Human mesenchymal stem cells inhibit the differentiation and effector functions of monocytes

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Abstract
Although monocytes represent an essential part of the host defence system, their accumulation and prolonged stimulation could be detrimental and may aggravate chronic inflammatory diseases. The present study has explored the less-understood immunomodulatory effects of mesenchymal stem cells on monocyte functions. Isolated purified human monocytes were co-cultured with human umbilical cord-derived mesenchymal stem cells under appropriate culture conditions to assess monocytes' vital functions. Based on the surface marker analysis, mesenchymal stem cells halted monocyte differentiation into dendritic cells and macrophages and reduced their phagocytosis functions, which rendered an inability to stimulate T-cell proliferation. The present study confers that mesenchymal stem cells exerted potent immunosuppressive activity on monocyte functions such as differentiation, phagocytosis and Ag presentation; hence, they promise a potential therapeutic role in down-regulating the unwanted monocyte-mediated immune responses in the context of chronic inflammatory diseases.

Keywords
Mesenchymal stem cells, monocytes, phagocytosis, antigen presentation, immunosuppression

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Introduction

The integrity of the host defence system is critically controlled by the balance of immune activation and immunosuppression. The fate or direction of the immune response is governed by many factors, within which monocytes play a critical role by not only being innate immune cells but also by delivering details of the inflammation or infection to the adaptive immune cells in the form of Ag presentation.[1] In contrast, monocytes contribute to the establishment of persistent infections and chronic inflammation.[2] The role of monocytes during inflammation through various models of bacterial, viral infections, cancer, atherosclerosis and autoimmunity has been widely studied and reported.[3]

During homeostasis and inflammation, monocytes leave the bloodstream and migrate to the tissues. Depending on the chemokines, growth factors, proinflammatory cytokines and microbial products, they differentiate into macrophages (MAC) and perform effector functions as phagocytes and APC. Recruitment of monocytes is essential for effective control and clearance of bacterial, fungal and protozoal

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infections. However, ungoverned recruitment and activation of monocytes contributes to the pathogenesis of many inflammatory and degenerative diseases.[2,3] The dual functions of monocytes as immune response initiators at the beginning of acute inflammation and contributors to detrimental chronic inflammation should be kept in control to ensure well-balanced immune responses. One of the potential tools that could check monocytes' activities are mesenchymal stem cells (MSC).

MSC are adult stem cells, often called multipotent stromal cells, found mainly in the bone marrow (BM) and several other tissues such as adipose tissues, blood, pancreas, dental pulp, umbilical cord and placenta.[4–6] In the stem cell niche of BM, MSC provide the necessary stimuli via physical and paracrine interactions to the haematopoietic stem cells for self-renewal and differentiation.[7] Apart from supporting haematopoiesis, MSC have been shown to modulate the activation, proliferation and effector functions of both innate and adaptive immune cells. The immunosuppressive function of MSC was well documented in both *in vitro* and animal model studies of T and B cells, NK cells and induced regulatory T-cell proliferation.[8–10] Although the proliferation phase of the immune response is a crucial step for adaptive immune cells, MSC also exert potent suppression of monocyte activation and the subsequent differentiation to the dendritic cells (DC) and MAC.[11,12] The present study explores the immunosuppressive functions of human umbilical cord-derived MSC on human primary monocytes, where differentiation, phagocytosis and, more importantly, the ability to stimulate T cells were deciphered in the presence of MSC.

Materials and methods

MSC cultures

Fully characterised human umbilical cord MSC were obtained from the Stem Cell & Immunity Research Group, Immunology Laboratory, Department of Pathology, Faculty of Medicine and Health Sciences, University Putra Malaysia.[13,14] Human samples were collected after obtaining written consent from the donors and the use of the human samples was approved by the Ethics and Research Committee of the Faculty of Medicine and Health Sciences, University Putra Malaysia. MSC were cultured in DMEM-F12 consisting of GLUTAMAX (Gibco, United Kingdom) and supplemented with 10% commercially available optimised MSC serum (Thermo Fisher Scientific, USE + A), 1% penicillin/streptomycin, 0.5% fungizone and 0.1% gentamycin (Gibco, UK). Early passages of MSC (P3–P8) were used in all

experiments. The inhouse produced MSC was characterised according to the minimal criteria defined by the International Society for Cellular Therapy (data not shown).[14,15]

Isolation of monocytes from human PBMC

After receiving informed consent, 20 ml of whole blood were collected from a healthy donor and immediately processed within 20 min by diluting in $1 \times$ PBS (Gibco, UK) without calcium and magnesium ions at the ration of 1:1. Diluted blood layered over 5 ml Ficoll-Paque solution (GE Healthcare, Life Sciences, Sweden) for gradient centrifugation for 30 min at $185\,g$ without deceleration. The PBMCs, which appeared as a white ring at the interface of plasma and Ficoll-Paque reagent, were collected and immediately suspended in 25 ml of $1 \times$ PBS buffer for monocyte separation. Cells were subjected for the monocyte isolation according to the manufacturer's instructions using a commercially available Monocyte Isolation Kit II (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany). Briefly, human monocytes were isolated by depletion of non-monocytes (negative selection). Non-monocytes were magnetically labelled with a cocktail of biotin-conjugated mAbs as a primary labelling reagent and anti-biotin mAbs conjugated to microbeads as a secondary labelling reagent. The magnetically labelled non-monocytes were depleted by being retained on a MACS® Column in the magnetic field of a MACS separator, while the unlabelled monocytes passed through the column. The percentage of CD14-positive cells was confirmed by flow cytometry analysis.

