

**ORIGINAL ARTICLE**

Iran J Allergy Asthma Immunol

March 2013; 12(1): 37-49.

## **Immunomodulatory Effects of Human Umbilical Cord Wharton's Jelly-Derived Mesenchymal Stem Cells on Differentiation, Maturation and Endocytosis of Monocyte-Derived Dendritic Cells**

**Mohsen Saeidi<sup>1,2</sup>, Ahmad Masoud<sup>2</sup>, Yadollah Shakiba<sup>2</sup>, Jamshid Hadjati<sup>2</sup>, Mandana Mohyeddin Bonab<sup>2</sup>,  
Mohammad Hossein Nicknam<sup>2,3</sup>, Mostafa Latifpour<sup>4</sup>, and Behrooz Nikbin<sup>2,3</sup>**

<sup>1</sup>*Department of Immunology, School of Medicine, Golestan University of Medical Sciences, Gorgan, Iran*

<sup>2</sup>*Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*

<sup>3</sup>*Molecular Immunology Research Center, Tehran University of Medical Sciences, Tehran, Iran*

<sup>4</sup>*Department of Anatomy, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*

*Received: 28 November 2011; Received in revised form: 20 February 2012; Accepted: 10 May 2012*

### **ABSTRACT**

The Wharton's jelly of the umbilical cord is believed to be a source of mesenchymal stem cells (MSCs) which can be therapeutically applied in degenerative diseases.

In this study, we investigated the immunomodulatory effect of umbilical cord derived-mesenchymal stem cells (UC-MSCs) and bone marrow-derived-mesenchymal stem cells (BM-MSCs) on differentiation, maturation, and endocytosis of monocyte-derived dendritic cells in a transwell culture system under laboratory conditions. Monocytes were differentiated into immature dendritic cells (iDCs) in the presence of GM-CSF and IL-4 for 6 days and then differentiated into mature dendritic cells (mDCs) in the presence of TNF- $\alpha$  for 2 days. In every stage of differentiation, immature and mature dendritic cells were separately co-cultured with UC-MSCs and BM-MSCs.

The findings showed that UC-MSCs and BM-MSCs inhibited strongly differentiation and maturation of dendritic cells at higher dilution ratios (1:1). The BM-MSCs and UC-MSCs showed more inhibitory effect on CD1a, CD83, CD86 expression, and dendritic cell endocytic activity, respectively. On the other hand, these cells severely up-regulated CD14 marker expression.

We concluded that UC-MSCs and BM-MSCs could inhibit differentiation, maturation and endocytosis in monocyte-derived DCs through the secreted factors and free of any cell-cell contacts under laboratory conditions. As DCs are believed to be the main antigen presenting cells for naïve T cells in triggering immune responses, it would be logical that their inhibitory effect on differentiation, maturation and function can decrease or modulate immune and inflammatory responses.

**Keywords:** Bone marrow; Dendritic cell; Endocytosis; Mesenchymal stem cells; Wharton's jelly

---

**Corresponding Authors:** Behrooz Nikbin, MD, PhD;  
Molecular Immunology Research Center and Department of  
Immunology, Tehran University of Medical Sciences, Tehran, Iran.

---

Tel: (+9821) 6641 9536, Fax: (+98 21) 6641 9536, E-mail:  
nikbinbe@sina.tums.ac.ir

## INTRODUCTION

Stem cells, according to their origin, are categorized to Embryonic and Adult Stem cells.<sup>1</sup> Mesenchymal stem cells (MSCs) are the most important source of adult stem cells and have attracted the attention of lots of researchers. These cells participate in regeneration of tissues with mesenchymal origin such as bone, cartilage, muscle, tendon and adipose tissue. In addition, they play a supportive role to bone marrow hematopoietic cells.<sup>2-4</sup> MSCs express negligible levels of MHC class I and no MHC class II, and are negative for hemopoietic and endothelial markers (CD45, CD34, CD31) and positive for other markers such as CD90, CD73, CD105, CD106 and CD44.<sup>5-8</sup> Bone marrow has been the most important source of MSCs during the recent years. Lately, scientists found out that MSCs can be collected from resources such as Wharton's jelly of the umbilical cord.<sup>9-12</sup>

The Wharton's jelly (WJ) is, in fact, a gelatin substance inside the umbilical cord which contains a significant amount of mucopolysaccharide (hyaluronic acid), chondroitin sulfate, and fibroblast cells. This mucoid tissue protects the veins of the umbilical cord.<sup>13,14</sup> The WJ secretes IGF (Insulin like growth factor), PDGF (platelet-derived growth factor), EGF (Epidermal growth factor) and TGF- $\beta$  (Transforming growth factor-beta) with capability to induce proliferation and growth of the other cells.<sup>15,16</sup> The Wharton's jelly of the umbilical cord derived-mesenchymal stem cells (UC-MSC) express MSC markers such as CD90, CD73, CD105, CD106 and do not express hemopoietic and endothelial markers such as CD45, CD34 and CD31.<sup>9, 10</sup> They can be differentiated in *in vitro* conditions to neuromuscular,<sup>17,18</sup> cardiac, hepatocyte,<sup>19,20</sup> skin,<sup>21</sup> cartilaginous and bone cells.<sup>22,23</sup> These cells, are therefore, classified as pluripotent cells.

The WJ of the umbilical cord comparing to mature tissues such as brain, bone marrow, adipose tissue and circulating blood is much more accessible for collection of MSCs. In addition, easy accessibility, low antigenicity, low ethical concerns, embryo-like properties, high capacity of proliferation and differentiation to other various cell lineages, tissue regeneration along with immunomodulatory effects on immune system, low risk of viral infectivity and pain-free collection prioritized UC-MSCs a better therapeutic application

compared to Bone marrow derived-MSCs (BM-MSCs).<sup>11,24,25</sup>

Dendritic cells are the major antigen presenting cells which originate from both CD34-derived and monocyte-derived dendritic cells by induction of Granulocyte Macrophage-Colony Stimulating Factor (GM-CSF) and Interleukine-4 (IL-4).<sup>26,27</sup> They particularly play indispensable role in priming and directing naïve T cell immune responses.<sup>28,29</sup> Regarding the produced cytokines and environmental stimuli, dendritic cells are able to initiate and drive different types of immune responses. Based on the maturation and growth conditions, they can promote primary or secondary responses as well as T-regulatory (T<sub>Reg</sub>) cell regeneration and T cell tolerance.<sup>28-33</sup> In the previous studies on laboratory models, BM-MSCs have been shown to possess suppressive effects on T cells and their immune responses through secretion of factors like TGF- $\beta$ , prostaglandin E2 (PGE2) and Hepatocyte growth factor (HGF) which can be the cause of host unresponsiveness, and therefore possible role in graft protection.<sup>6,34,35</sup> The immunomodulatory effect of MSCs on immune responses can be contributed to the capacity of dendritic cells in producing tolerogenic antigen presenting cells thorough functional alterations.

Accordingly, the main aim of this study was to investigate immunomodulatory effects of Human UC-MSCs on monocyte-derived dendritic cells (indirect contact with Transwell culture systems).

