Murine mesenchymal stem cells suppress dendritic cell migration, maturation and antigen presentation

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Abstract

Mesenchymal stem cells (MSC) possess a wide range of immunosuppressive functions. Among these is the ability to inhibit CD4^{+} T cell proliferation. Dendritic cells (DC) play a role in initiating cell-mediated immunity; however, the immunosuppressive influence of MSC on professional antigen presenting cells remains unclear. DC exposed to TNF-α and cultured with murine MSC failed to show regular upregulation of maturation markers. Similarly, the presence of MSC abrogated the capacity of ovalbumin-pulsed DC to support antigen specific CD4^{+} T cell proliferation, or for DC to display an MHC class II- peptide complex recognizable by specific antibody. Interestingly, culture of MSC with DC resulted in reduced expression of CCR7 by DC following stimulation. Likewise, DC matured in the presence of MSC, showed significantly less migration to CCL19. In contrast, murine MSC prevented loss of expression of the tissue anchoring protein E-cadherin by DC. Modulation of DC maturation and function was not permanent and could be restored after removal of MSC. These data demonstrate that MSC modulate the three cardinal features of DC maturation, providing the first demonstration of MSC interference with DC migration.
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1. Introduction

Adult mesenchymal stem cells (MSC) are multipotent cells able to self-renew under controlled conditions. MSC may differentiate into multiple lineages including bone, fat and cartilage [1,2]. Usually, MSC reside in the bone marrow, and provide support for hematopoietic stem cells through the production of growth factors [3]. However, the primary function of MSC is to repair and replace damaged tissues [4–6]. These characteristics make MSC an attractive source for tissue engineering and regenerative medicine.

MSC possess an array of immunosuppressive capabilities. Various groups have demonstrated that both murine and human MSC suppress alloantigen and mitogen driven T cell proliferation in vitro [7–10]. Some of these functions are dependent on exogenous IFN-γ [7,11,12]. Comparable studies, investigating MSC immunosuppression of B cells, have demonstrated that MSC physically hindered proliferation [9,13], likewise, MSC can inhibit NK cell proliferation and cytotoxicity [11,14]. Whilst these are important data, a deeper understanding of how MSC modulate T cell responses will be central to the development of acceptable therapies involving MSC.

Dendritic cells (DC) arise in the bone marrow and play a pivotal role in the induction of adaptive immunity. DC have the ability to initiate a primary adaptive immune response through the capture, processing and presentation of antigen to naïve CD4^{+} T cells, however differences in these capacities are linked to the developmental maturity of the DC. Immature DC (iDC), resident in tissue, efficiently capture antigen [15]. Exposure to stimuli such as pathogen associated molecules including LPS, bacterial DNA, or pro-inflammatory cytokines (e.g. TNF-α), initiate a process termed maturation [16]. Maturing DC alter the expression of chemokine receptors including CCR7, thus becoming responsive to the lymph node derived chemokine CCL19 [17]. In addition, mature DC (mDC) down-regulate E-cadherin expression, allowing migration to regional lymph nodes [18]. In the lymph node, mDC express high levels of MHC class II, CD80 and CD86 which play a well-described

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role in antigen presentation to CD4+ T cells [15]. Thus, DC maturation plays a key role in initiating T cell responses, and not surprisingly, many pathogens have exploited this process to evade immunity [19,20].

A number of recent studies have focused on the influence of MSC on DC function [21–24]. However, data have been confounded by differences in models, technical approaches and in particular differences between MSC and DC isolation protocols [21]. At least five different murine MSC populations have been described and subtle differences in their function have been observed [10,13,25–27].

In the present study, a well-characterised MSC population and a clearly defined DC population were used to probe the interaction between these two cell types. This study demonstrates that MSC disrupt the three major functions that characterise the transition of DC from immature to mature stages; namely the upregulation of antigen presentation and co-stimulatory molecule expression, the ability to present defined antigens, and the capacity to migrate to CCL19. Collectively, these data support the hypothesis that MSC profoundly influence host immunity by modulating DC function suggesting that allogeneic MSC may not require profound immunosuppression during clinical application.