Differentiation of monocytes towards DCs and MAC

Isolated CD14$^+$ monocytes were cultured in the presence or absence of MSC in a six-well tissue culture plate (3×10^5 monocytes per well and 3×10^4 MSC per well; monocytes: MSC = 10: 1) for 7 d in DC and MAC differentiation media. Immature DC (i-DC) were generated by culturing monocytes in Roswell Park Memorial Institute (RPMI) complete media, supplemented with Granulocyte-macrophage colony-stimulating factor (GM-CSF) (50 ng/ml) and IL-4 (20 ng/ml) for 5 d. To induce DC maturation, mature DC (m-DC) TNF-α (12 ng/ml) was added at d 5 for the subsequent 2 d. However, MAC were generated by culturing monocytes in RPMI complete media, supplemented with GM-CSF (50 ng/ml) for 7 d. Medium change was performed every 3 d with a freshly prepared cytokine. Differentiated cells (DC and MAC) were immunophenotyped using a panel of cell surface markers for monocytes, DC and MAC at d 5 and 7. Approximately, 1×10^5 cells in 200 μl $1 \times$ PBS

buffer was transferred in a FACS tube and stained with 1.5 µl of CD14-FITC, CD11B-APC, HLA-DR,DP, DQ-FITC, CD80-FITC, CD83-FITC, CD86-PE, CD1A-APC Abs (Becton Dickinson, San Diego). The labelled samples were incubated in the dark at 4°C for 15–20 min, and the staining period was terminated with adding 1–2 ml of 1 × PBS buffer. After washing of the samples (514 g for 5 min at room temperature (RT)), cells were re-suspended in 500 µl of 1 × PBS and analysed in LSR Fortessa (BD Biosciences, USA) flow cytometer; a minimum of 10^4 events was acquired and interpreted using the FACS Diva software. The population gate of monocytes/monocyte-derived cells in culture with differentiation media was set based on the side and forward scatters of flow cytometry during the acquisition. Based on the size and granularity, the population of interested cells was defined to avoid the contamination of residual MSC. Expression of surface markers was determined based on respective population gates.

Isolation of total RNA

To further explore the inhibitory effect of MSC on monocyte activation and differentiation, the quantitative PCR (qPCR) technique was employed. Before conducting qPCR of selected genes, the isolation of total RNA was performed, followed by cDNA synthesis and, finally, cDNA transcripts were used as templates to conduct qPCR. Total RNA was isolated from monocytes, monocytes cultured with MSC, monocyte-derived m-DC and monocyte-derived MAC (m-MAC), which were co-cultured with and without MSC for 7 d. All cell preparations were pelleted by centrifugation before lysis. Total RNA was isolated using the Qiagen RNAeasy Mini Kit (Qiagen, Hilden, Germany). The amount of total RNA was quantified using a spectrophotometer at an OD of 260 nm. The RNA was immediately converted into cDNA using Roche Transcriptor First Strand cDNA Synthesis Kit (Mannheim, Germany). The suitable template concentration used was within the range of 5–10 µg total RNA in a final reaction mixture volume of 20 µl according to the manufacturer's protocol. The cDNA transcripts were then stored at −25°C in the freezer as recommended.

Quantitative RT-PCR

Two-step quantitative RT-PCR (RT-qPCR) of selected gene products was performed using Roche LightCycler® 480 DNA SYBR Green I Master qPCR kit (Mannheim, Germany). The PCR primers and probes were designed according to the published cDNA sequences at GenBank for two genes of interest,

namely TNF receptor superfamily member 11a (*Tnfrsf11a*), TGF-α, fibroblast growth factor receptor 1 (*Fgfr1*) and complement factor 3 (*C3*). All primers were custom synthesised by Integrated DNA Technologies (Coralville, IA). Triplicate PCR reactions for each sample were conducted in 96-well PCR plates in 20 µl final reaction volume as recommended by the manufacturer's protocol. The PCR reaction mixtures at a final volume of 20 µl that contained reaction buffer, deoxyribonucleotide triphosphate (dNTP) mix (with dUTP instead of dTTP), SYBR Green I dye, and $MgCl_2$ with a specific primer set, then 5 µl cDNA aliquot was added. The cycling conditions were 2 min 50°C, 10 min 95°C, followed by 40 cycles of 15 s, 95°C, for denaturation and 1 min, 60°C, for combined annealing and extension. For analysis of RT-qPCR data, $2^{-\Delta\Delta C_T}$ method was utilised. For normalisation of RT-qPCR, GAPDH housekeeping gene was used.