## MATERIALS AND METHODS

### Collection and Primary Cultivation of MSCs Extracted from the Wharton's Jelly of the Umbilical Cord by Collagenase Assay

Umbilical cord samples were obtained after the delivery of normal-term babies with informed consent of the healthy mothers. Then the human umbilical cords were stored in sterile Hank's Balanced Salt Solution (HBSS) supplemented with 100 IU/ml penicillin, 100  $\mu$ g/ml streptomycin, and 0.025  $\mu$ g/ml Amphotericin B to be transported to cell culture laboratory. Next, the umbilical cords were washed with 75% ethylic alcohol for 30 seconds and the blood vessels were scraped off and then the Wharton's jellies cut up in 2-3mm pieces. The pieces then were centrifuged in Dulbecco's Modified Eagle Medium (DMEM) media (Gibco) at 250g for 5

## Effects of Mesenchymal Stem Cells on Monocyte-Derived Dendritic Cells

minutes and the isolated cells were incubated in DMEM media containing antibiotic-antimycotic (Invitrogen), and collagenase (1mg/ml) for 16-18 hours in 5% CO<sub>2</sub> in a 37 °C incubator. The adhered cells were trypsinized (0.25%) and transferred to the flasks containing DMEM-F12 media with fetal bovine serum (FBS) 10% (Gibco) and incubated for 1-2 weeks in 5% CO<sub>2</sub> at 37°C. The media were changed every 3-4 days. At this stage, three tests were applied: 1) Viability test (more than 95%), 2) Differentiation to adipocyte and osteocyte, 3) Cell surface marker expression (CD45, CD34, CD90, CD105, CD73, and HLA-DR) through flow cytometry analysis (Becton Dickinson) to confirm MSC cell profile. Finally, we froze the UC-MSCs cryogenically (10% DMSO and 90% FBS) for later use.

### Collection and Primary Cultivation of BM-MSCs

We used BM-MSCs as control to be compared to UC-MSCs. Bone marrow samples were obtained after informed consent from harvests of healthy donors for allogeneic transplantation. Mononuclear cells were centrifuged at 400g for 5 minutes in ficoll (1.077 g/ml; Invitrogen). Next, the cells were washed with phosphate-buffered saline (PBS) and cultured at 25cm<sup>2</sup> SPL flasks in DMEM-LG (Gibco) supplemented with 10% FBS, antibiotic-antimycotic (Invitrogen) for 2 weeks in 5% CO<sub>2</sub> in a 37°C incubator. After 72 hours, unadhered cells were washed away with PBS and the culture medium was refreshed. The medium was changed every 3-4 days to eventually obtain 80% confluent cells. The medium was then washed with PBS and the adhered MSC cells were treated with Trypsin-EDTA 0.25% and washed with PBS and FBS and cultured at 75 and 175 cm<sup>2</sup> flasks. Then, at this stage, three tests were applied: 1) Viability test (more than 95%), 2) Differentiation to adipocyte and osteocyte, 3) Cell surface marker expression (CD45, CD34, CD90, CD105, CD73, and HLA-DR) through flow cytometry analysis (Becton Dickinson) to confirm MSC cell profile. Finally, we froze the BM-MSCs cryogenically for later use.

### The Differentiation of the UC-MSCs and BM-MSCs to Adipose Cells

The isolated and cultivated mesenchymal stem cells from bone marrow and the Wharton's jelly of the umbilical cord (80% confluent at passage 2) were

used to be differentiated to adipocytes. The cultured cells in DMEM and FBS 10% were considered as control. The MSCs were washed with PBS and incubated in DMEM adipogenic differentiation medium supplemented with 10% FBS, ascorbate 1-phosphate (50mg/ml), dexamethasone (10<sup>-7</sup> M), indomethacine (50mg/ml) and 5µg/ml insulin in 5% CO<sub>2</sub> at 37°C for 15 days. The medium was changed every 4 days. On the day 15, the cells were washed with PBS two times and then fixed in 10% formaldehyde for 1 hour. Next, the fixed cells were re-washed with water and exposed to Oil Red O (0.5% in 99% isopropanol) for 15 minutes. Finally, the cells were washed with water and examined under inverted phase contrast microscope immediately to show adipogenic differentiation.

### The Differentiation of UC-MSCs and BM-MSCs to Osteocytes

The isolated and cultivated MSCs from bone marrow and the Wharton's jelly of the umbilical cord (80% confluent at passage) were used to be differentiated to osteocytes. The cultured cells in DMEM and FBS 10% were considered as control. The MSCs were washed with PBS and incubated in DMEM osteogenic differentiation medium supplemented with 10% FBS, ascorbate 2-phosphate (50mg/ml), dexametasone 10<sup>-8</sup>M, B-glycerol phosphate (10 mM) in 5% CO<sub>2</sub> at 37°C for 21 days. The medium was changed every 4 days. On the day 21, the cells were washed with PBS two times and then fixed in methanol for 10 minutes. Next, the fixed cells were stained with 1% alizarin red stain in 25% ammonia for 10-15 minutes, and washed with water two times. Finally, the cells were dried in air and examined under inverted phase contrast microscope immediately to detect intracellular calcium deposition (orange red color) and thereby osteogenic differentiation.

### The Isolation of Peripheral Blood Monocytes (PBMC) by Using the Magnetic-activated cell sorting (MACS) Monocyte Isolation Kit

The blood samples were obtained with heparinized sterile syringes from 15 healthy donors. Then, the samples were mixed and diluted with the equal volume of PBS and PBMC obtained using Ficoll-Hypaque (1.077g/ml; Invitrogen). Monocytes (more than 96%) were purified from PBMC by using the MACS monocyte Isolation kit (Miltenyi Biotec).

Different ratios (1:1, 1:2 and 1:10) of the isolated cells were co-cultured with the UC-MSCs and BM-MSCs in six-compartment culture plates.

### **The Cultivation of PBMC and Differentiation to Immature and Mature Dendritic Cells *in vitro***

CD14 Microbead acquired monocytes were cultured in three series of six-compartment culture plates containing whole RPMI-1640 medium (Gibco) supplemented with 10% FBS, streptomycin (100µg/ml), and penicillin (100u/ml). Then, GM-CSF (1000u/ml) and IL-4 (500u/ml; R&D) were added to all plates and incubated for 6 days in 5% CO<sub>2</sub> at 37° C to be differentiated to immature dendritic cells (iDCs). On day 3, fresh medium with the similar content of GM-CSF and IL-4 was added to the plates. On day 7, the cells in plate 1 and 2 were examined for immature dendritic cell surface markers (CD1a, CD14, CD80, CD83, CD86 and HLA-DR) through flowcytometry and endocytic activity through Fluorescein isothiocyanate dextran, respectively. On day 6, TNF- α (10ng/ml; R&D) as a dendritic cell maturation factor was added to the third plate. On day 9, the cells in the third plate (mature dendritic cells surface markers), were analyzed through flowcytometry.

### **The Co-cultivation of UC- and BM-MSCs with Dendritic Cells**

The six-compartment transwell plates with 0.4 µm pore sizes were used to assure cell contacts through their secretions rather than any direct physical contacts. With respect to different dilution ratios (1:1, 1:2, and 1:10), the collected monocytes were added to the lower chambers and the upper chambers were occupied with UC-MSCs or BM-MSCs. To assess surface marker expression profile, two separate plates were considered for co-cultivation of UC-MSCs and BM-MSCs with immature dendritic cells. To assess endocytic activity, two other separate plates were considered for co-cultivation of UC-MSCs and BM-MSCs with iDCs. Two more plates were used to co-cultivate UC-MSCs and BM-MSCs with mature dendritic cells (mDCs) to analyze surface marker expression profiles. RPMI-1640 supplemented with 10% FBS was used as the growth medium.