2. Materials and methods

2.1. Animals

Six-to-eight-week-old female BALB/c (H-2^d), C57BL/6 (Harlan, Oxon, U.K.) and DO11.10 (H-2^d) mice [28] were used for experiments under the guidelines of the Irish Department of Health and the approval of the research ethics committee of the National University of Ireland Maynooth.

2.2. Isolation and culture of bone marrow derived mesenchymal stem cells

Murine MSC were isolated and expanded using an in-house modification [7] of the method of Peister et al. [29]. Cells retained differentiation capacity as previously described [29]. All MSC were MHC I$^+$, Sca-1$^+$, CD44^{low}, CD106^{low}, MHC II$^-$, CD11b$^-$, CD11c$^-$, CD34$^-$, CD45$^-$ and CD117$^-$. Stem cells were used between passages 3 and 10 and rigorous purification and quality control were performed to ensure MSC purity as previously described [7].

2.3. Dendritic cell culture

Isolation and culture of bone marrow derived DC was carried out as previously described with minor modifications [30,31]. Briefly, immature DC were prepared by culturing cells taken from murine bone marrow (femur and tibia) in RPMI 1640 medium (Gibco-Invitrogen, Paisley, UK) supplemented with 10% (v/v) heat inactivated, endotoxin low fetal calf serum, 1% (v/v) penicillin/streptomycin (Gibco-Invitrogen) and 1% (v/v) L-glutamine (Gibco-Invitrogen) with either 95 ng/ml recombinant GMCSF/300 pg/ml IL-4 (Peprotech EC, London, UK) or by recombinant J558 supernatant previously shown to be equivalent in supporting DC culture [31]. Cells were cultured for 3d at 37 °C, and culture medium was carefully replaced with fresh medium at this time. Cells were harvested at 6d by gentle aspiration, counted, and analysed by flow cytometry. DC populations used were >70% CD11c+ with no detectable expression of Sca-1, a marker found on MSC but not DC. In maturation experiments, immature DC were seeded at 7.5×10^5/ml and cultured in 2 ml cRPMI +/− 25 ng/ml TNF-((R&D systems, Abington, UK) as maturing agent, then co-cultured in the presence or absence of MSC or control MLE-12 cells (2.5×10^5/ml) for 24 h or 48 h. Differences in adherence to tissue culture plastic by the two populations allowed a simple but reproducible technique to recover DC from co-cultures. DC were harvested by gentle aspiration from strongly adherent MSC or MLE-12 cells after 24 or 48 h. Isolated DC were re-analysed by flow cytometry using antibodies against CD11c and Sca-1. DC re-isolated by this method were >70% CD11c+ but did not express Sca-1 (<1%). For return of function studies, DC were similarly re-isolated from MSC co-culture and re-stimulated in fresh medium with TNF-α for 48 h before use in chemotaxis assays.

2.4. CD4+ T cell proliferation

Immature DC (1×10^5/ml) were cultured with 20 μg/ml Ovalbumin (Sigma–Aldrich) in the presence or absence of MSC (1:3 MSC: DC) for 24 h. DC were isolated by gentle aspiration, and washed twice in culture medium. DC were then co-cultured with 4×10^5/ml naïve OVA-specific, I-A^d-restricted CD4$^+$ DO11.10 T cells purified by negative selection (R&D Systems). Cells were cultured for 72 h in total with [^{3}H]thymidine (Amersham Biosciences, Buckinghamshire, England) (5 μCi/ml) added for the final 6 h as previously described [32]. Cells were harvested and Thymidine incorporation quantified as mean counts per minute (±S.E.) by liquid scintillation [32].

2.5. Flow cytometry

Cell phenotype was characterised by flow cytometry (FACSCalibur), analysed using CellQuest software (BD Biosciences, Oxford, UK). Cells were labelled with combinations of FITC or PE-conjugated antibodies against Sca-1, CD11b, CD11c, CD34, CD40, CD44, CD45, CD80, CD86, CD106, CD117, CCR7, MHC I and II or appropriate species and isotype-matched controls (eBiosciences, San Diego, CA).