Phagocytosis assay

Phagocytosis assay was performed using the Cayman's Phagocytosis Assay Kit (IgG FITC) (Cayman Chemical, USA) according to the manufacturer's instructions with slight modifications. Primary monocytes with and without MSC, m-DC and m-MAC generated in the presence or absence of MSC were seeded in a six-well plate (3×10^5 monocyte, DC and MAC per well) and cultured with latex beads coated with fluorescently labelled rabbit-IgG and LPS 40 ng/ml (Sigma, Germany) in a final volume of 3 ml for 3 and 5 d. At the respective time points, cultured cells were fluorescently labelled with the rabbit-IgG probe in each well and incubated for 4 h. Cells harvested and collected into FACS tubes and centrifuged for 5 min at 400 g at RT. The supernatant was discarded, the pellet was dislodged and 1 ml of assay buffer was added to each tube and centrifuged again for 5 min at 400 g at RT. The supernatant was discarded, 500 µl of the assay buffer was added to each tube and the samples were immediately acquired in an LSR Fortessa (BD Biosciences, USA) flow cytometer and 10^4 events were acquired and analysed using the FACS Diva software.

Isolation of human T cells

The T cells were isolated from 20 ml of peripheral blood using a human Pan T Cell Isolation Kit (Macs Miltenyi, USA) according to the manufacturer's instructions. The PBMCs were separated as described above. Briefly, PBMCs re-suspended in 40 µl of buffer per 10^7 total cells together with 10 µl of Pan T Cell Biotin-Ab Cocktail. The mixture was incubated for 5 min in the refrigerator (2–8°C). The reaction was

stopped by adding 30 μl of buffer to 20 μl of Pan T Cell MicroBead Cocktail for another 10 min. The LS column was placed in the magnetic field of a midi MACS separator and rinsed with 3 ml of cold $1 \times$ PBS. Up to 10^8 cells in 500 μl of the cell suspension were passed through the column and the effluent (unlabelled cells) collected are T cells.

Ag presentation assay. The ability of monocytes, m-DC and m-MAC to efficiently present the Ag to the isolated T cells was assessed by T cell proliferation assay. Primary monocytes were first differentiated into DC and MAC in the absence and presence of MSC for 7 d and later cultured with autologous T cells. Responder T cells were cultured in a 96-well plate (5×10^4 T cells per well) together with stimulator cells (monocytes, m-mDC and m-MAC) at a 1:1 ratio (5×10^4 cells per well) in a final volume of 200 μl. The responder T cells were stimulated with and without PHA (10 μg/ml) (Sigma Aldrich, USA) for 3 d. At the end of each time point, cells were harvested and T cell proliferation was analysed using tritiated thymidine assay.

Statistical analysis

Data are expressed as mean ± standard deviation. Differences were considered significant at $P \leq 0.05$. Statistical analyses were conducted with Microsoft Office 2013 (Excel) using the student T test.

Results

MSC cells inhibit monocyte differentiation into MAC and DC

Immunophenotyping of monocytes, monocyte-derived DC and MAC using a panel of cell surface markers namely CD14, CD1A, HLA-DR/DP/DQ, CD80, CD86 and CD83 was performed at d 5 and 7 in standard and respective differentiation media. In monocytes, the classical monocyte marker CD14 was highly expressed, followed by HLA-DR/DP/DQ and CD86 at 5 and 7 d (Figure 1a-i and 1b-i). In the presence of MSC, the expression of HLA-DR/DP/DQ and CD86 significantly reduced ($P \leq 0.05$), but CD14 expression remained unaltered at both days. On induction with appropriate differentiation media, monocyte differentiated into MAC and DC. Monocyte induced with i-DC differentiation media, highly expressed CD1a, HLA-DR/DP/DQ and CD86 on d 5. However, in the presence of MSC expression of these markers was significantly reduced (Figure 1a-ii). In m-DC culture, the expressions of CD80, CD86, HLA-DR/DP/DQ and CD83 increased. However, in co-cultures, MSC significantly inhibited the aforementioned expressions, except CD14 whose expression was enhanced in the presence of MSC (Figure 1b-ii). In the MAC differentiation assay, the expression of CD80, CD1a and CD83 was scarce at d 5 and 7; however, co-culture of MSC neither increased nor decreased the expression of the respective markers. It was noted that the differentiated MAC highly expressed CD14, CD86 and HLA-DR/DP/DQ markers, but adding MSC into the culture reduced the expression of CD86 and HLA-DR/DP/DQ significantly on both days (Figure 1a-iii and 1b-iii).

MSC cells down-regulate the gene expression of monocytes, monocyte derived-DC and MAC

RT-qPCR was conducted to validate monocyte differentiation at a molecular level. For this study, *Tnfrsf11a, Tgf-a, Fgfr1* and *C3* were selected. These genes were differentially expressed in monocyte-derived DC and MAC and involved in cellular activation of monocyte-derived DC and MAC.[16–18] It showed *Tnfrsf11a* was highly expressed, remarkably in DC compared to in monocyte and MAC (Figure 2a). However, the co-culture of MSC had significantly (*$P \leq 0.05$) reduced the expression of *Tnfrsf11a* in mDC (Figure 2a). *Tgf-a* was sparsely expressed in MAC, followed by m-DC and monocytes (Figure 2b). In the presence of MSC, the expression of *Tgf-a* was significantly down-graded (*$P \leq 0.05$) (Figure 2b). *Fgfr1* was highly expressed in MAC compared to in monocytes and m-DC (Figure 2C). However, co-culture of MSC significantly reduced *Fgfr1* expression (*$P \leq 0.05$) (Figure 2c). *C3* was also scantly expressed in MAC followed by monocytes and m-DC (Figure 2d). In the presence of MSC, the expression was significantly (*$P \leq 0.05$) reduced (Figure 2d).