### **Dendritic Cell Endocytosis**

With respect to the different dilution ratios (1:1,

1:2, and 1:10), the collected monocytes were first cultured in two transwell plates containing the medium supplemented with IL-4 (20ng/ml) and GM-CSF (30ng/ml) for 6 days to generate iDCs. Next, the immature cells were separately co-cultured in transwell plates with UC-MSCs and BM-MSCs and incubated for 3 days. Then, the cultured iDCs were isolated and incubated at 37°C and at 4°C (as control) for 1 hour exposed to FITC-Dextran (1 mg/mL; molecular weight 40000 MW; Sigma). Next, the cells were washed with cold PBS two times to stop endocytosis. Finally, they were fixed in 1% formalin and analyzed through flowcytometry immediately.

### **Statistical Analysis**

Analytical statistics were performed using the SPSS 16. According to the quantitative data, Shapiro- Wilk test for normality of data distribution was assessed. Comparisons between different groups with control were performed using the paired sample *t* test. *P* values less than 0.05 were considered statistically significant.

## **RESULTS**

### **Comparison between UC-MSC and BM-MSC in Proliferation and Size**

We observed that UC-MSCs in addition to higher proliferation rate were larger in size and diameter compared to BM-MSCs and with increasing the passage number (P20) the proliferation rate remained unchanged although decreased in BM-MSCs (Figure 1).

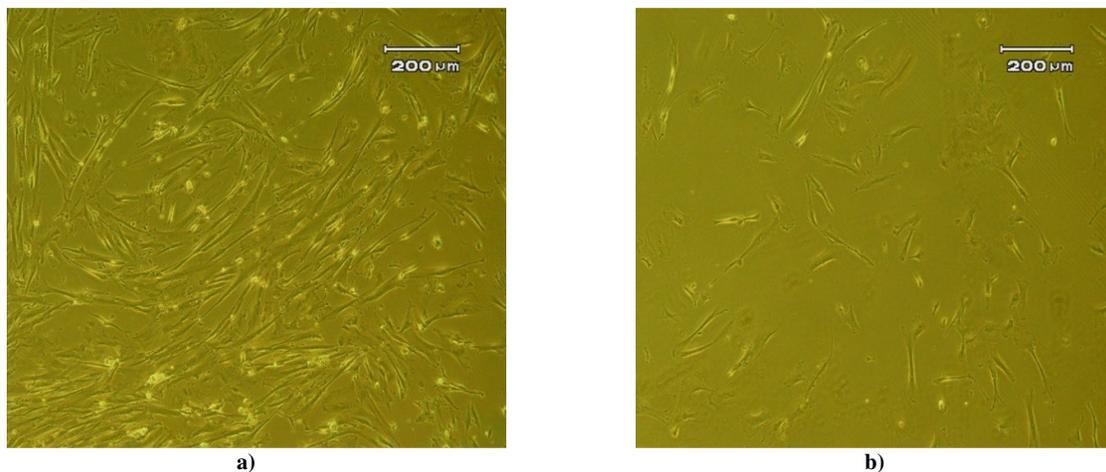
### **Osteogenic Differentiation of UC-MSCs and BM-MSCs**

The cells in UC-MSC and BM-MSC (date not shown) were separately cultured in osteogenic differentiation medium for 21 days. The cultured cells in DMEM and 10% FBS were considered as negative control. On day 21, the cells (calcium deposition) were stained with Alizarin Red dye to confirm osteogenic patterning (Figure 2a, b).

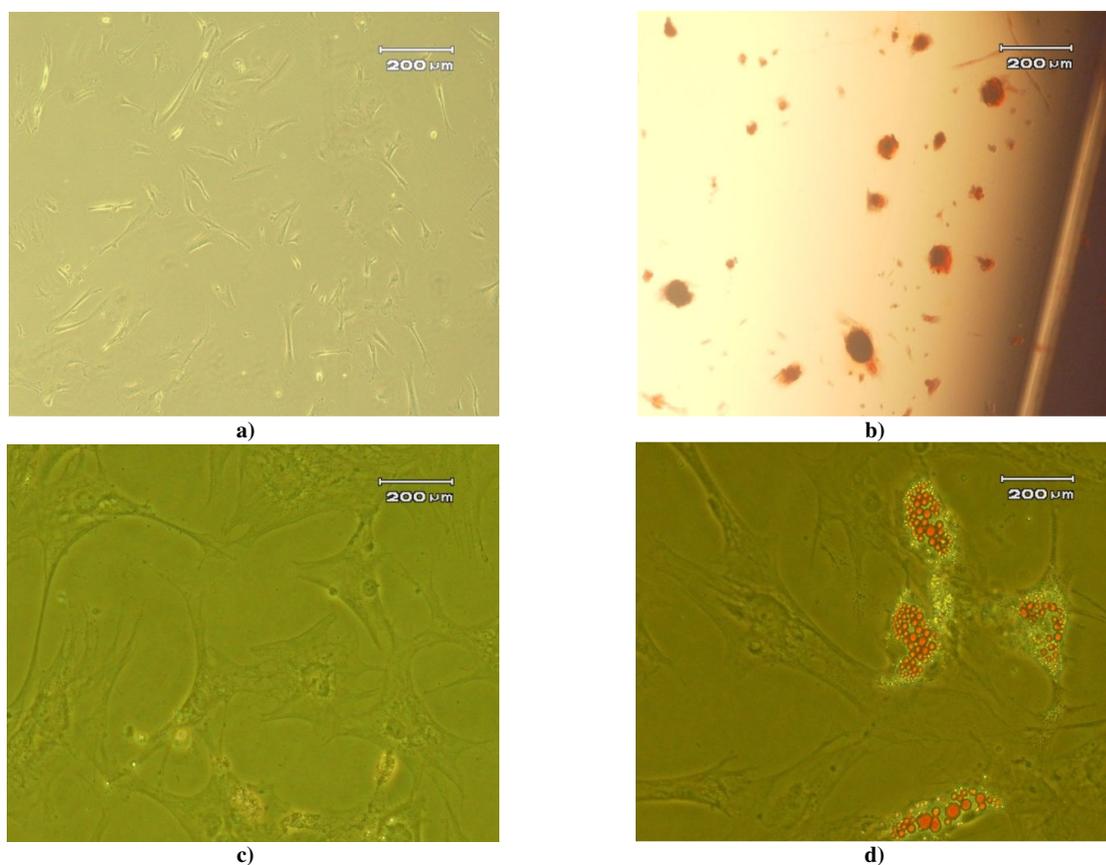
### **Adipogenic Differentiation of UC-MSCs and BM-MSCs**

The cells in UC-MSC and BM-MSC were separately cultured in adipogenic differentiation medium for 15 days. The cultured cells in DMEM and 10% FBS were considered as negative control.