2.6. Chemotaxis

Cell migration was performed in 24-well Transwell^{TM} chambers (Corning Costar, Cambridge, MA) using 5 μm-pore size polycarbonate membranes as described [33] with minor modifications. 1×10^5 mature DC in serum free medium (100 μl) were loaded into the upper chamber. CCL19 (100 ng/ml) (R & D Systems) in sterile serum-free RPMI, 1% (v/v) BSA was added to the lower chamber. After 1.5 h incubation at 37 °C, 5% CO_2, chemo-

taxed cells were counted in three random fields of view from digital images generated using an inverted microscope (Olympus CK40, Centre valley, PA). Each assay was performed in duplicate, and repeated three times. The lower compartment of control wells contained medium alone. A chemotactic index was calculated against the basal level of DC migration to medium alone.

2.7. Eα:I-A^b MHC class II complex antigen presentation assay

DC were incubated alone or in the presence of 10 µg/ml I-Eα peptide 52–68 (ASFEAQGALANIAVDKA) (Anaspec, San Jose, CA) or vehicle buffer control in the presence or absence of MSC for 48 h. Peptide binding was detected using the biotin-conjugated anti-I-A^b:Eα complex-specific antibody, YAe (eBioscience) as previously described [34]. Binding of biotin-conjugated antibody was detected by PE-conjugated streptavadin (Sigma–Aldrich) and was analysed by flow cytometry (BD Biosciences). Data analysis was carried out using CellQuest software (BD Biosciences).

2.8. Semi-quantitative RT-PCR

DC were seeded at 6×10^5 cells/ml in 24-well cultures (Nunc, Rorsklide, Denmark) and either non-stimulated, or matured with TNF-α (25 ng/ml) in the presence, or absence of MSC (2×10^5/ml) and harvested at 24 h for RNA isolation using TRI Reagent™ (Molecular Research Centre, Cincinnati, OH) according to manufacturer's instructions. cDNA from iDC, mDC or DC cultured with MSC were probed for GAPDH, CCR7 or E-cadherin. Primers pairs were: GAPDH: 5′-GGTG-AAGGTCGGAGTCAACG-′3 and 5′-CAAAGTTGTCATGG-ATGACC-′3; E-cadherin: 5′-GGCTGGACCGAGAGAGTT-′3 and 5′-CTGCTTGGCCTCAAAATC-′3; CCR7: 5′-AGTCTT-CCAGCTGCCCTACA-′3 and 5′-CAGCCCAAGTCCTTGAA-GAG-′3.

2.9. Quantitative RT-PCR

cDNA were analysed for the expression of murine CCR7 and E-cadherin by fluorogenic 5′- nuclease PCR assay (MJ Research Inc., Waltham, MA). cDNA (500 ng) were amplified in the presence of SYBR® Green PCR mastermix (Qiagen, West Sussex, UK). Standard curves for CCR7 and E-cadherin expression were generated by amplifying 10-fold serial dilutions of known quantities of CCR7 and E-cadherin PCR product standards. Quantification of target gene expression was obtained using sequence detector system software (MJ Research Inc., Waltham, MA).

2.10. Western blot

Cells were lysed in RIPA buffer (Sigma–Aldrich) and lysate resolved by 12% SDS/PAGE, then electro-transferred to nitrocellulose membranes (Amersham Biosciences, Buckinghamshire, U.K.) and blocked for 1h in 5% (w/v) non-fat milk powder/PBS with 0.05% (v/v) Tween 20. Membranes were probed with anti-mouse E-cadherin (BD Biosciences, Oxford, UK) or matched control antibody. Binding of antibody to E-cadherin was detected by horseradish peroxidase conjugated anti-mouse secondary antibody (Sigma–Aldrich) and detected by Amersham ECL Western Blotting analysis system (Amersham Biosciences).

2.11. Statistical methods

Results are expressed as mean $\pm$ (S.E.). A Student's paired t-test was used to determine significance between the groups, $p < 0.05$ was considered significant. Statistical analyses and graphical representations were performed using GraphPad Prism™ software (GraphPad, San Diego, CA).