MSC inhibit the phagocytosis of monocytes, monocyte-derived DC and MAC

Phagocytosis assay was conducted to assess the functional phagocytic ability of monocytes, monocyte-derived m-DC and MAC in the presence and absence of MSC. It was noticed that monocytes, DC and MAC were able to perform the phagocytosis function effectively at d 3 and 5. As expected, MAC displayed the highest phagocytic capacity followed by monocyte and DC at both days (3 and 5) (Figure 3a and b). It was further observed that the magnitude of phagocytosis in monocytes, mDC and MAC was notably highest at d 5 (Figure 4 and 3b). However, phagocytosis was significantly inhibited in monocytes, DC and MAC when co-cultured with MSC at both days (3 and 5) as shown in Figures 3a and b.
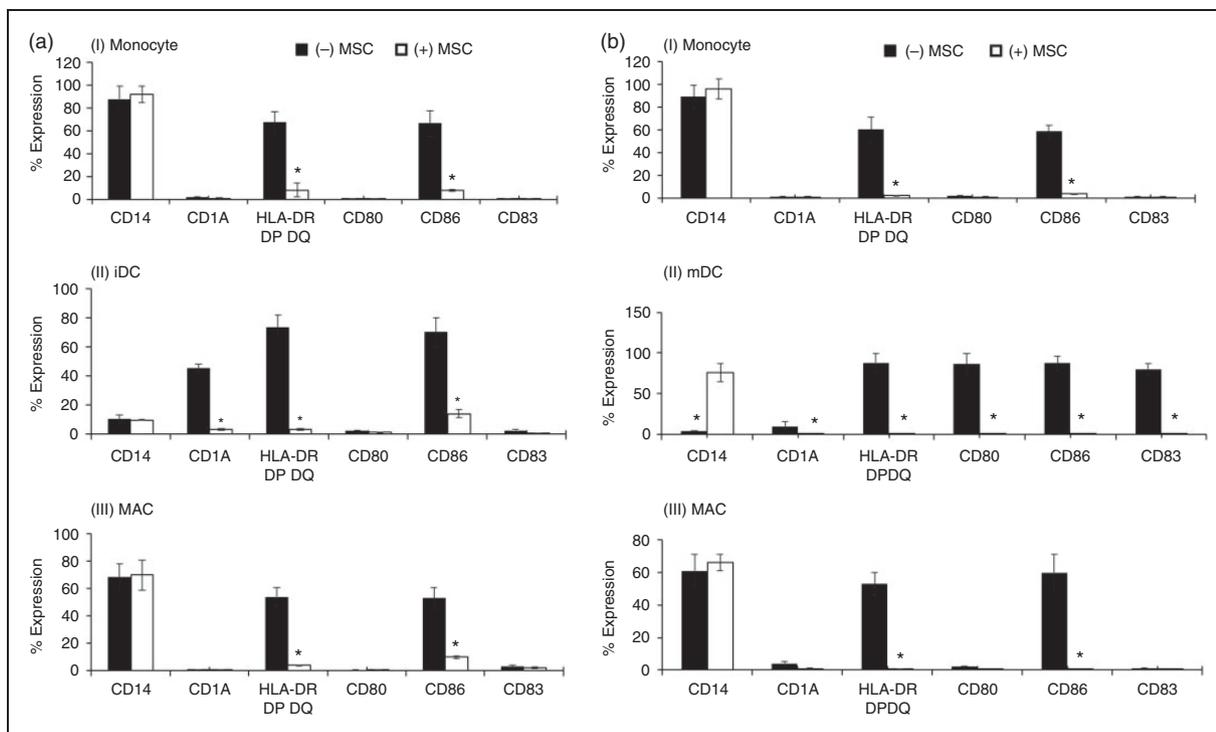
Figure 1. The impact of mesenchymal stem cells (MSC) on monocyte differentiation at d 5 and 7. Monocytes (3×10^5 cells) were cultured in either immature dendritic cell (i-DC) or macrophage (MAC) differentiation media in presence and absence of MSC for 5 (a) and 7 (b) d. (i) Monocytes were cultured in normal media (control), (ii) i-DC differentiation media and (iii) MAC differentiation media. Cells harvested and subjected for immunophenotyping using a flow cytometer. MSC significantly (*$P \leq 0.05$) inhibited the monocyte differentiation towards i-DC and MAC at d 5 and 7 as compared to cells cultured without MSC. A *T* test was used to conduct the statistical analysis. This result is an average of three repeated individual experiments with mean $\pm$ SD (*$P \leq 0.05$).

MSC inhibit the Ag-presenting ability of monocytes, monocyte-derived DC and MAC

The T cell proliferation was assessed to confirm the Ag-presenting capacity of monocytes, monocyte-derived DC and MAC cultured in the presence or absence of MSC for 3 d. The resting T cells co-cultured with monocytes, DC and MAC regardless of MSC's presence did not proliferate at d 3 (Figure 4a, b and c). With PHA stimulation, monocyte, monocyte-derived DC and MAC efficiently presented PHA and induced a remarkable T cell proliferation. As expected, the T cell proliferation was highest in culture with DC with PHA (Figure 4b), therefore showing efficient Ag presentation by mature DC in stimulating T cell proliferation *in vitro* compared to monocytes and MAC. Notably, the presence of MSC significantly diminished T cell proliferation (Figure 4a, b and c). Among the cells employed as Ag presenting, MSC exerted the least inhibition on the Ag-presentation ability of monocytes as compared with DC and MAC. Furthermore, the magnitude of T cell proliferation seemed to be high in the culture when mature DC and MAC served as APC.

