]

The present work aims in one hand to isolate the mesenchymal stem cells from human adipose tissue and studied their differentiation ability to osteocytes **and on the other hand to** study in vitro some immunological properties of human adipose-derived mesenchymal stem cells (HAMSCs) to assess their suitability for allogeneic transplantation.

Materials and Methods

Isolation and expansion of HAMSCs

Samples of human adipose tissue were collected during surgery from 3 donors (40–60 years old) after an informed signed consent and according to the requirements of the Mansoura University Hospital, Mansoura, Egypt. Wither of 2 h the samples were delivered in the laboratory and processed.

Human adipose-derived stem cells (HAMSCs) were isolated according to method performed by Kern *et al*. [12] The lipoaspirate (50–100 ml) was washed intensely with phosphate buffer saline (PBS). Thereafter the lipoaspirates were digested with an equal volume of 0.075% collagenase type I (Sigma-Aldrich, USA) for 30–60 minutes at 37°C with gentle agitation. The activity of the collagenase was neutralized with Dulbecco's Modified Eagle Medium-low glucose (DMEM-lg) containing 10% fetal calf serum (FCS). To obtain the high-density stromal vascular fraction (SVF) pellet, the digested lipoaspirate was centrifuged at 1200 g (gravitational acceleration) for 10 minutes. The pellet was resuspended in DMEM-lg containing 10% mesenchymal stem cell growth medium (MSCGM) and filtered through a 100 μm nylon cell strainer (Falcon). The filtered cells were centrifuged at 1200 g for 10 minutes. The resuspended SVF cells were plated at a density of $1 \times 10^6/cm^2$ into T75 or T175 culture flasks. Nonadherent cells were removed wither 12–18 hours after initial plating by intensely washing the plates. The resulting adipose tissue-derived fibroblastoid adherent cells (AT-FACs) were cultivated at 37°C at a humidified atmosphere containing 5% CO2. The expansion medium consisted of Dulbecco's modified Eagle's medium-low glucose (DMEM-lg) containing 10% mesenchymal stem cell growth supplements (MSCGS) (Cambrex, http://www.cambrex.com). Adipose tissue-derived fibroblastoid adherent cells were maintained in DMEM-lg + 10% MSCGS until they reached 70% to 90% confluency. Cells were harvested at subconfluence using Trypsin (PromoCell, Heidelberg, Germany, http://www.PromoCell.com). Cells at the second passage and thereafter were replated at a mean density of $3.1 \times 10^3/cm^2$. The cell cultures were expanded for 2–8 passages, with the medium being changed every 4days.

Flow cytometry

For immunophenotypic characterization HAMSCs at 3rd passage, were trypsinized (0.05% trypsin/1.0mM EDTA at 37°C for 3min), harvested, washed once with and resuspended in PBS. Cells (1×10^5 per sample) were treated at room temperature for 30min

with the isotype control monoclonal antibodies (mAbs) and with the following specific anti-human antibodies: anti-CD90-PE, -CD29-PE, -CD34-FITC, -CD105-PE, -CD14-PE, -CD13-PE (Becton Dickinson, USA). After washing twice in PBS, the cells were fixed in fix solution (Becton Dickinson, USA) as recommended by the manufacturer. The specific fluorescent labeling was analyzed by FACS Calibur flow cytometer (Becton Dickinson, USA) using the Cell Quest software.

Osteogenic Differentiation

Human adipose tissue-derived MSCs from the 3rd to 5th passages were used at concentration 1×10^4 cells/well as a control. The cells were cultured in 24-well flat-bottom plates (Orange Scientific, Belgium) in a total volume of 1 ml in complete culture medium (DMEM- LG) supplemented with 10% FCS per well.

To promote osteogenic differentiation, cells (4th passage) were replated at a density of 1×10^4 cells/cm^2 into 24-well plates in triplicate and were cultured in DMEM- LG supplemented with 10% FCS, 100 nM (40 ng/ml) dexamethasone (Sigma-Aldrich), 0.2 mM ascorbic acid-2-phosphate (Sigma-Aldrich) and 10 mM β-glycerophosphate (Sigma-Aldrich). The osteogenic induction medium was replaced every 3 days for 3weeks. At the end of the induction period, osteogenic differentiation was determined by measuring the staining for alkaline phosphatase (ALP) activity, following the protocol of Leskelä *et al*. [13] For mineralized deposits detection, Alizarin red staining was performed as described by Caplan. [14]

Isolation and culturing of PBMCs

Peripheral blood mononuclear cells (PBMCs) were isolated from leukocyte concentrates from healthy donors kindly supplied by the Experiemental Research Center, Mansoura University. The leukocyte concentrate (50ml) was centrifuged at 1200 rpm for 10min and the supernatant blood plasma was discarded. The pelleted cells were resuspended in 60ml DMEM-LG and over layered on Ficoll-Hypaque (Pharmacia-LKB, Sweden). After centrifugation at 1750 rpm for 30min, the mononuclear layer was collected, cells were washed twice in PBS, counted using a hemocytometer and resuspended in culture medium. All cell cultures were performed in DMEM-LG supplemented with 10% FCS.

PBMCs at a concentration 1×10^6 cells/ml were cultured (control culture) in 6-well flat-bottom plates (Orange Scientific, Belgium) in the presence of GM-CSF (Granulocyte-macrophage colony-stimulating factor; 100 ng/ml) and IL-4 (Interleukin-4; 50ng/ml) in a total volume of 5 ml DMEM-LG containing 10% FCS per well. Each sample was repeated in 6 wells. After 7days, LPS (Lipopolysaccharide; 50 ng/ml) was added in fresh media then after 3 other days, supernatants were collected and studied by enzyme-linked immunosorbent assay (ELISA).

Monocytes Purification using immunomagnetic beads

After isolation of the PBMCs by the density centrifugation, the CD14+ population was separated using magnetic beads (MACS, Miltenyi Biotec, Germany) as recommended by the producer. Briefly: 5×10^7 PBMCs were centrifuged (250 g for 10 min) and then resuspended carefully in 400 µl sterile MACS buffer (PBS, pH 7.2; 2mM EDTA; 0.5% bovine serum albumin). Microbeads (100 µl) for direct separation (CD14 MicroBeads; Miltenyi Biotec, Germany) were added to the sample and incubated for 15 min at 4 °C. After the incubation cells were washed in 10 ml MACS buffer and again centrifuged at 250 g for 10 min. The cellular pellet was resuspended in 500µ cooled MACS buffer and loaded onto a separation column (MS MACS Column; Miltenyi Biotec, Germany) in a magnetic field . The flow through cell fraction was preserved and the column was washed 3 times with 500 µl of MACS buffer. The separation column was removed from the magnetic stand and the CD14+ positive cell fraction was eluted with 1ml MACS buffer. The eluted cells were analyzed by flow cytometry and used in further experiments.