3. Results

3.1. Murine MSC prevent TNF-α driven dendritic cell maturation

DC maturation is a critical feature for the induction of adaptive immune responses, aspects of alloantigen elimination and transplant rejection. Maturation is accompanied by phenotypic changes in DC surface protein expression associated with APC competence. The capacity of MSC to modulate DC maturation driven by a robust stimulus was examined. Immature bone marrow derived CD11c^+ DC (iDC) expressed low levels of MHC class II and the co-stimulatory molecule CD86 (Fig. 1A), as expected, maturation by TNF-α resulted in increased surface expression of these markers (Fig. 1A). DC are susceptible to maturation by relatively gentle manipulation, importantly co-culture of immature DC with MSC did not lead to increased expression of CD86 or MHC class II, and therefore manipulation or contact between these cell types did not result in anomalous DC maturation. DC cultured with TNF-α were separated from strongly adherent MSC in co-cultures by gentle aspiration after 48 h. Unlike DC exposed to TNF-α alone, DC cultured with TNF-α in the presence of MSC failed to upregulate either marker post TNF-α stimulation (Fig. 1A), rather DC co-cultured with MSC showed significantly reduced surface expression of MHC class II and CD86 (Fig. 1B). It was possible that co-culture of DC with MSC led to exhaustion of nutrients in cell culture medium. Therefore it might be argued that MSC modulation of DC maturation marker expression was not MSC specific and/or was merely a result of nutrient exhaustion in the culture medium. To rule out this possibility, co-cultures were repeated in which rapidly dividing transformed epithelial cells (MLE-12) replaced MSC. Co-culture of DC with MLE-12 cells did not prevent upregulation of MHC class II or CD86 suggesting that competition for nutrients or medium exhaustion was unlikely to be the mechanism of modulation (Table 1.). The presence of MSC in cultures did not increase DC apoptosis or alter cell viability. Furthermore DC removed from co-culture with MSC and re-stimulated with TNF-α for 48 h showed regular upregulation of maturation markers as indicated by MHC class II (Fig. 1C). These data are consistent with the hypothesis that MSC induce
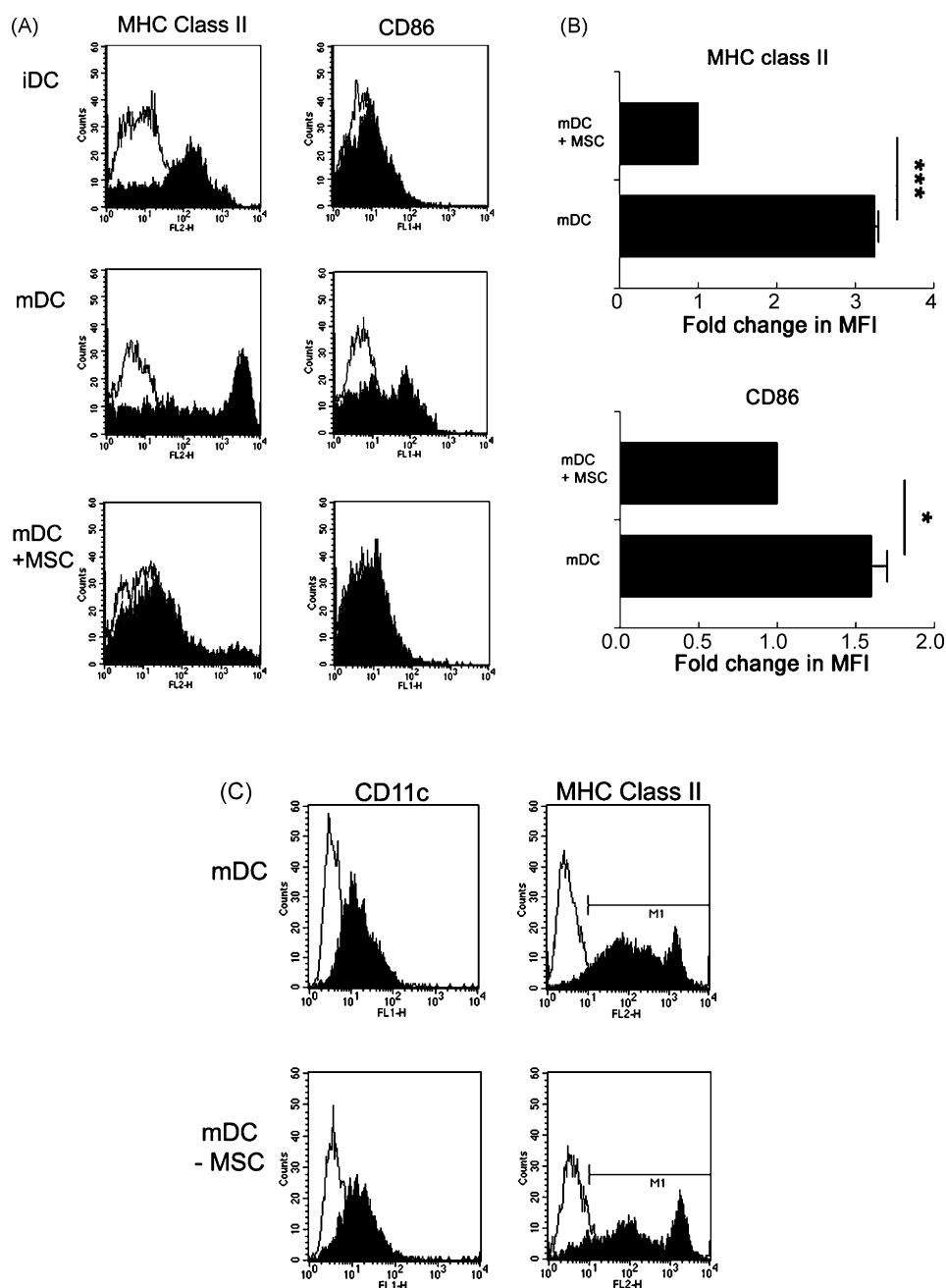
Fig. 1. Murine MSC prevent upregulation of DC maturation markers. Immature DC (iDC) were stimulated with TNF-α (mDC) and cultured in the presence or absence of MSC for 48 h. DC stimulated with TNF-α were separated from adherent MSC in co-cultures by gentle aspiration after 48 h and maturation marker (MHC class II or CD86) expression (shaded) analysed by flow cytometry and compared to species and isotype-matched controls (open). Data were expressed as histograms (A) or mean fluorescence intensity (MFI) change (B). DC cultured with MSC showed significantly reduced MHC class II MFI (***, $p < 0.001$), and CD86 (*, $p < 0.05$). DC previously co-cultured with MSC, were harvested from MSC co-cultures, re-stimulated with TNF-α and examined for the capacity to upregulate surface expression of MHC class II. (C). Expression of MHC class II by DC in response to TNF-α could be restored if MSC were removed. Results are mean (±S.E.) of triplicate data ($n = 3$), from a minimum of three experiments. Cells remained viable throughout culture and no induction of apoptosis was detected in this or subsequent experiments.