Discussion

Monocytes and MSC are two different cell types that originate from distinct cell sources. Haematopoietic stem cells reside in the BM and produce mature monocytes. However, MSC are non-haematopoietic cells in the BM that supply cardinal signals and resources to support uninterrupted haematopoiesis.[10] Although the direct interaction of MSC and maturing monocytes in the BM niche is not fully elucidated, the assistance of MSC in the form of the stromal compartment, extracellular matrix and paracrine secretion provide insight on their positive effects on cell expansion and differentiation. Taking this notion into consideration, the present paper investigated the impact of human umbilical cord-derived MSC on differentiation and effector functions of monocytes as a tool to control the unwanted immune response mediated by monocytes.

Based on the immunophenotyping and morphological assessment (not shown) results of monocytes, when co-cultured with MSC in the appropriate differentiation media, did not display the common surface Ags markers or the morphologies that represented DC or MAC. MSC blocked monocyte differentiation into i-DC by inhibiting CD1A expression and interfered
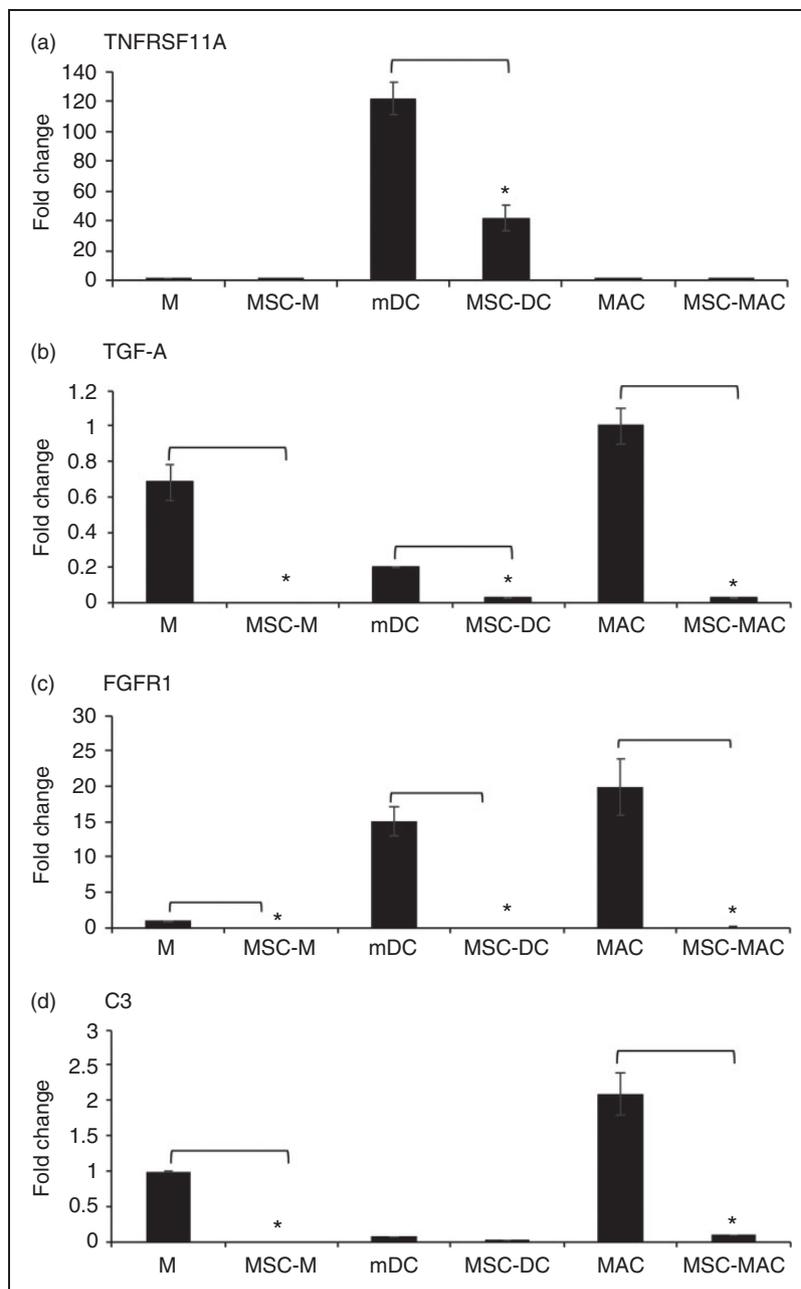
Figure 2. Evaluation of gene expression by quantitative RT-PCR (RT-qPCR). Monocyte (M) alone, monocytes cultured in the dendritic cell (DC) and macrophage (MAC) differentiation media in the presence and absence of MSC for 7 d were subjected for quantification of genes (a) *TNFRSF11A*, (b) *TGF-A*, (c) *FGFR1* and (d) *C3* using RT-qPCR. The differential gene expression was analysed using the $2^{-\Delta\Delta C_T}$ method and results are expressed as fold change. The following genes were differentially expressed in monocyte, mature DC (m-DC) and MAC and in the presence of MSC the expression of the selected genes was significantly (*$P \leq 0.05$) down-regulated. The *T* test was used to conduct the statistical analysis. This result is representative of two biological replicates with mean ± SD (*$P \leq 0.05$).