The isolated CD14+ cells (1×10^6/ml) were distributed at 1ml volume in triplication in 24-well flat-bottom plates (Orange Scientific, Belgium) and cultured for 6 days in Roswell Park Memorial Institute media (RPMI 1640; PAA Laboratories GmbH, Pashing, Austria) with 10% fetal bovine serum (A 15-151, PAA Laboratories GmbH, Pashing, Austria) and antibiotics in the presence of 100 ng/ml GM-CSF (Immunex Corp., USA) and 50 ng/ml IL-4 (14269-5UG, Sigma–Aldrich, USA). Maturation of DCs was induced on day 6th by

stimulation with 50 ng/ml of LPS (L-43-91, Sigma–Aldrich, St. Louis, MO 63103, USA) in fresh complete medium for 3 days then the cells were analyzed by flow cytometry and the supernatants were harvested and immediately stored at −20 ˚C until tested.

Co-culture of HAMSCs with monocytes and flow cytometry analysis of monocytes (CD 14, HLA-DR class II)

CD14+ monocytes at a concentration 1×10^6 cells/ml were cocultured in 24-well flat-bottom plates (Orange Scientific, Belgium) with allogeneic HAMSCs (1×10^4 cells/well) both in a total volume of 1 ml in complete culture medium per well. Each sample was repeated in 3 wells. On day 6^{th} day LPS (50 ng/ml) was added in fresh medium for 3 days.

On the 9^{th} day dendritic cells from each well were collected and their numbers were counted in a hemocytometer. The cells were washed by PBS (pH 7.4), centrifuged at 1200 rpm for 10 min and adjusted at 1×10^6 cells. Monoclonal anti-CD14 FITC and anti-HLA-DR-FITC antibodies (Becton Dickenson, USA) were added and incubated for 30 min, in the dark. After double washing in a CellWash solution the cells were fixed in FIX solution as recommended by the producer company. The specific fluorescent labeling was analyzed at FACSCalibur flow cytometer and 10 000 events were counted and analyzed using flowing software version 2.5.0 program by Pertu Terho, Turku centre for biotechnology , university of Turku, Finland.

Cytokine quantification (IL-10) in cell culture supernatants using Enzyme Linked Immuno-Sorbent Assay (ELISA)

Interleukin-10 in cell culture supernatants from mDCs, HAMSCs and co-cultured DCs and MSC from both types were tested using the Quantikine Human ELISA kit (Bender MedSystems, Burlingame, CA94010, USA). Tests were performed according to the manufacture's instructions.

Quantitative data of each parameter of all experimental groups were expressed as means ± standard deviation (SD). Statistical significance of difference between diverse data sets was assessed by one-way analysis of variance (ANOVA) with significant difference test and by Student's *t*-test, where appropriate. A level of $P<0.05$ was considered significant.

Results

Assay of osteogenic differentiation

To evaluate mineral deposition, osteogenic differentiation was performed in monolayer culture for 3 weeks. The growing HAMSCs for at least 2 passages in culture were morphologically defined by their fibroblast-like appearance **Fig.(1)**. After reaching confluency, HAMSCs were induced towards osteogenesis with the induction medium. The induced HAMSCs changed from a fibroblastic appearance to a more polygonal appearance with nodules **(Fig. 2)**.

Fig. 1: hAMSCs at passage three (P3) were morphologically defined by the fibroblast– like appearance (× 100).

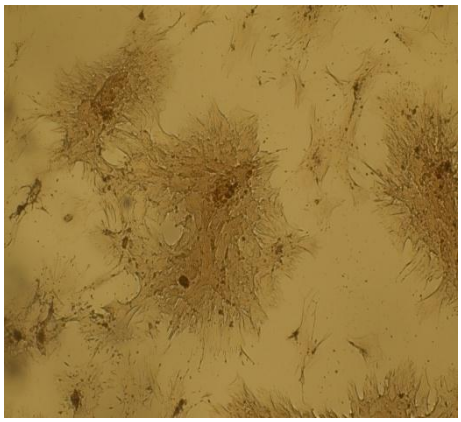

Fig. 2: hAMSCs differentiation to osteoblasts (× 100).

Phenotypic analysis

To characterize the cell-surface antigen profile of HAMSCs, the expression of surface markers was analyzed by flow cytometry at passage four to eight **(Fig. 3)**.

Results of the flow cytometry analysis showed that cells positively expressed CD29 (94%) , CD 90 (92%), , CD 105 (92%) and CD13 (89%) and negatively expressed CD14 (4%) and CD34 (6%).