or maintain DC with an immature or "suppressor" phenotype, but this condition is not permanent and regular DC maturation can be restored in the absence of MSC (Fig. 1C).

3.2. Murine MSC interfere with dendritic cell antigen presentation

DC maturation involves a switch from antigen acquisition to antigen processing and presentation, a process that is broader than merely MHC class II, and CD86 upregulation. Interference in this process is likely to hamper antigen presentation, since co-stimulatory molecules are essential for optimal antigen presentation to naive CD4^{+} T cells. In order to investigate the influence of MSC on antigen presentation, an antigen-specific proliferation assay was performed using DO11.10 CD4^{+} T cells bearing the transgenic T cell receptor specific for the ovalbumin (OVA) peptide 323–339. This model was chosen to allow defined epitope effects to be studied and to avoid confounding effects

Table 1
MSC but not transformed epithelial cells prevent DC upregulation of maturation markers

Marker	Expression (mean fluorescence intensity)[a]			
	iDC	mDC	mDC + MSC	mDC + MLE
CD11c	48	63	69	52
MHC II	539	601	410	990
CD86	44	68	46	78

[a] CD11c, MHC class II and CD86 expression were measured by flow cytometry of iDC, mDC, and TNF-a stimulated DC co-cultured with either MSC (mDC+MSC) or the transformed epithelial cell line MLE-12 (mDC+MLE). Data are presented as mean fluorescent intensity from a representative experiment of three replicates, 10,000 events were sampled in each case.

of allogenicity possible in other systems. Whole OVA pulsed DC derived from non-transgenic haploidentical bone marrow, efficiently supported antigen-specific DO11.10 CD4^{+} T cell proliferation (Fig. 2A). However, when DC were cultured in the presence of antigen and MSC, CD4^{+} T cell proliferation was partially but significantly reduced (Fig. 2A). The use of whole OVA protein, as opposed to specific peptide in these studies indicated that full antigen processing is occurring and eliminated the possibility of auto-presentation of peptide in a T cell to T cell manner.