## Effects of Mesenchymal Stem Cells on Monocyte-Derived Dendritic Cells

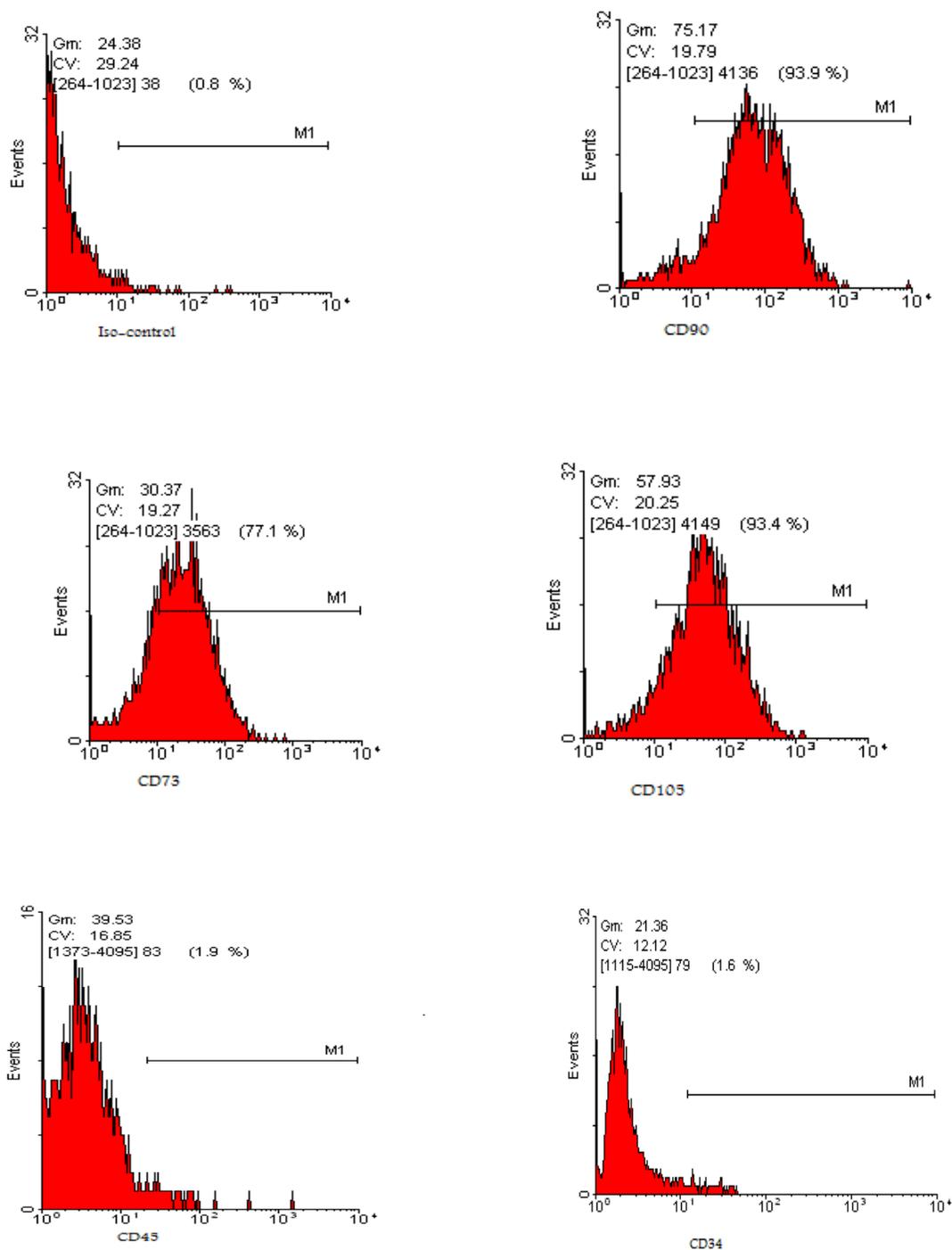


**Figure 1. Characteristics of UC-MSCs (a) and BM-MSCs (b). The cells possess fibroblast-like morphology.**



**Figure 2. Osteogenic and Adipogenic differentiation of UC-MSCs.**

**a) UC-MSCs after 21 days in DMEM-F12 medium with no color change under Alizarin Red S staining (Negative control, 40x). b) Osteogenic induction in UC-MSCs after 21 days in DMEM-F12 supplemented with growth factors, intracellular calcium deposition (orange red) under Alizarin Red S staining in osteocytes (40X). c) UC-MSCs after 15 days in DMEM-F12 lacking of fat droplets under Oil Red O staining (Negative control, 40x). d) Adipogenic induction in UC-MSCs after 15 days in DMEM-F12 supplemented with growth factors, intracellular vacuoles containing fat droplets (dark red) under Oil Red O staining in adipocytes (40x).**



**Figure 3.** Flow cytometric analysis of UC-MSCs' surface markers. UC-MSCs were cultured in DMEM-F12 supplemented with 10% FBS. Then, the cells were analyzed by flowcytometry. Similar results were obtained with BM-MSCs (data not shown).

## Effects of Mesenchymal Stem Cells on Monocyte-Derived Dendritic Cells

Fat vacuoles were microscopically observed in BM-MSC (data not shown) and the WJ of the umbilical cord in adipogenic differentiation medium over 3-4 days after cultivation and increased in number with time such that the cytoplasm of some cells were accumulated with such vacuoles at the end of the second week. On day 15, the cells were stained with Oil Red O to confirm adipogenic patterning (Red cytoplasm, Figure 2c, d).

### Flow Cytometric Analysis of Surface-marker Expression on UC-MSCs and BM-MSCs

The UC-MSCs and BM-MSCs were cultured in DMEM-F12 supplemented with 10% FBS (Passage 2). The surface markers including CD73, CD90, CD105, CD34, and CD45 were measured by flowcytometry. Our data showed that UC-MSCs and BM-MSCs had the same surface marker profiles (Figure 3).

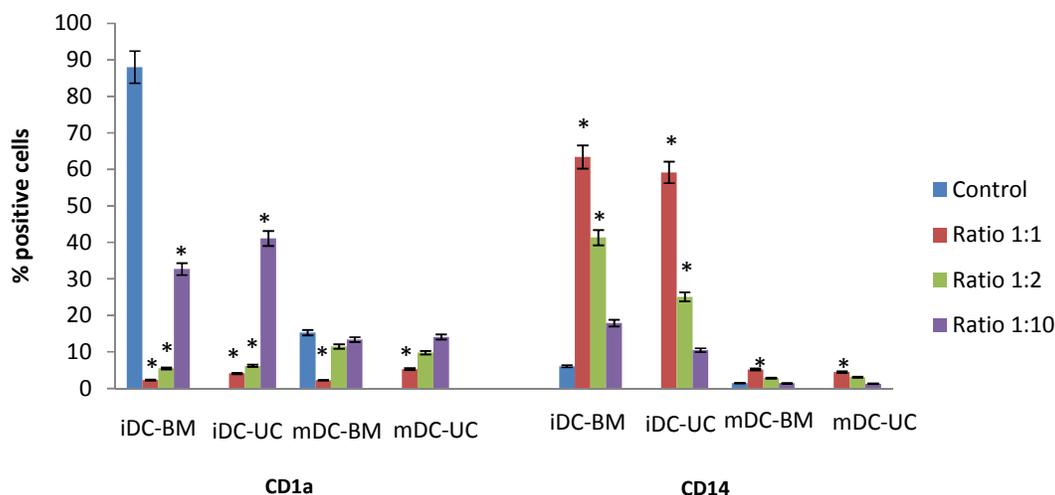
### UC-MSC Inhibitory Effect on Differentiation and Maturation Markers in Monocyte-Derived Dendritic Cells

Monocytes were cultured in medium supplemented with GM-CSF and IL-4 for 6 days and then TNF- $\alpha$  for 2 days at 37°C and 5% CO<sub>2</sub> to differentiate to immature

and mature dendritic cells, respectively. We observed that UC-MSCs down-regulated maturation and differentiation markers on immature and mature dendritic cells and by increasing the cell dilution ratio, the inhibitory effect also increased. CD1a surface marker was down-regulated in immature and mature dendritic cell differentiation stages particularly in 1:1, 1:2 and 1:10 ratio and 1:1 ratio, respectively, so that with rise in cell dilution ratio, the effect augmented and vice versa (Figure 4). Also, CD14 surface marker expression was up-regulated in immature and mature dendritic cell differentiation stages particularly in 1:1, 1:2 ratio and 1:1 ratio, respectively (Figure 4).