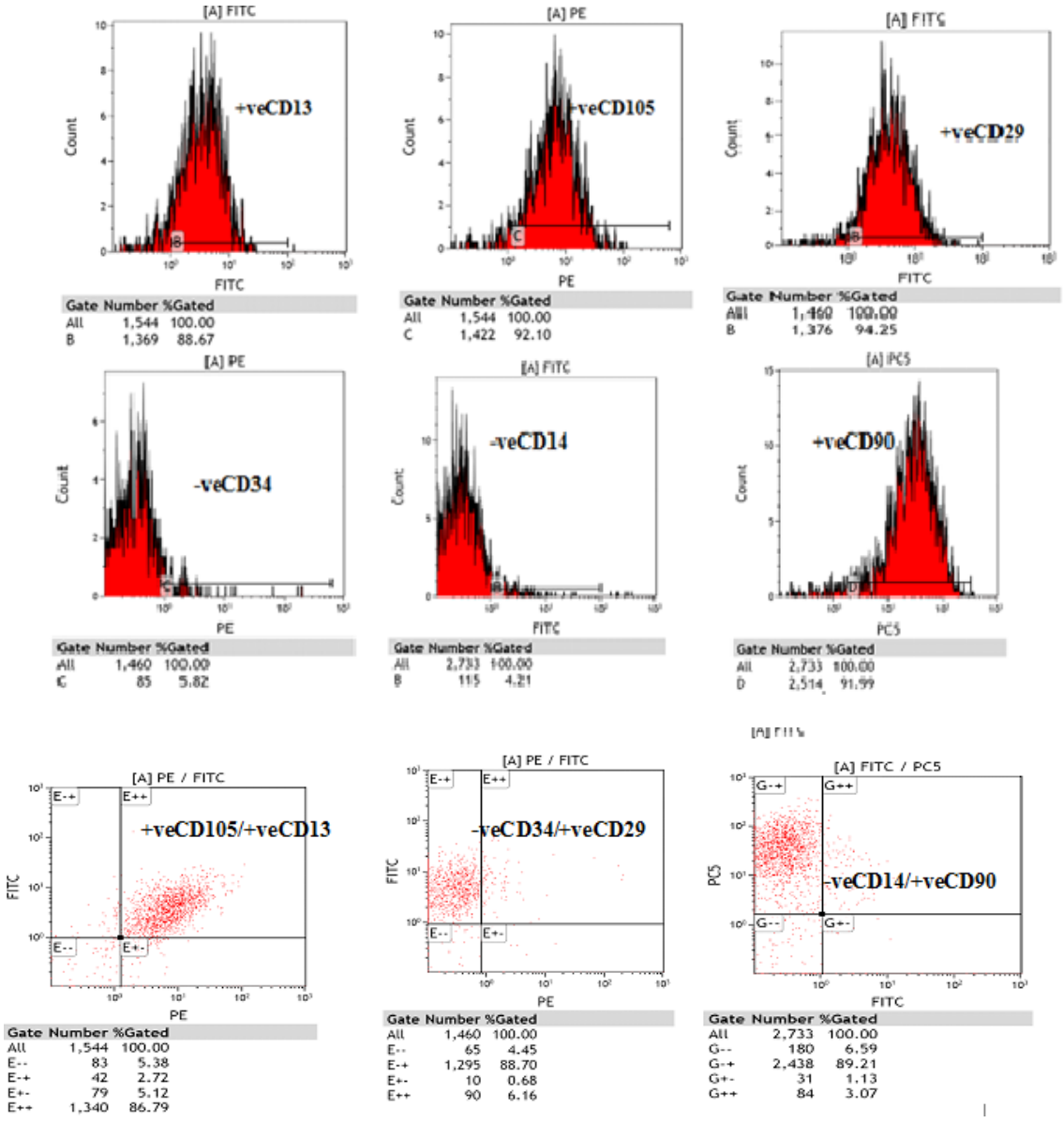

Fig. 3: Flow cytometry analysis of human adipose tissue mesenchymal stem cells revealed expression of surface antigens such as CD29 (94%), CD 90 (92%), CD 105 (92%) and CD13 (89%). (Passage 4:8) was strongly positive; while CD14 (4%) and CD34 (6%) was showed a negative results.

Effect of HAMSCs on monocytes differentiation:

CD14+ monocytes at a concentration 1×10^6 cells/ml were co-cultured in 24-well flat-bottom plates with allogeneic HAMSCs (1×10^4 cells/well). Both were in a total volume of 1 ml in complete culture medium per well containing 100 ng/ml GM-CSF and 50 ng/ml IL-4.

Each sample was repeated in 3 wells. On the 6th day LPS (50 ng/ml) was added in fresh medium for 3 days, the cells shows much lower proliferation rate than the control monocyte cultures reflecting the inhibition effect of HAMSCs **(Fig. 4).**

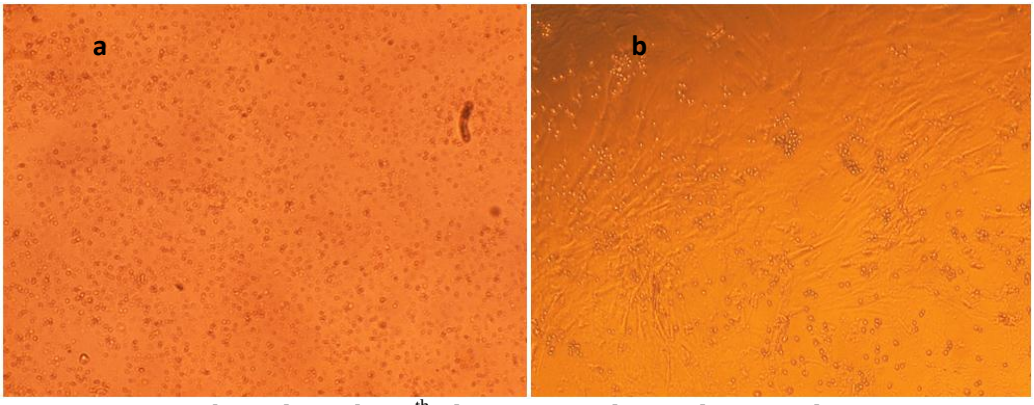

Fig 4a: Monocytes showed at the 7th day in complete culture media containing LPS (10x).**b :**Monocytes and HAMSCs co-culture at the 10th day in complete culture media containing GM-CSF and IL-4(10x).

At the 9th day dendritic cells from each well were collected and analyzed at FACSCalibur flow cytometer. In control cultures monocytes expressed 6.9 % CD14 positive (Fig.5), a much higher percentage (40.44 %) of monocytes HLA-DR class II positive (Fig.6).

Monocytes co-cultures with HAMSCs only recorded 6.44% HLA-DR class II positive (Fig. 7).

When comparing flow cytometry analysis in control and mixed cultures, we found that there is a highly significant difference in the means of monocytes (CD14+ cells) numbers ($P<0.003$) in control culture and in HAMSCs co-culture **(Fig. 8)**, also when comparing percent of monocytes cells positive for HLA-DR class II in control culture and in HAMSCs coculture, it showed a highly significant difference in the percent of positive cells ($P<0.001$) **(Fig. 9)**; this explains the inhibition effect of HAMSCs on the maturation of monocytes to differentiate in to dentritic cells.

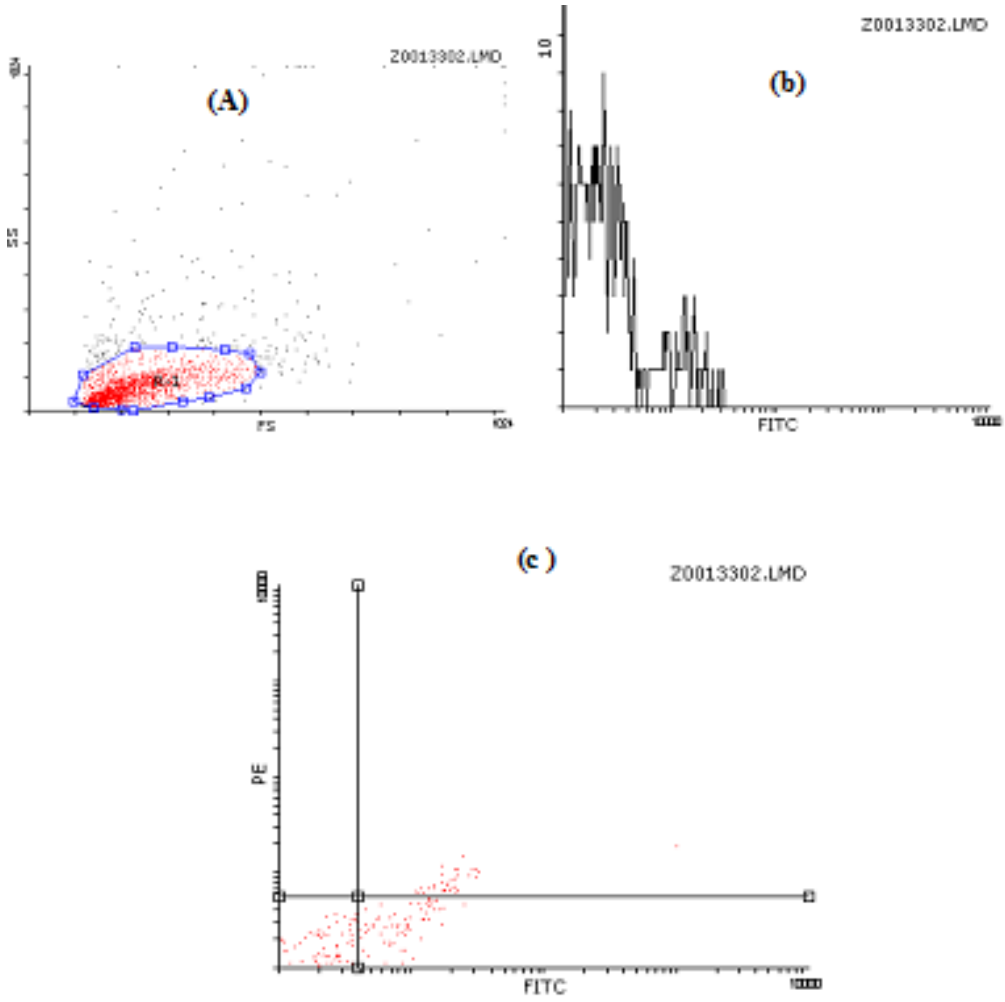

Fig.5 ; a: Dot plot of monocyte cells (control culture) showing fluorescence of CD14 positive monocyte cultured cells. **b:** Histogram showing number of cells in region 1 from the above analysis. **c:** Dot plot with quadrant showing positive cells (6.29%) for CD14 (right bottom is the positive region)