with DC maturation by supressing CD83 expression *in vitro*. Similarly, MSC blocked monocyte differentiation into MAC evidenced by lack of CD80, CD86 and HLA-DR/DP/DQ expression while preserving CD14 expression in monocytes. This indicates that MSC interfere with monocyte maturation and activation *in vitro*. Our results have revealed this sort of inhibition was not limited to differentiation, but extended further by affecting the effector functions of those cells. These outcomes support the results reported by Jiang and team where MSC co-cultured with monocytes strongly inhibited the initial differentiation of monocytes into
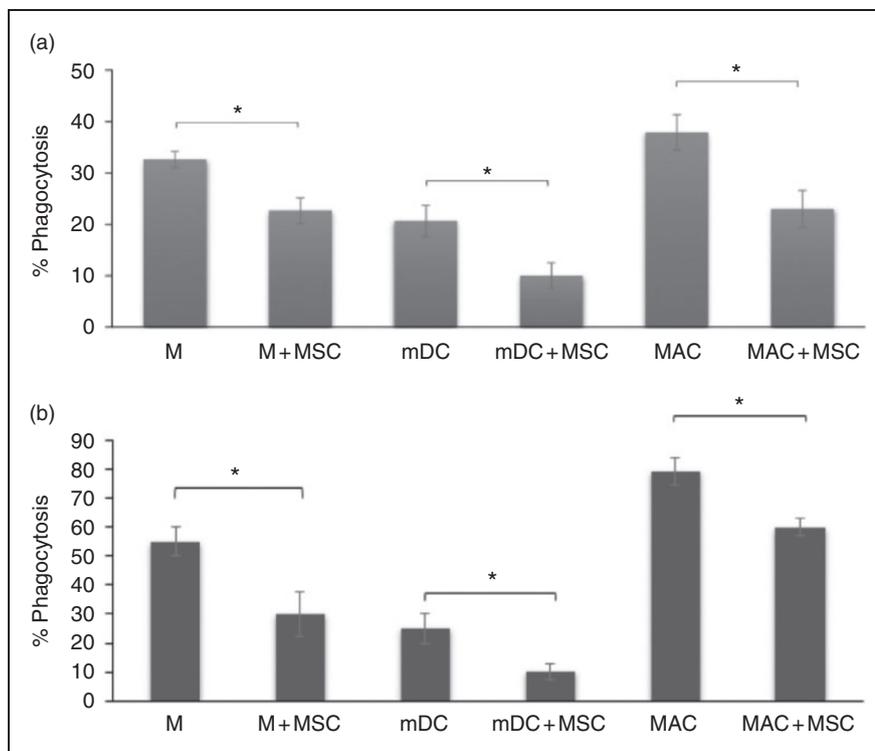
Figure 3. Mesenchymal stem cells (MSC) inhibit the phagocytosis of monocyte, monocyte-derived dendritic dells (DC) and macrophages (MAC). In total 300,000 monocytes were cultured in a standard or differentiation media for MAC and DC for 3 (a) and 5 (b) d, and assessed for phagocytosis. MSC significantly (*$P \leq 0.05$) inhibited phagocytosis in monocytes, DC and MAC at d 3 and 5 compared to the control cells that were cultured alone. The *T* test was used for the statistical analysis. The result is an average of four repeated individual experiments with mean $\pm$ SD (*$P \leq 0.05$).

DC and MAC.[19] These findings were further supported by Zhang and colleagues through secretome analysis.[20] Most likely, cells generated from monocytes and MSC co-culture in the respective induction media transformed into anti-inflammatory cells that were arrested in the midst of the differentiation process. Cytokine profiling of monocytes cultured in induction media in the presence and absence of MSC via cytokine array will provide a better understanding of the type of cells generated. Hence these transformed cells can play a powerful regulatory role in multiple anti-inflammatory mechanisms, and therefore explain its clinical benefits in immunotherapy. Melief et al. have reported that multipotent stromal cells (also known as MSC) skew monocyte function towards an anti-inflammatory response. It was further deciphered that multipotent stromal cells induce monocytes to secrete IL-10 via IL-6 and HGF, hence suppressing inflammation.[21] Furthermore, it is unsure of defining the transformed monocytes in the differentiated media as MAC or DC as these cells are lacking the putative surface markers. It has been reported that MSC can induce polarization towards M2 MAC.[22] M2 MAC not only have a regulatory function but also participate in the

wound healing process.[23] To date, not much clinical data are available assessing the immunosuppressive function of MSC on MAC. However, it has been shown in an animal model that MSC are able to stabilise the atherosclerotic plaques by affecting the activities of MAC.[24] Atherosclerotic plaques with a thin fibrous cap, large lipid core and a large number of infiltrating mononuclear MAC are unstable, prone to rupturing and eventually lead to acute cardio-cerebrovascular events. Employing a rat model of atherosclerosis, Wang and colleagues had reported that MSC transplantation elevates the anti-inflammatory factors such as Il-10, diminishes apoptosis and possibly down-regulates the NF-κB signalling pathway as a tool to reduce the infiltration of neutrophils and MAC into plaques.[24] Hence, MSC stabilises vulnerable atherosclerotic plaques by its anti-inflammatory properties.[25]