The costimulatory molecules of CD86/CD80 expression were down regulated significantly in 1:1 ratio and 1:1, 1:2 ratio in immature and mature differentiation stages, respectively. So that with rise in cell dilution ratio the effect augmented and vice versa (Figure 5).

The expression of CD83 and HLA-DR molecules were down-regulated significantly particularly in 1:1 ratio and 1:1, 1:2 ratio in immature and mature differentiation stage, respectively, so that with rise in cell dilution ratio, the effect augmented and vice versa (Figure 6).

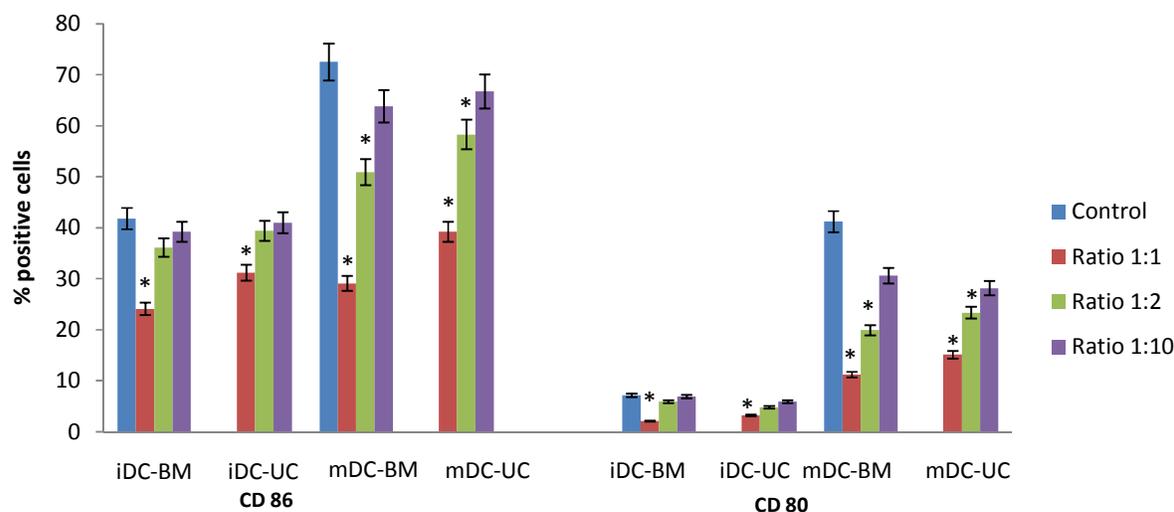


**Figure 4.** The effect of UC-MSCs and BM-MSCs on CD1a and CD14 markers in iDCs and mDCs.

UC-MSCs and BM-MSCs are compared to the control group (in blue). Statistical analysis revealed significant differences in UC-MSC and BM-MSC inhibitory effects on CD1a marker on iDCs at dilutions 1:1 ( $p=0.001$ ), 1:2 ( $p=0.007$ ) and 1:10 ( $p=0.008$ ) and also on mDCs at dilution 1:1 ( $p=0.01$ ). Differences in UC-MSC enhancing effect on CD14 marker on iDCs at dilutions 1:1 ( $p=0.007$ ), 1:2 ( $p=0.02$ ) and also on mDCs at dilution 1:1 ( $p=0.01$ ) were statistically significant. Bars represent the mean  $\pm$ Standard Error (S.E).

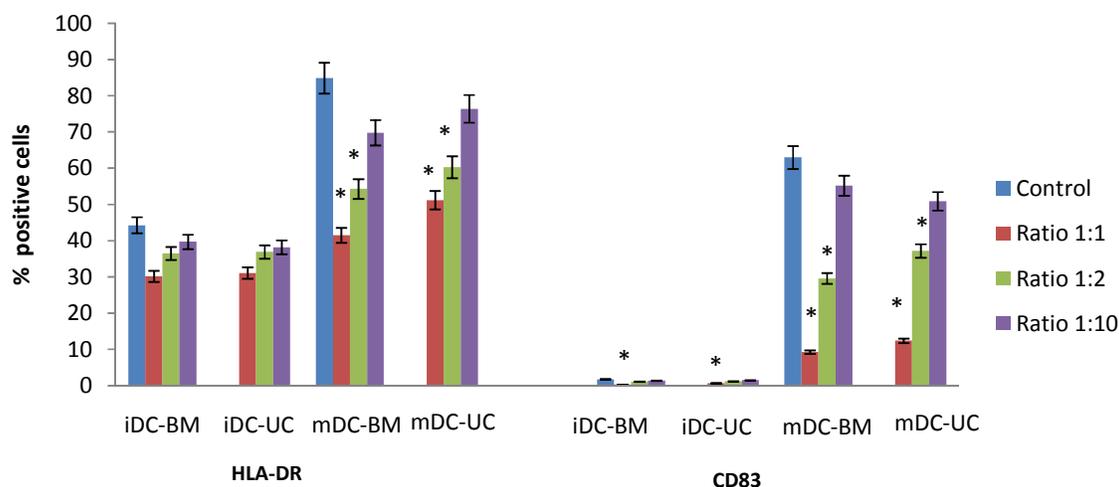
iDC (immature dendritic cell); mDC (mature dendritic cell); BM (Bone marrow); UC (Umbilical cord)

\* indicates  $p < 0.05$



**Figure 5.** The effect of UC-MSCs and BM-MSCs on CD80 and CD86 markers in iDCs and mDCs. UC-MSCs and BM-MSCs are compared to the control group (in blue). Differences in UC-MSC and BM-MSC inhibitory effect on CD80 marker on iDCs at dilution 1:1 ( $p=0.04$ ) and on mDCs at dilutions 1:1 ( $p=0.005$ ) and 1:2 ( $p=0.025$ ) were statistically significant. Differences in UC-MSC inhibitory effect on CD86 marker on iDCs at dilution 1:1 ( $p=0.01$ ) and on mDCs at dilutions 1:1 ( $p=0.001$ ) and 1:2 ( $p=0.03$ ) were statistically significant. Bars represent the mean  $\pm$ standard Error (S.E).