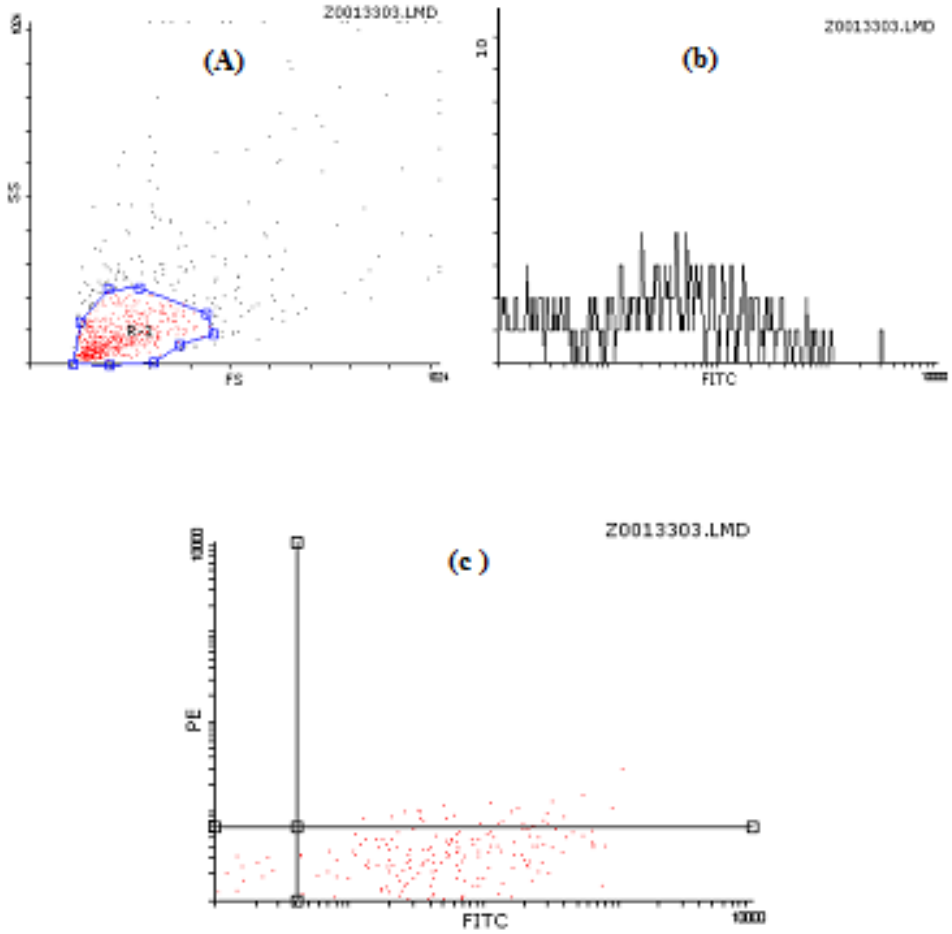

Fig. 6; a: Dot plot of monocyte cells (control culture) showing fluorescence of HLA-DR. **b:** Histogram showing number of cells in region 1 from the above analysis. **c:** Dot plot with quadrant showing positive cells (40.44%) for HLA-DR (right bottom is the positive region.

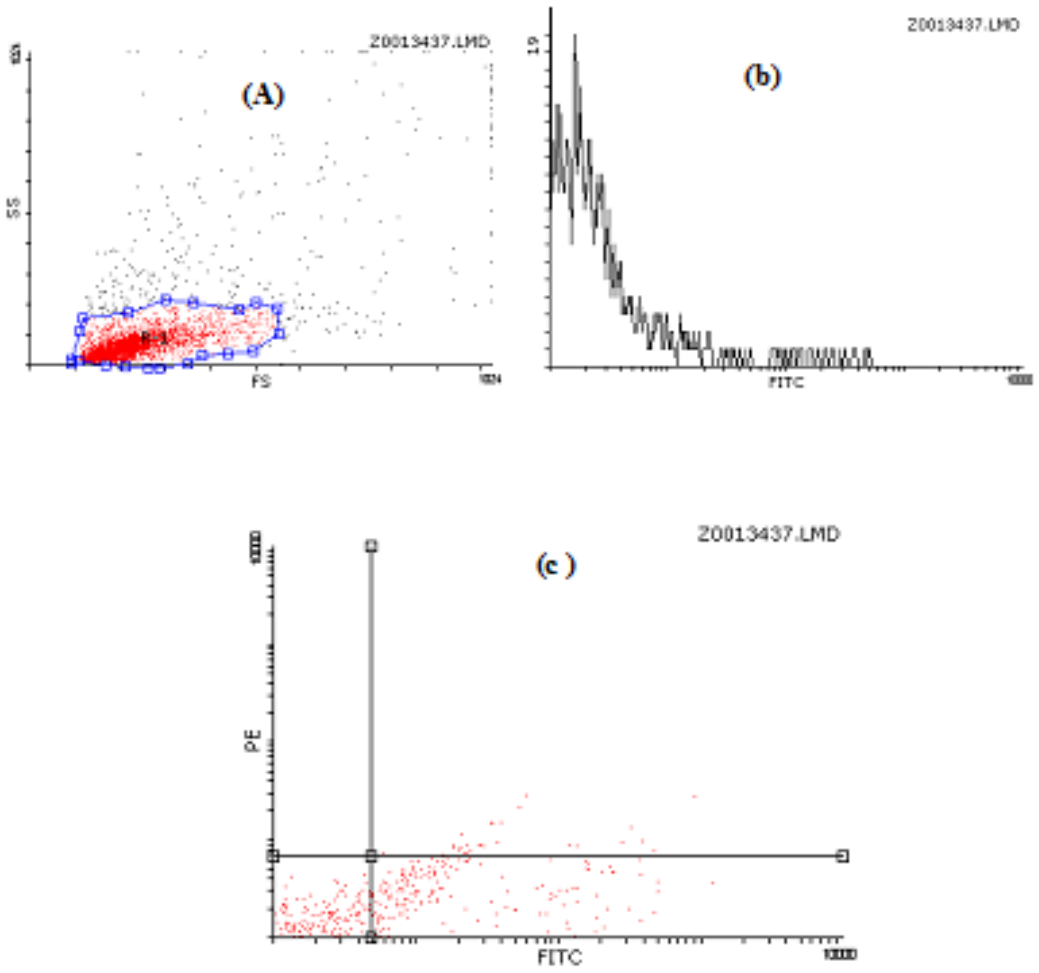

Fig.7 ; a: Dot plot of monocytes from HAMSCs- monocytes coculture, showing fluorescence of HLA-DR. **b:** Histogram showing number of cells in region 1 from the above analysis. **c:** Dot plot with quadrant showing positive cells (6.44%) for HLA-DR from monocyte culture (right bottom is the positive region)