The immunosuppressive activity of MSC on monocyte differentiation toward DC and MAC was also further deciphered through a gene expression study. A qPCR was conducted to determine the expression of *Tgf-a*, *Tnfrsf11a*, *Fgfr1* and C3 as a number of studies have shown the above-selected genes are differentially regulated in monocyte-derived DC and MAC.[16–18]
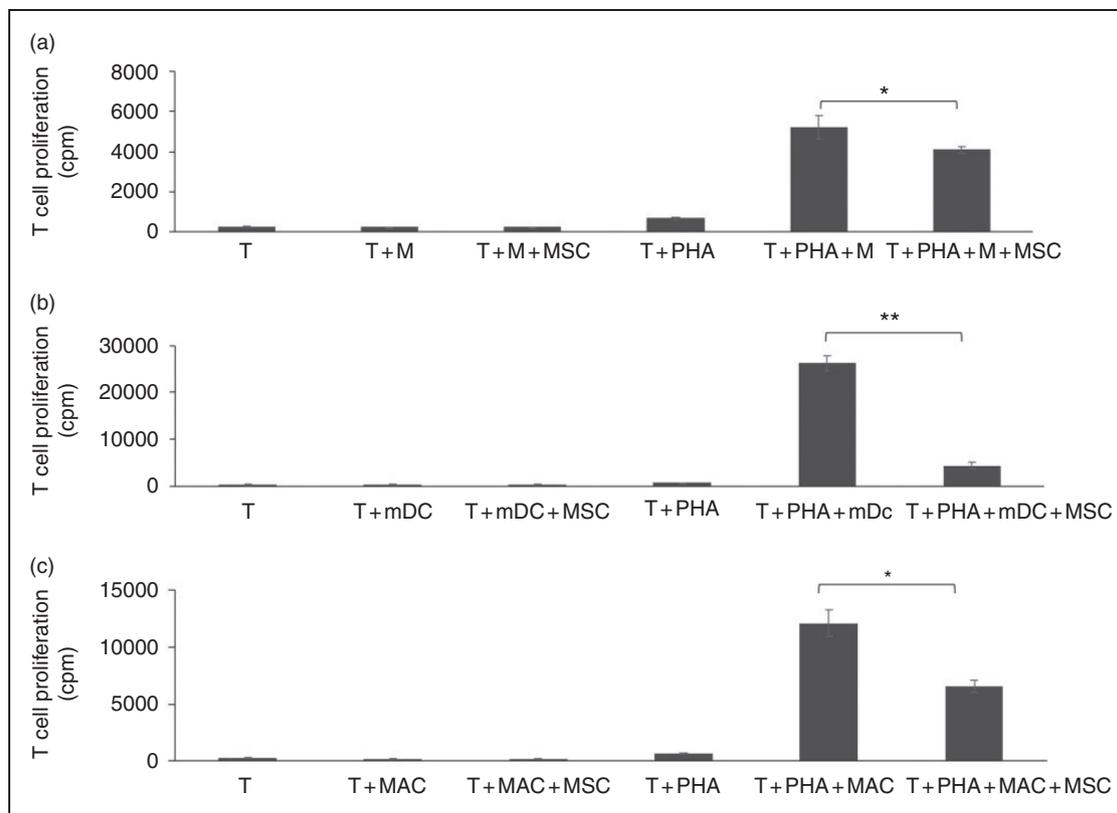
Figure 4. Mesenchymal stem cells (MSC) inhibit the Ag-presenting ability of monocytes, monocyte-derived dendritic cells (DC) and macrophages (MAC). Monocytes and monocyte-derived MAC and DC (5×10^4 cells) in the presence or absence of MSC were co-cultured with autologous T cells at a 1:1 ratio, and stimulated with PHA (5 μg/ml) in a 96-well plate for 3 d. The T cell proliferation was measured using the tritiated thymidine assay. The MAC and DC generated using monocytes with the influence of MSC failed to stimulate T cells sufficiently as compared to monocytes (a), DC (b) and MAC (c) alone. The T test was used for statistical analysis. This result is representative of two repeated individual experiments with mean ± SD (*$P \leq 0.05$).

Hence qPCR was performed using monocytes, monocyte-derived DC and MAC and monocytes that co-cultured with MSC in normal, DC and MAC inductive media. The data showed the highest expression of *Tnfrsf11a* (TNFR superfamily, membrane 11a, NF-κB activator) found in DC (Figure 2a) as expected because TNFRSF-11a is a receptor activator of NK-kβ, encoding an essential co-stimulatory molecule regulating DC-T cell interactions.[17] The expression of *Fgfr1* (fibroblast growth factor receptor 1) was up-regulated in both DC and MAC but was significantly (*$P \leq 0.05$) high in MAC (Figure 2c). This gene has multiple regulatory functions and has been reported to regulate the pro-tumour functions of MAC and wound healing.[26] The expression of *Tgf-a* and *C3* was minimal in monocytes, and MAC and were negligible in DC (Figure 2b and d). For *Tgf-a* and *C3,* a similar pattern of expression was reported by Lehtonen and colleagues.[17] The expression of the selected genes was dramatically down-regulated in monocytes, DC and MAC in the presence of MSC. This further supports that MSC

prevent monocyte differentiation into DC and MAC. However, in the physiological condition, the possibility of monocyte differentiation into DC at tissue microenvironment is still elusive as most likely monocytes would have become MAC and the tissue niches are patrolled by BM-derived DC. Although, it is impossible to associate monocytes and their direct conversion into DC in a tissue vicinity, yet other studies also demonstrated MSC are capable of interfering with BM-derived DC maturation.