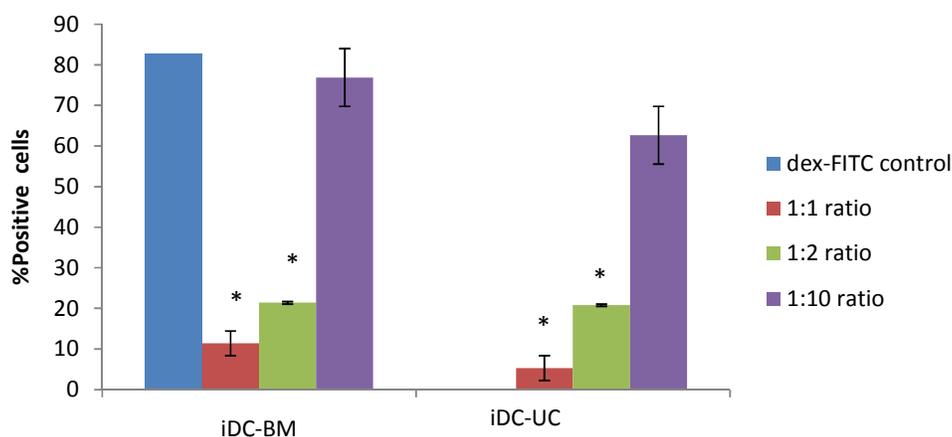
\* indicates  $p < 0.05$ .



**Figure 6.** The effect of UC-MSCs and BM-MSCs on CD83 and HLA-DR markers in iDCs and mDCs. UC-MSCs and BM-MSCs are compared to the control group (in blue). Differences in UC-MSC and BM-MSC inhibitory effect on CD83 marker on iDCs at dilution 1:1 ( $p=0.04$ ) and on mDCs at dilution 1:1 ( $p=0.007$ ) and 1:2 ( $p=0.015$ ) were statistically significant. Differences in UC-MSC inhibitory effect on HLA-DR marker on mDCs at dilutions 1:1 ( $p=0.006$ ) and 1:2 ( $p=0.03$ ) were statistically significant. Bars represent the mean  $\pm$ standard Error (S.E).

\* indicates  $p < 0.05$ .

## Effects of Mesenchymal Stem Cells on Monocyte-Derived Dendritic Cells



**Figure 7.** The effect of UC-MSCs and BM-MSCs on endocytic activity in iDCs. Differences in UC-MSC and BM-MSC inhibitory effects on iDCs at dilution ratio 1:1 ( $p=0.006$ ) and 1:2 ( $p=0.02$ ) were statistically significant. Bars represent the mean  $\pm$  standard Error (S.E).

\* indicates  $p < 0.05$ .

### The Effect of Secreted Factors from UC-MSCs and BM-MSCs on Dendritic Cell Endocytic Activity

We used transwell culture system (pore size  $0.4\mu\text{m}$ ) to co-culture iDCs in the absence and presence of UC-MSCs and BM-MSC. As expected, UC-MSCs were capable of declining endocytic activity of iDCs (particularly in 1:1 cell dilution ratio) so with increasing the dilution ratio, the endocytic activity was positively affected and vice versa (Figure 7).

## DISCUSSION

UC-MSCs due to their easy preparation, lack of expression of the surface antigenic markers such as HLA-class II, the ability to undergo sustained proliferation and the potential to give rise to multiple mesenchymal cell lineages, received much more attention over the recent years. It has been suggested that these cells could be applied in cell therapy and regeneration of the damaged biological structures.<sup>36-38</sup> These cells, in spite of their distribution and poor quantity in the Wharton's jelly of the umbilical cord, can secrete large amount of growth factors such as EGF, TGF- $\beta$ , IGF-1 and Glial cell-derived neurotrophic factor (GDNF) to influence proliferation and growth of the other cell populations.<sup>15, 24, 39</sup>

Some studies demonstrated that BM-MSCs modulate maturation markers and also T lymphocytes<sup>35</sup>

and dendritic cell functions<sup>47-52</sup> via cell-cell contact or secreted factors. This modulatory effect has been slightly studied in UC-MSCs rather than BM-MSCs.

In the present study, we investigated the immunomodulatory effects of BM-MSCs and UC-MSCs on monocyte-derived dendritic cells. In this system, the secreted factors from MSCs could cross the transwell plate and penetrate to the chamber designated for dendritic cells. Our data showed that UC-MSCs like BM-MSCs have the same surface marker profile (Figure 1). Also UC-MSCs like BM-MSCs can differentiate in *in vitro* conditions to osteocytes and adipocytes over 2-3 weeks (Figure 2).

Interestingly, we observed that UC-MSCs like BM-MSCs can easily grow and proliferate in DMEM-F12 medium supplemented with 10% FBS. UC-MSC proliferation was clearly much more evident than BM-MSCs so that we sometimes had to culture the cells every 3 days. Although the BM-MSCs changed morphologically and their dedifferentiation compromised over frequent passages, we cultured UC-MSCs up to 20 times with no detectable changes in shape and their differentiation to osteocytes and adipocytes (Figures 2, 3).

Therefore UC-MSCs may have an extended culture capacity compared to BM-MSCs, specifically greater than 20 passages. In fact, it has been shown that UC-MSCs are transitional between embryonic and adult

stem cells and possess higher proliferation and differentiation rate than BM-MSCs and therefore can be helpful in research and clinical applications.<sup>11, 25</sup>

In this study, we showed that Co-cultivation of UC-MSCs and BM-MSCs with monocytes during dendritic cell differentiation and maturation period inhibited strongly the initial differentiation of CD14<sup>+</sup> monocytes into CD1a<sup>+</sup> DCs via soluble factors which indicate that UC-MSCs and BM-MSCs impeded reasonably the differentiation of monocytes to immature dendritic cells (Figure 4).

In the case of mature DCs, our data showed that UC-MSCs were able to down-regulate expression of costimulatory molecules CD80, CD86 and CD83 (a key marker in mature dendritic cells) particularly at higher dilutions. Hence, modulation of DC differentiation and function is relevant to the immunomodulatory effects of UC-MSCs. Therefore, their down-regulation caused a defect in differentiation of immature dendritic cells to mature ones and eventually a deficiency in antigen presentation (Figures 5, 6). Nevertheless, the modulatory effect led to down-regulation of the other dendritic cells' surface markers which was consistent with other studies.<sup>47-50, 52</sup>

Also, we found that modulatory effects of UC-MSCs and BM-MSCs on markers of differentiation, maturation and endocytic activity of dendritic cell was dose dependent so that with rise in cell dilution ratio from 1:1 to 1:10 this effect showed statistically significant difference (Figures 4,5,6,7).

As we know, the main function of DCs is to take up and process antigen material and present it on the surface to naïve T lymphocytes and other cells of the immune system to generate immune responses. Immature DCs display a high ability for antigen uptake and processing.<sup>31, 51, 52</sup>

In this study, we co-cultured monocyte-derived iDCs with UC-MSCs and BM-MSCs and analyzed their ability to take up fluorescently labeled dextran (endocytic activity). We found that UC-MSCs and BM-MSCs can modulate them to large extent. The immunomodulatory effects of UC-MSCs sounded much stronger compared to BM-MSCs so that with increase in cell dilution ratio, the endocytic activity of iDCs decreased and vice versa (Figure 7).

The ability of DCs to initiate an immune response depends on their transition from antigen processing to antigen-presenting cells, during which they up-regulate class II major histocompatibility complex II (HLA-DR)

and T-cell costimulatory molecules (CD80,CD86) on the cell surface, a process referred to as DC maturation.<sup>53,54</sup> This transition constitutes an important checkpoint in mounting an immune response because iDCs not only fail to prime T cells effectively but also serve to promote tolerance induction.<sup>55,56</sup> Thus, DCs are critical in the initiation of primary immune responses, becoming an important target for immunosuppression to prevent allograft rejection.<sup>57,58</sup>

In general, our data showed that UC-MSCs like BM-MSCs possess significant inhibitory effects on differentiation and maturation markers and also endocytic activity of immature and mature dendritic cells. In addition, the isolation of UC-MSCs is easier compared to BM-MSCs. These cells proliferate rapidly in cell culture flasks to high passage numbers which can be eventually frozen.