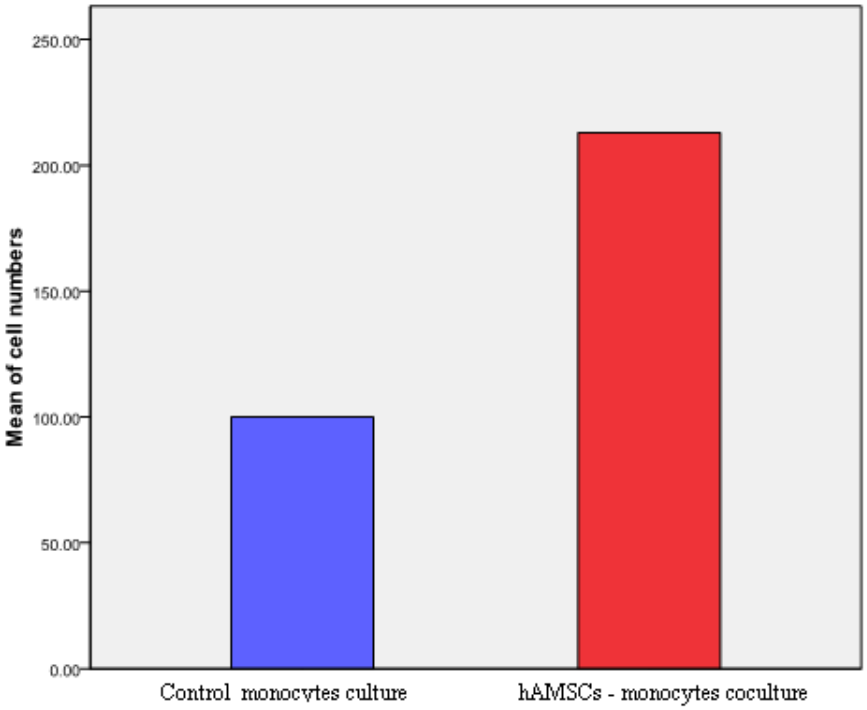

Fig. 8: Histogram comparing mean of monocytes cell numbers positive for CD14 in control culture and in HAMSCs co-culture (P<0.003).

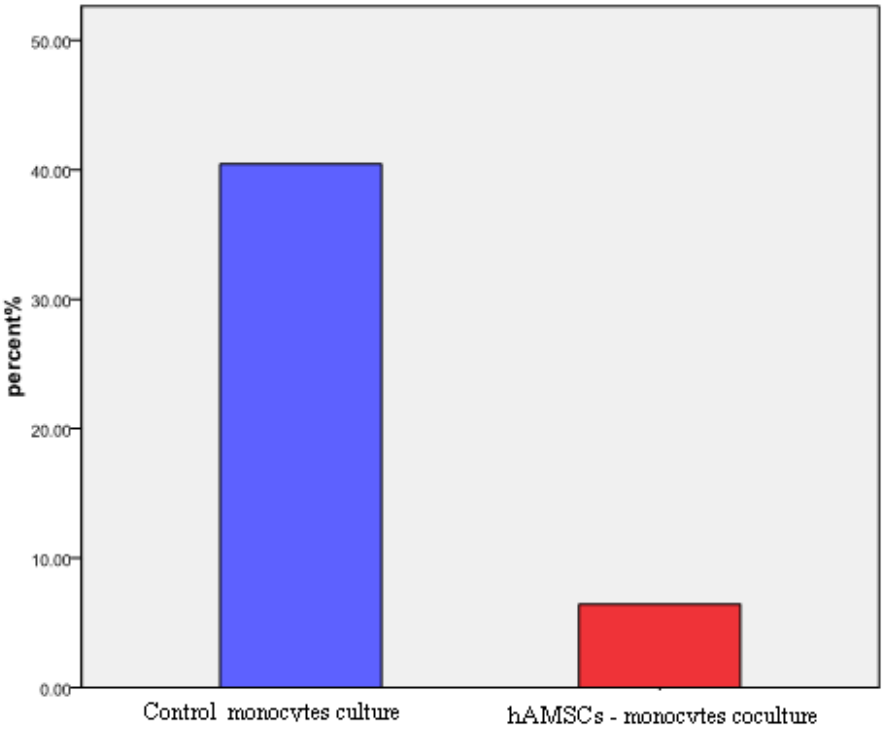

Fig. 9: Histogram comparing percentage of monocytes cells positive for HLA-DR class II in control culture and in HAMSCs coculture (P<0.001).

Cytokine quantification in cell culture supernatants :

Interleukin-10 (IL-10) was detected in measurable concentrations in supernatants of DCs control cultures and DCs co-cultures with HAMSCs. It should be pointed out that IL-10 could not be detected in supernatants from HAMSCs. The concentrations of IL-10 in supernatants from DCs co-cultured with human adipose tissue derived MSCs were significantly higher than the control cultures DCs **(Table 1, Fig. 10)**. The mean values of three independent experiments were analyzed and the differences were found to be statistically significant ($p < 0.05$).

Table 1:Concentrations of IL-10 measured by ELISA in supernatants of DCs control cultures and DCs co-cultures with HAMSCs

Culture supernatant	N	Mean	Std. Deviation	Std. Error Mean	One-Sample Test (0.05)
DCs	3	243.4333	5.45355	3.14861	0.05^{-5}*
DCs+ HAMSCs	3	273.2967	1.12429	.64911	0.05^{-5}*

* significant difference (P<0.001).