T cell proliferation is a complex system and reductions might be occurring through other mechanisms. The effect of MSC on specific antigen presentation by DC was therefore analysed by a well-defined antigen presentation system taking advantage of the YAe antibody [35]. This antibody recognises a complex composed of I-Eα peptide bound to I-A^b MHC class II molecules. C57/BL6 DC were chosen for these studies because this strain express I-A^b but not I-E and therefore would not constitutively express the I-Eα: I-A^b MHC class II complex [36]. C57/BL6 DC pulsed with I-Eα peptide displayed the peptide: MHC II complex at the cell surface as determined by recognition of the fluorescently labelled YAe antibody (Fig. 2B). However, this was reduced when DC had been co-cultured with MSC (Fig. 2B), whereas controls consisting of iDC alone or iDC pulsed with vehicle buffer in the presence of MSC did not display the peptide: MHC II complex. Taken together these data confirm a role for MSC interference in antigen presentation by DC.

3.3. Murine MSC prevent dendritic cell migration ability to CCL19

DC maturation is accompanied by an alteration in the expression of chemokine receptors and other surface markers reflecting the requirement of maturing DC to migrate to regional lymph nodes. The three most striking of these alterations are: (1) the acquisition of CCR7 surface expression by mature DC; (2) a consequent capacity to migrate to CCL19 not seen in immature DC [17]; and (3) the loss of expression of the epithelial anchoring protein E-cadherin. Consequently, the effect of MSC on CCR7 expression and chemotactic ability by DC was investigated. DC cultured in the presence of MSC and stimulated with TNF-α exhibited reduced CCR7 mRNA (Fig. 3A), and reduced surface protein expression (Fig. 3B) when compared to regular TNF-α matured DC. Similarly, an examination of the chemotactic
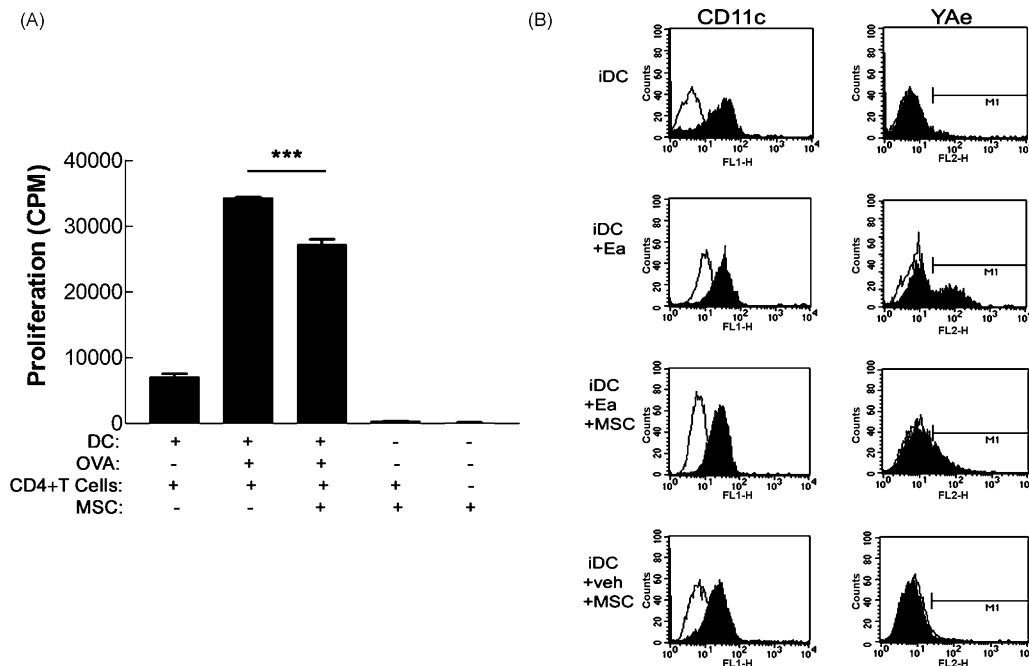