In summary, Our results show that UC-MSCs are able to on cell differentiation, expression of surface markers of monocyte-derived dendritic cell and antigen uptake have a significantly impact.

On the other hand, isolation and cultivation of UC-MSCs compared to BM-MSCs is easier and with less ethical problems. Therefore, UC-MSCs as a new source of MSCs could be advantageous for future therapeutic and immunomodulatory applications in recovery and reduction of autoimmune and inflammatory diseases.

#### ACKNOWLEDGEMENTS

We thank research deputy management of Tehran University of Medical Sciences for assistance and financial support. We also thank the specialists, stem cell transplant center staff, Mr. Javanmardi and Mrs. Raoufi in Tehran Shariati hospital who helped us in bone marrow and umbilical cord sample preparation.

#### REFERENCES

1. Kørbling M, Estrov Z. Adult stem cells for tissue repair - a new therapeutic concept? *N Engl J Med* 2003; 349 (6):570-82.
2. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, et al. Multilineage potential of adult human mesenchymal stem cells. *Science* 1999; 284(5411):143-7.

## Effects of Mesenchymal Stem Cells on Monocyte-Derived Dendritic Cells

3. Chamberlain G, Fox J, Ashton B, Middleton J. Mesenchymal stem cells: their phenotype, differentiation capacity, immunological features, and potential for homing. *Stem Cells* 2007; 25(11):2739-49.
4. Uccelli A, Moretta L, Pistoia V. Mesenchymal stem cells in health and disease. *Nat Rev Immunol* 2008; 8(9):726-36.
5. Frank P, Barry, Raymond E, Boynton, Stephen Haynesworth, J, Mary Murphy, and Joseph Zaia. The Monoclonal Antibody SH-2, Raised against Human Mesenchymal Stem Cells, Recognizes an Epitope on Endoglin (CD105). *Biochemical and Biophysical Research Communications*. 1999; 265, 134-139
6. Bartholomew A, Sturgeon C, Siatskas M, et al. Mesenchymal stem cells suppress lymphocyte proliferation in vitro and prolong skin graft survival in vivo. *Exp Hematol* 2002; 30(1):42-8.
7. Oswald J, Boxberger S, Jorgensen B, Feldmann S, Ehninger G, Bornhäuser M, et al. Mesenchymal stem cells can be differentiated into endothelial cells in vitro. *Stem Cells* 2004; 22(3):377-84.
8. Conconi MT, Burra P, Di Liddo R, Calore C, Turetta M, Bellini S, et al. CD105(+) cells from Wharton's jelly show in vitro and in vivo myogenic differentiative potential. *Int J Mol Med* 2006; 18(6):1089-96.
9. Troyer DL, Weiss ML. Wharton's jelly-derived cells are a primitive stromal cell population. *Stem Cells* 2008; 26(3):591-9.
10. Wang HS, Hung SC, Peng ST, Huang CC, Wei HM, Guo YJ, et al. Mesenchymal stem cells in the Wharton's jelly of the human umbilical cord. *Stem Cells* 2004; 22(7):1330-7.
11. Zeddou M, Briquet A, Relic B, Josse C, Malaise MG, Gothot A, et al. The umbilical cord matrix is a better source of mesenchymal stem cells (MSC) than the umbilical cord blood. *Cell Biol Int* 2010; 34(7):693-701.
12. Romanov YA, Svintsitskaya VA, Smirnov VN. Searching for alternative sources of postnatal human mesenchymal stem cells: candidate MSC-like cells from umbilical cord. *Stem Cells* 2003; 21(1):105-10.
13. McElreavey KD, Irvine AI, Ennis KT, McLean WH. Isolation, culture and characterisation of fibroblast-like cells derived from the Wharton's jelly portion of human umbilical cord. *Biochem Soc Trans* 1991; 19(1):29S.
14. Kobayashi K, Kubota T, Aso T. Study on myofibroblast differentiation in the stromal cells of Wharton's jelly: expression and localization of alpha-smooth muscle actin. *Early Hum Dev* 1998; 51(3):223-33.
15. Carlin R, Davis D, Weiss M, Schultz B, Troyer D. Expression of early transcription factors Oct-4, Sox-2 and Nanog by porcine umbilical cord (PUC) matrix cells. *Reprod Biol Endocrinol* 2006; 4:8.
16. Sobolewski K, Małkowski A, Bańkowski E, Jaworski S. Wharton's jelly as a reservoir of peptide growth factors. *Placenta* 2005; 26(10):747-52.
17. Mitchell KE, Weiss ML, Mitchell BM, Martin P, Davis D, Morales L, et al. Matrix cells from Wharton's jelly form neurons and glia. *Stem Cells* 2003; 21(1):50-60.
18. Zhang HT, Fan J, Cai YQ, Zhao SJ, Xue S, Lin JH, et al. Human Wharton's jelly cells can be induced to differentiate into growth factor-secreting oligodendrocyte progenitor-like cells. *Differentiation* 2010; 79(1):15-20.
19. Zhang YN, Lie PC, Wei X. Differentiation of mesenchymal stromal cells derived from umbilical cord Wharton's jelly into hepatocyte-like cells. *Cytotherapy* 2009; 11(5):548-58.
20. Anzalone R, Lo Iacono M, Corrao S, Magno F, Loria T, Cappello F, et al. New emerging potentials for human Wharton's jelly mesenchymal stem cells: immunological features and hepatocyte-like differentiative capacity. *Stem Cells Dev* 2010; 19(4):423-38.
21. Schneider RK, Püllen A, Kramann R, Bornemann J, Knüchel R, Neuss S, et al. Long-term survival and characterization of human umbilical cord-derived mesenchymal stem cells on dermal equivalents. *Differentiation* 2010;79(3):182-93.
22. Xu HH, Zhao L, Detamore MS, Takagi S, Chow LC. Umbilical cord stem cell seeding on fast-resorbable calcium phosphate bone cement. *Tissue Eng Part A* 2010; 16(9):2743-53.
23. Caballero M, Reed CR, Madan G, van Aalst JA. Osteoinduction in umbilical cord- and palate periosteum-derived mesenchymal stem cells. *Ann Plast Surg* 2010; 64(5):605-9.
24. Raio L, Cromi A, Ghezzi F, Passi A, Karousou E, Viola M, et al. Hyaluronan content of Wharton's jelly in healthy and Down syndrome fetuses. *Matrix Biol* 2005; 24(2):166-74.
25. Prasanna SJ, Gopalakrishnan D, Shankar SR, Vasandan AB. Pro-inflammatory cytokines, IFN $\gamma$  and TNF $\alpha$ , influence immune properties of human bone marrow and Wharton jelly mesenchymal stem cells differentially. *PLoS One* 2010; 5(2):e9016.
26. Romani N, Gruner S, Brang D, Kämpgen E, Lenz A, Trockenbacher B, et al. Proliferating dendritic cell progenitors in human blood. *J Exp Med* 1994; 180(1):83-93.