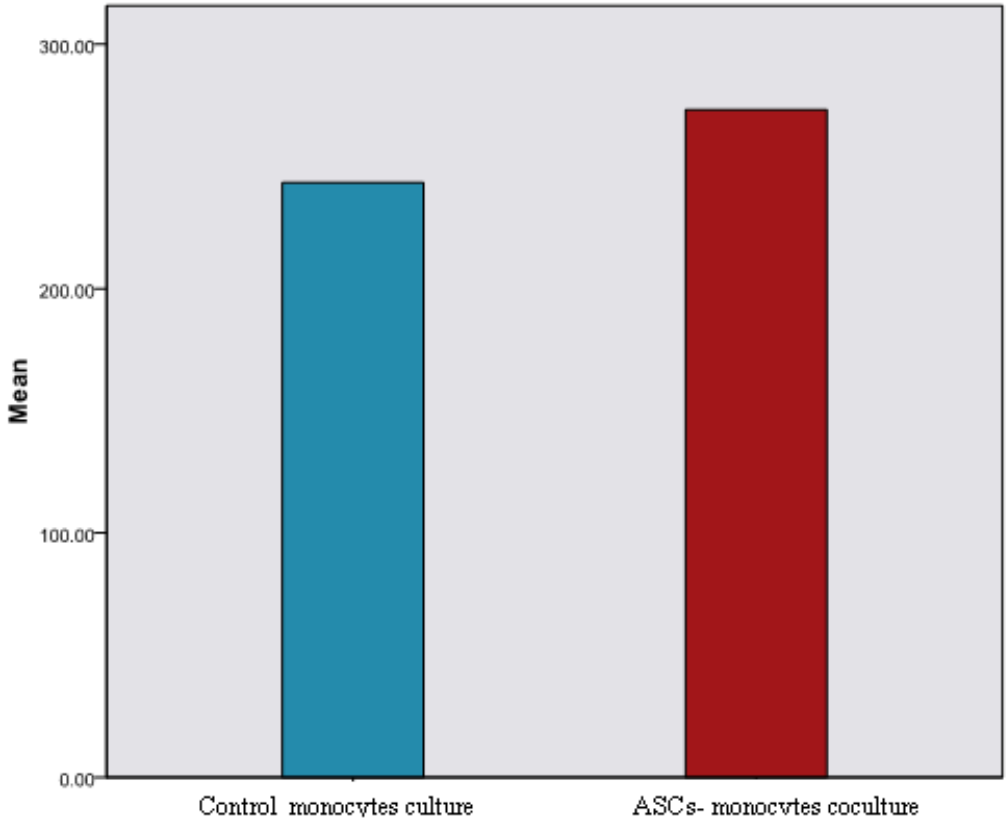

Fig. 10: Concentrations (Pg/ml) of IL-10 measured by ELISA in supernatants, of control culture and in HAMSCs co-culture.

Discussion

The major properties of the mesenchymal stem cells (MSCs) are based on their plasticity, self-renewal and differentiation capacity and these characteristic features define the MSCs as a very promising means for the purposes of the regenerative medicine. **[14,15,16]**

In this work, mesenchymal stem cells (MSCs) were harvested from human adipose tissue, purified, and grown in culture. They were morphologically defined by a fibroblast-like appearance.

Abundant numbers of HAMSCs can be derived from lipoaspirate, the waste product of liposuction surgery. Processing 300 mL of lipoaspirate routinely yields between 1×10^7 and 6×10^8 HAMSCs with >90% cell viability. **[3, 17-20]** The yield compares favourably with a bone marrow aspirate, **[21]**

when Comparing with BMSC it was proved that HAMSCs are more easily cultured and grow more rapidly. [22] They can also be cultured for longer than BMSC before becoming senescent. [10] All of these qualities make HAMSCs a useful source of mesenchymal stem cells.

The stromal vascular fraction (SVF) pellet found in the lipoaspirate contains an unpurified population of stromal cells, which includes the HAMSCs. The other cell types that may be present in SVF, namely endothelial cells, smooth muscle cells, pericytes, fibroblasts, and circulating cell types such as leucocytes, haematopoietic stem cells or endothelial progenitor cells. [23] Many authors use the entire unpurified SVF in their experiments, on the basis that the HAMSCs are adherent to the plastic tissue cultureware, so they are self-selected out of the SVF during subsequent tissue culture passages. [24] As few as one in 30 of the SVF cells adhere to the plastic, there is progressive loss of haematopoietic lineage cell markers (such as CD11, CD14 and CD45) with successive cultures of HAMSCs. [20] Adherence to plastic tissue cultureware, however, is not a feature that is specific to HAMSCs, fibroblast cells also behave in this manner. Some critics have suggested that even a low fraction of contaminating cells such as haematopoietic stem cells could be the source of differentiation seen in HAMSCs experiments. [25]

Our data revealed that cultured HAMSCs in osteogenic medium for two weeks, cells changed from a fibroblastic appearance to a more polygonal appearance and formed nodules. After 3 weeks culture time, the induced cells were stained positive with Alizarin Red S stain for mineral deposition in their newly formed matrix and most of cells became alkaline phosphatase positive. Undifferentiated mesenchymal stem cells have no extracellular calcium deposits, whereas differentiated osteoblasts have extracellular calcium deposits. Calcium deposits are therefore an indication of successful differentiation of MSC into osteoblasts and in vitro bone-formation. Calcium deposits can specifically be stained bright orange-red using Alizarin Red staining. Undifferentiated HAMSCs (without extracellular calcium deposits) were slightly reddish, whereas hAMSC-derived osteoblasts (with extracellular calcium deposits) were bright orange-red.Most studies agree that dexamethasone is a requirement for stimulation of osteogenesis in vitro, [26-27] although its exact mechanism of action is yet unknown, and one study replaces this factor with 1,25-dihydroxyvitamin D3. [28] In vitro, osteogenic differentiation takes at least 28 days, after which time calcified extracellular matrix can be seen after staining with 2% Alizarin red stain.

Definitive cell surface markers of HAMSCs would help not only distinguish them from other cell populations in cell culture, but would also enable purification of HAMSCs from SVF. To characterize the cell-surface antigen profile of HAMSCs in this study, the expression of surface markers were analyzed by flow cytometry at passage four to eight. Results of the flow analysis showed that cells positively expressed CD29 (94%) , CD 90 (92%), , CD 105 (92%) and CD13 (89%) and negatively expressed CD14 (4%) and CD34 (6%).

Extensive research efforts have been concentrated on the ability of the MSCs to modulate the immune response. [29-31] It has been reported that MSCs can suppress the proliferation of T cells in vitro induced by cellular and non-specific mitogenic stimuli [32,33] and it can prolong significantly the survival time of skin transplant in mouse model in vivo. [34] The mechanisms underlying the suppressive effects seem to be quite various including induction of cell division arrest causing anergy [35,36]

Similar mechanisms have been reported to be involved in the suppression of B cell functions. **Corcione *et al*.** [37] have reported that B cell proliferation was suppressed by human MCS's through an arrest in the G0/G2 phase of the cycle.

Natural killer (NK) cells are also targets of the suppressive effect of MSCs as it has been reported that co-culture of MSCs and NK cells caused a change in the NK cell phenotype and inhibit proliferation, cytokine secretion and cytotoxicity. Most of these effects require direct cellular interactions but some of the effects are mediated through secreted factors. [38]. One of the mechanisms of the immunosuppression exerted by the MSCs has been shown to be the influence on the differentiation and maturation of the dendritic cells from monocyte origin (mDCs). [5,6]

The dendritic cells are the most potent antigen-presenting cells and parallel to that possess quite powerful immunoregulatory activities. Monocyte-derived DCs originate from the blood monocytes and differentiate passing through the stage of the immature dendritic cells and their later maturation. Every differentiation and/or maturation stage can be strictly identified by the expression of surface markers (CD14, CD80, CD86, CD83, HLA-DR) analyzed through immunophenotyping. [7-9] The immunomodulatory activity of MSCs brings forward the question for their possible application in autoimmune diseases.

The results obtained from this study showed that HAMSCs were immune-modulators of differentiation of human DCs. The percentages of CD14+ cells were increased in co-cultures of HAMSCs and DCs and at the same time down regulation the expression of HLA-DR. These results revealed higher potential of the adipose tissue-derived MSCs to inhibit the differentiation and expression of functionally important co-stimulatory molecules on the surface of monocyte-derived dendritic cells.