The ability of Ag presentation, especially extracellular Ags, requires the Ag intake in the form of phagocytosis. Although many innate immune cells such as granulocytes are phagocytes, the Ag-presenting function is limited to MAC and DC. The present study has demonstrated that MSC exerted a profound inhibition of phagocytosis in monocytes, m-DC and MAC. Amongst, MAC showed the highest phagocytosis on d 5 compared to monocytes and DC. Previously, studies have shown that MSC interfere with the phagocytosis ability of DC and MAC.[11,19,20,27] The noted reduction

in phagocytosis could be a reflection of mal-differentiation of monocytes in contact with MSC. Most likely MSC retain monocytes in the immature form of MAC and DC by halting at differentiation, which can affect phagocytosis. It could be possible that MSC affect the cytoskeletal rearrangement of the cells, which is an essential process during cell motility and proliferation. Although the current study is unable to supply additional data, the inhibition of phagocytosis by MSC may occur due to the suppression of cytoplasmic projection during the formation of pseudopodia, which is crucial in phagocytosis.

The present study also demonstrated that in MSC co-culture, monocytes, monocyte-derived DC and MAC failed to induce a significant level of T-cell proliferation when induced with PHA-L, a T cell mitogen. Their ability to present PHA was extremely compromised in the presence of MSC to elicit T cell proliferation. Hence MSC render the monocytes in an 'unfit' state for effective Ag presentation, and therefore T cells remain in an unstimulated or undifferentiated state or possibly collapsed into anergy. Zhao et al. also reported that DC generated in the presence of MSC strongly hampered their ability to induce T-cell activation. They suggested that MSC suppressed the differentiation of DC and resulted in the formation of immature DC, which eventually displayed the suppressor or inhibiting phenotype.[28] Beyth and the team also reported that MSC alter APC maturation and induce T-cell un-responsiveness by a unique immunoregulatory mechanism in which human MSC induce regulatory APC. The functional properties of DC are strictly dependent on their maturation state[30] and DC maturation is a critical step in the presentation of Ags to T cells, therefore in the initiation of an adaptive immune response.[31] The current research brought to light that MSC markedly suppress the differentiation of monocytes into MAC, where it renders the functional inability of MAC to activate T cells. Moreover, auxiliary to the existing data on DC and MSC, the present study revealed a similar immunosuppressive activity that was extended towards MAC.

It is noteworthy that phagocytosis and ability to stimulate T cells via Ag presentation in monocytes and monocyte-derived DC and MAC were not entirely abrogated as some considerable activity of phagocytosis and T cell stimulation was noted. This, in turn, affirms the notion that in the presence of MSC, only a limited number of cells were undergoing the differentiation process, whereas the significant fraction of cells remained arrested. Or, it could be possible the number of MSC required to abrogate the differentiation, phagocytosis and Ag presentation fully is higher than what had been consumed in the current experimental settings. However, one should not assume the ultimate inhibition of adaptive immune response is exclusively due to the alteration of monocytes or monocyte-derived cells. MSC have been shown to suppress T and B cell functions, surpassing the needs of monocytes by directly affecting adaptive immune cells.[32,33] We have also shown that MSC can render the inhibitory activity of CD3/CD28 microbead-activated T cells where the microbeads stimulate T cells without undergoing MAC presentation by a direct ligation with T-cell receptors.[34]

The immunosuppressive function of MSC towards monocytes can be achieved through a contact-dependent manner or via secretion of a plethora of soluble factors that includes cytokines. However, in the present study, the mode of monocyte inhibition was not deciphered. The monocytes and MSC were co-cultured to provide an optimum interaction between these cells. It could be possible that multiple soluble factors and receptor-ligand interaction between MSC and target cells are responsible for the observed inhibition of monocytes, although the inhibition of monocytes differentiation towards DC by MSC is not a new finding. However, the current study investigated the impact of umbilical cord-derived MSC on monocytes and monocyte-derived cells on the grounds of differentiation and effector functions. In regard to immunosuppression, most of the studies use BM-derived MSC because MSC play a supportive role in haematopoiesis in the stem cell niche of BM and the focus is more on monocyte-derived DC. Whereas our study provides a complete scenario of MSC mediated immunomodulation on monocytes differentiation towards i-DC, m-DC, MAC and their effector functions such as phagocytosis and Ag presentation.

Conclusions

The current data revealed that MSC interfere with the differentiation of monocytes in normal, DC and MAC differentiation media, where APCs profoundly lose their functional properties in terms of phagocytosis and effective Ag presentation. These results suggest MSC may modulate the immune system, not only through acting directly on T cells but also at the first step of the immune response through the inhibition of DC and MAC differentiation and maturation phases. Overall the study revealed that MSC can modulate monocytes by interfering with its fundamental function, which is the terminal differentiation into DC and MAC and therefore inhibits phagocytosis and Ag presentation ability.

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Declaration of conflicting interests

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