27. Chapuis F, Rosenzweig M, Yagello M, Ekman M, Biberfeld P, Gluckman JC. Differentiation of human dendritic cells from monocytes in vitro. *Eur J Immunol* 1997; 27(2):431-41
28. Banchereau J, Briere F, Caux C, Davoust J, Lebecque S, Liu YJ, et al. Immunobiology of dendritic cells. *Annu Rev Immunol* 2000; 18:767-811.
29. Liu YJ, Kanzler H, Soumelis V, Gilliet M. Dendritic cell lineage, plasticity and cross-regulation. *Nat Immunol* 2001; 2(7):585-9.
30. Steinman RM, Hawiger D, Nussenzweig MC. Tolerogenic dendritic cells. *Annu Rev Immunol* 2003; 21:685-711.
31. Smits HH, de Jong EC, Wierenga EA, Kapsenberg ML. Different faces of regulatory DCs in homeostasis and immunity. *Trends Immunol* 2005; 26(3):123-9.
32. Steinman RM, Banchereau J. Taking dendritic cells into medicine. *Nature* 2007; 449(7161):419-26.
33. Nauta AJ, Westerhuis G, Kruisselbrink AB, Lurvink EG, Willemze R, Fibbe WE. Donor-derived mesenchymal stem cells are immunogenic in an allogeneic host and stimulate donor graft rejection in a nonmyeloablative setting. *Blood* 2006; 108(6):2114-20.
34. Di Nicola M, Carlo-Stella C, Magni M, Milanese M, Longoni PD, Matteucci P, et al. Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. *Blood* 2002; 99(10):3838-43.
35. Yang SH, Park MJ, Yoon IH, Kim SY, Hong SH, Shin JY, et al. Soluble Mediators from Mesenchymal Stem Cells Suppress T cell Proliferation by inducing IL-10. *Exp Mol Med* 2009; 41(5):315-24.
36. Bailey MM, Wang L, Bode CJ, Mitchell KE, Detamore MS. A comparison of human umbilical cord matrix stem cells and temporomandibular joint condylar chondrocytes for tissue engineering temporomandibular joint condylar cartilage. *Tissue Eng* 2007; 13(8):2003-10.
37. Jomura S, Uy M, Mitchell K, Dallasen R, Bode CJ, Xu Y. Potential treatment of cerebral global ischemia with Oct-4+ umbilical cord matrix cells. *Stem Cells*. 2007; 25(1):98-106.
38. Tian X, Fu R, Deng L. [Method and conditions of isolation and proliferation of multipotent mesenchymal stem cells]. *Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi* 2007; 21(1):81-5.
39. Sobolewski K, Małkowski A, Bańkowski E, Jaworski S. Wharton's jelly as a reservoir of peptide growth factors. *Placenta* 2005; 26(10):747-52.
40. Janderova L, McNeil M, Murrell AN, Mynatt RL, Smith SR. Human mesenchymal stem cells as an in vitro model for human adipogenesis. *Obes Res* 2003 11(1):65-74.
41. Le Blanc K, Tammik L, Sundberg B, Haynesworth SE, Ringdén O. Mesenchymal stem cells inhibit and stimulate mixed lymphocyte cultures and mitogenic responses independently of the major histocompatibility complex. *Scand J Immunol* 2003; 57(1):11-20.
42. Krampera M, Glennie S, Dyson J, Scott D, Laylor R, Simpson E, et al. Bone marrow mesenchymal stem cells inhibit the response of naive and memory antigen-specific T cells to their cognate peptide. *Blood* 2003; 101(9):3722-9.
43. Tse WT, Pendleton JD, Beyer WM, Egalka MC, Guinan EC. Suppression of allogeneic T-cell proliferation by human marrow stromal cells: implications in transplantation. *Transplantation* 2003; 75(3):389-97.
44. Djouad F, Ponce P, Bony C, Tropel P, Apparailly F, Sany J, et al. Immunosuppressive effect of mesenchymal stem cells favors tumor growth in allogeneic animals. *Blood* 2003; 102(10):3837-44.
45. Meisel R, Zibert A, Laryea M, Göbel U, Däubener W, Dilloo D. Human bone marrow stromal cells inhibit allogeneic T-cell responses by indoleamine 2,3-dioxygenase-mediated tryptophan degradation. *Blood* 2004; 103(12):4619-21.
46. Potian JA, Aviv H, Ponzio NM, Harrison JS, Rameshwar P. Veto-like activity of mesenchymal stem cells: functional discrimination between cellular responses to alloantigens and recall antigens. *J Immunol* 2003; 171(7):3426-34.
47. Jiang XX, Zhang Y, Liu B, Zhang SX, Wu Y, Yu XD, et al. Human mesenchymal stem cells inhibit differentiation and function of monocytederived dendritic cells. *Blood* 2005; 105(10):4120-6.
48. Nauta AJ, Kruisselbrink AB, Lurvink E, Willemze R, Fibbe WE. Mesenchymal stem cells inhibit generation and function of both CD34 + -derived and monocyte-derived dendritic cells. *J Immunol* 2006; 177(4):2080-7.
49. Zhang W, Ge W, Li C, You S, Liao L, Han Q, et al. Effects of mesenchymal stem cells on differentiation: maturation, and function of human monocyte-derived dendritic cells. *Stem Cells Dev* 2004; 13(3):263-71.
50. Ramasamy R, Fazekasova H, Lam EW, Soeiro I, Lombardi G, Dazzi F. Mesenchymal stem cells inhibit dendritic cell differentiation and function by preventing entry into the cell cycle. *Transplantation* 2007; 83(1):71-6.

## Effects of Mesenchymal Stem Cells on Monocyte-Derived Dendritic Cells

51. Antony J. Cutler, Vasanti Limbani, John Girdlestone, and Cristina V. Navarrete. Umbilical Cord-Derived Mesenchymal Stromal Cells Modulate Monocyte Function to Suppress T Cell Proliferation. *The Journal of Immunology*, 2010, 185: 6617–6623.
52. Lutz MB, Suri RM, Niimi M, Ogilvie AL, Kukutsch NA, Rossner S, et al. Immature dendritic cells generated with low doses of GM-CSF in the absence of IL-4 are maturation resistant and prolong allograft survival in vivo. *Eur J Immunol* 2000; 30(7):1813–22.
53. Mellman I, Steinman RM. Dendritic cells: specialized and regulated antigen processing machines. *Cell* 2001; 106(3):255–8.
54. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. *Nature* 1998; 392(6673):245–52.
55. Hackstein H, Morelli AE, Thomson AW. Designer dendritic cells for tolerance induction: guided not misguided missiles. *Trends Immunol* 2001; 22(8):437–42.
56. Fu F, Li Y, Qian S, Lu L, Chambers F, Starzl TE, et al. Costimulatory molecule-deficient dendritic cell progenitors (MHC class II+, CD80dim, D86-) prolong cardiac allograft survival in nonimmunosuppressed recipients. *Transplantation* 1996; 62(5):659–65.
57. Josien R, Heslan M, Brouard S, Souillou JP, Cuturi MC. Critical requirement for graft passenger leukocytes in allograft tolerance induced by donor blood transfusion. *Blood* 1998; 92(12):4539–44.
58. Sallusto F, Lanzavecchia A. Mobilizing dendritic cells for tolerance, priming, and chronic inflammation *J Exp Med* 1999; 189(4):611–4.