Monocyte-derived DCs represent the final stage of the differentiation of blood monocytes. [39] Immunomodulatory activities of monocyte-derived DCs comprise the major characteristics since these cells are capable of triggering the differentiation of naive T helpers in Th2 cells, [40,41] inducing immune tolerance and controlling the immune response. [42] The existence of very sophisticated networks of regulations between the cell populations presumes that the DCs themselves are objects of regulations. The results from this study showed that human MSCs and particularly adipose tissue-derived MSCs can influence the differentiation of monocyte-derived DCs as well as the secretion of cytokines by DCs. In our experiments the culture of monocytes in the presence of factors triggering their differentiation and maturation (IL-4, GM-CSF, LPS) lead to development of mature dendritic cells demonstrated by immunophenotyping. It is known that in the course of differentiation the monocytes could loose the expression of CD14 under the influence of IL-4 [43] and the same effect was observed in our experiments. However, co-culturing of differentiating DCs with HAMSCs caused significant changes. These results demonstrated that the MSC blocked effectively the differentiation of the greater part of the monocytes and the cells remained at the level of CD14-expressing monocytes. A similar effect was observed with expression of HLA-DR marker as in co-cultured DCs which were significantly diminished. The molecular mechanisms of HAMSCs to inhibit the differentiation of monocytes into dendritic cells have not been clarified, so far, although some data revealed that TGF is directly related to the increased expression of CD14 and decreased expression of HLA-DR. [44]

It has been reported that one of the major ways of the HAMSCs to control the development of the DCs is secretion of cytokines such as TGF and IL-10 [44] and IL-10 by monocyte-derived DCs. [6] It is known that IL-10 is the major anti-inflammatory cytokine which deviates the immune response to Th2 bias and induces tolerance and apoptosis. [45, 46] It has been reported that IL-10 influences various processes related to monocytes and monocyte-derived DCs by controlling the cytokine secretion, [47] their maturation and function [48] and up-regulates their apoptosis. [49] IL-10 can induce tolerance by triggering anergy of the T lymphocytes by simultaneous inhibition of the surface expression of CD28 and surface expression of CD80/CD86 by the dendritic cells. [50]

These results demonstrated a significant up-regulation of the IL- 10 secretion by monocyte-derived DCs co-cultured with ASCs. IL-10 could be detected in the supernatants of independently cultured DCs but was not found neither in supernatants of activated HAMSCs nor resting HAMSCs cultured alone. Based both on literature data and the present results our hypothesis is that the ASCs up-regulate the secretion of IL-10 by DCs and the secreted cytokine in an autocrine manner suppression of the co-stimulatory molecules such as CD80 and CD86, this inhibiting the capacity of the DCs to stimulate effectively the T lymphocytes could lead to a lowing or absence of immune response or even anergy of the T cells. The present results are in agreement with the results Jiang *et al*. [5] and Ivanova-Todorovaa *et al.* [1] who reported an increase of CD14 and decrease of CD83, CD80 and CD86 by monocyte-derived DCs under the influence of MSCs. The new findings in our study refer to the data that adipose tissue-derived MSC are more potent suppressors of the expression of these molecules and better up-regulators of the IL-10 secretion.

In Conclusion, the results from this study clearly demonstrate that adipose tissue-derived mesenchymal stem cells are effective suppressors of monocyte-derived dendritic cells differentiation. These findings together with the other characteristics of adipose tissue MSCs are supposed an immunomodulatory agents for clinical applications. Given the ease of harvest, isolation and culture of HAMSCs, as well as their relative abundance, it is likely that HAMSCs will find widespread application in clinical practice in the future.

References

1. Ivanova-Todorovaa E, Bochevb I, Mourdjevab M, Dimitrovb R, Bukarevc D, Kyurkchievb S, Tivchevc P, Altunkovaa I and Kyurkchieva DS (2009): Adipose tissue-derived mesenchymal stem cells are more potent suppressors of dendritic cells differentiation compared to bone marrow-derived mesenchymal stem cells. Immunology Letters, 126: 37–42.

2. Patel SA, Sherman L, Muno J, Rameshwar P (2008): Immunological properties of mesenchymal stem cells and clinical implications. Arch. Immunol. Ther. Exp., 56, 1–8.

3. Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, Benhaim P, Lorenz HP, Hedrick MH (2001): Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng., 7: 211–28.

4. Kern S, Eichler H, Stoeve J, Kluter H, Bieback K (2006): Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood or adipose tissue. Stem Cells,24:1294e301.

5. Maccario R, Podestà M, Moretta A, Cometa A, Comoli P, Montagna D, Daudt L, batici A, Piaggio G, Pozzi S, Frassoni F, Locatelli F (2005): Interaction of human mesenchymal stem cells with cells involved in alloantigen-specific immune response favors the differentiation of CD4+ T-cell subsets expressing a regulatory/suppressive phenotype Haematologica, 90:516-525.

6. Bassi EJ, Aita CA, Câmara NS (2011): Immune regulatory properties of multipotent mesenchymal stromal cells: Where do we stand?. World J Stem Cells, 3(1): 1-8.

7. Jiang X-X, Zhang Y, Liu B, Zhang S-X, Wu Y, Yu X-D, Mao N (2005): Human mesenchymal stem cells inhibit differentiation and function of monocyte derived dendritic cells. Blood,105:4120–6.

8. Groh ME,Maitra B, Szekely E, Koc ON (2005): Human mesenchymal stem cells require monocyte-mediated activation to suppress alloreactive T cells. Exp Hematol,33:928–34.

9. Bancheraeau J, Briere F, Caux C, Davoust J, Lebeeque S, Liu YJ, Pulendran B, Palucka K (2000): Immunobiology of dendritic cells. Annu Rev Immunol,18:757–811.

10. Thery C, Amigorena S (2001): The cell biology of antigen presentation in dendritic cells. Curr Opin Immunol,13:45–57.

11. Hart DJ (1997): Dendritic cells: unique leukocyte population which controls the primary immune response. Blood,90:3245.

12. Leskela¨ HV, Olkku A, Lehtonen S, Mahonen A, Koivunen J, Turpeinen M (2006): Estrogen receptor alpha genotype confers interindividual variability of response to estrogen and testosterone in mesenchymal-stem-cell-derived osteoblasts. Bone,39:1026e34.

13. Caplan AI (2008): Cell Stem Cell, 3(3):229-30.

14. Pittenger MF,Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR (1999): Multilineage potential of human mesenchymal stem cells. Science,284:143–7.

15. Bianco P, Gehron P, Robey P (2000): Marrow stromal stem cells. J Clin Invest,105:1663–8.

16. Jiang Y, Jahagirdar BN, Reinhardt RL (2002): Pluripotency of mesenchymal stem cells derived from adult marrow. Nature,418:41–9.

17. Aust L, Devlin B, Foster SJ, Halvorsen YD, Hicok K, du Laney T, Sen A, Willingmyre GD, Gimble JM (2004): Yield of human adipose-derived adult stem cells from liposuction aspirates. Cytotherapy, 6: 7–14.

18. Boquest AC, Shahdadfar A, Brinchmann JE, Collas P (2006): Isolation of stromal stem cells from human adipose tissue. Methods Mol. Biol., 325: 35–46.

19. Parker AM, Katz AJ (2006): Adipose-derived stem cells for the regeneration of damaged tissues. Expert Opin. Biol. Ther., 6: 567–78.

20. Mitchell JB, McIntosh K, Zvonic S, Garrett S, Floyd ZE, Kloster A, Di Halvorsen Y, Storms RW, Goh B, Kilroy G, Wu X, Gimble JM (2006): Immunophenotype of human adipose-derived cells: temporal changes in stromal-associated and stem cell-associated markers. Stem Cells, 24: 376–85.

21. Sekiya I, Larson BL, Smith JR, Pochampally R, Cui J-G, Prockop DJ (2002): Expansion of human adult stem cells from bone marrow stroma: conditions that maximize the yields of early progenitors and evaluate their quality. Stem Cells, 20: 530–41.

22. Sommer B, Sattler G (2000): Current concepts of fat graft survival: histology of aspirated adipose tissue and review of the literature. Dermatol. Surg., 26: 1159–66.

23. Tholpady SS, Llull R, Ogle RC, Rubin JP, Futrell JW, Katz AJ (2006): Adipose tissue: stem cells and beyond. Clin. Plast. Surg., 33: 55–62.

24. Ogawa R (2006): The importance of adipose-derived stem cells and vascularized tissue

regeneration in the field of tissue transplantation. Curr. Stem Cell Res. Ther., 1: 13–20.

25. Safford KM, Rice HE (2005): Stem cell therapy for neurologic disorders: therapeutic potential of adipose-derived stem cells. Curr. Drug. Targets, 6: 57–62.

26. Lee JA, Parrett BM, Conejero JA, Laser J, Chen J, Kogon AJ, Nanda D, Grant RT, Breitbart AS (2003): Biological alchemy: engineering bone and fat from fat-derived stem cells. Ann. Plast. Surg., 50: 610–17.

27. Kim DH, Je CM, Sin JY, Jung JS(2003): Effect of partial hepatectomy on in vivo engraftment after intravenous administration of human adipose tissue stromal cells in mouse. Microsurgery, 23: 424–31.

28. Aggarwal S, Pittenger MF (2005): Humanmesenchymal stem cells modulate allogeneic immune cell responses. Blood,105:1815–22.

29. Rasmusson I (2006): Immune modulation by mesenchymal stem cells. Exp Cell Res,312:2169–79.

30. Uccelli A, Moretta L, Pistoia V (2006): Immunoregulatory function of mesenchymal stem cells. Eur J Immunol,36:2566–73.

31. Di Nicola M, Carlo-Stella C, Magni M (2002): Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. Blood,99:3838–43.

32. Krampera M, Glennie S, Dyson J (2003): Bone marrow mesenchymal stem cells inhibit the response of naive and memory antigen-specific T cells to their cognate peptide. Blood,101:3722–9.

33. Bartholomew A, Sturgeon C, Siatskas M, Ferrer K (2002): Mesenchymal stem cells suppress lymphocyte proliferation in vitro and prolong skin graft survival in vivo. Exp Hematol,30:42–8.

34. Beyth S, Borovsky Z, Mevorach D (2005): Human mesenchymal stem cells alter antigen-presenting cell maturation and induce T-cell unresponsiveness. Blood,105:2214–9.

35. Glennie S, Soeiro I, Dyson PJ, Lam EW, Dazzi F (2005): Bone marrow mesenchymal stem cells induce division arrest anergy of activated T cells. Blood,105:2821 7.

36. Corcione A, Benvenuto F, Ferretti E, Giunti D, Cappiello V, Cazzanti F, Risso M, Gualandi F, Mancardi GL, Pistoia V, Uccelli A (2006): Human mesenchymal stem cells modulate B-cell functions. Blood,107:367–72.

37. Sotiropoulou PA, Perez SA, Gritzapis AD, Baxevanis CN, Papamichail CN (2006): Interactions between human mesenchymal stem cells and natural killer cells. Stem Cells,24:74–85.

38. Palucka KA, Taquet N, Sanchez-Chapuis F, Glukman JC (1998): Dendritic cells as the terminal stage of monocyte differentiation. J Immunol,160:4587.

39. Banchereau JR, Steinman M (1998): Dendritc cells and the control of immunity. Nature,292:245.

40. Tanaka H, Demeure CE, Rubio M, Delespesse G, Sarfati M (2000): Human monocytederived dendritic cells induce naive T cells differentiation into T helper cell type2 (Th2) or Th1/Th2 effectors: role of stimulator/responder ratio. J Exp Med,192:405–12.

41. Van Duivenvoorde LM, van Mierlo GJD, Boonman ZFHM, Rene EM (2006): Toes dendritic cells: vehicles for tolerance inducton and prevention of autoimmune diseases. Immunobiology,211:627–32.

42. Diliouglou S, Cruse MJ, Lewis RE (2003): Function of CD 80 and CD 86 on monocytes and stem cell-derived dendritic cells. Exp Mol Pathol,75:217–27.

43. Ruau D, Xin-Sheng J., Zenke M (2006): Genomics of TGF_ signalling in stem cell commitment and dendritic cell development. Cell Immunol,244:116–20.

44. Hagiwara E, Gourley M, Lee S, Klinman DM (1996): Disease severity in patients with systemic lupus erythematosus correlates with the increased ratio of IL-10: IFNgamma secreting cells in the peripheral blood. Arthritis Rheum,39:379–86.

45. Bailey DP, Kashyar M, Bouton LA, Murray PJ, Ryan JJ (2006): Interleukin-10 induces apoptosis in developing mast cells and macrophages. J Leuk Biol,80:581–9.

46. De Waal MR, Abrams J, Bennet B, Figdor CG, De Vries JE (1991): Interleukin 10 (IL- 10) inhibits cytokine synthesis by human monocites: an autoregulatory role of IL-10 produced by monocytes. J Exp Med,174:1209–20.

47. De Smedt T, Van Mechelen M, De Becker G, Urbain J, Leo O, Moser M (1997): Effect of interleukin 10 on dendritc cell maturation and function. Eur J Immunol,27:1229–35.

48. Schmidt M, Lugering N, Pauels HG, Schulze-Osthoff K, Domschke W, Kucharzik T (2000): IL-10 induces apoptosis in human monocytes involving the CD95 receptor/ ligand pathway. Eur J Immunol,6:1769–77.

49. Morel AS, Quarantino S, Douek DC, Londei M. (1997): Split activity of interleukin-10 on antigen capture and antigen presentation by human dendritic cells: definition of maturative step. Eur J Immunol,27:26–34.

50. Shusen Y, LiW,LiuW,Gao C, Zhou B, Wang Z, Zhang RH, Kong YH, Li Y, Han W, Gan RT, Xue HJ, Geng JQ, Yang SS, Shao Q, Zhang M (2006): Il-10 gene modified dendritic cells induced antigen-specific tolerance in experimental myocarditis. Clin Immunol,121:63–73.