Fig. 2. MSC interfered with DC antigen presentation. Immature DC pulsed with OVA in the presence or absence of MSC for 24 h were harvested and washed twice before culture with CD4^{+} DO11.10 T cells (A). After 72 h, [³H]thymidine incorporation was measured. MSC significantly decreased the ability of OVA pulsed DC to support D011.10 CD4^{+} T cell proliferation (***, $p < 0.001$). Results are mean (±S.E.) of triplicate data ($n = 3$), from a minimum of three experiments. Antigen presentation was also measured using an alternative display system (B). iDC alone or iDC pulsed with Eα peptide (10 μg/ml) or vehicle buffer control in the presence or absence of MSC for 48 h were harvested as described in Fig. 1. Labelling by biotinylated YAe, coupled to streptavidin-PE was detected by flow cytometry. MSC reduced DC presentation of I-Eα:I-A^b MHC class II peptide complex detected by of YAe. Results are representative of two replicates in this instance.
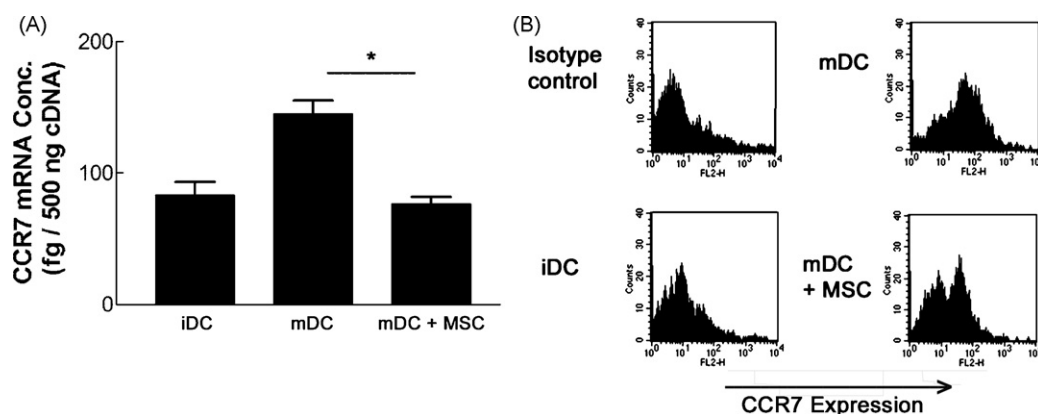
Fig. 3. Murine MSC downregulated CCR7 mRNA and surface protein expression by mature DC. (A) CCR7 mRNA was determined by quantitative real time RT-PCR at 24 h from immature DC or TNF-α stimulated DC cultured in the presence or absence of MSC. CCR7 mRNA expression by stimulated DC was significantly lower when cultured with MSC (*, $p < 0.05$). (B) CCR7 expression by iDC, TNF-α stimulated DC (mDC) or mDC cultured with MSC (mDC + MSC) was examined by flow cytometry with isotype-matched controls for comparison. MSC reduced CCR7 surface expression by TNF-α matured DC at 48 h. Results are mean (±S.E.) of triplicate data ($n = 3$), from a minimum of three experiments.

response revealed that DC co-cultured with MSC displayed significantly reduced capacity to migrate to CCL19, the lymph node expressed chemokine ligand for CCR7 (Fig. 4A). This reduction was not permanent, as removal of the DC from MSC co-cultures and subsequent re-stimulation with TNF-α restored the capacity of DC to migrate to CCL19 (Fig. 4B) and again confirmed that these were not affects attributable to medium exhaustion.

For effective relocation, mature DC must not only become responsive to lymph node derived signals, but must also downregulate tissue anchoring proteins [18,37,38]. The most prominent of such proteins is E-cadherin. E-cadherin has been reported to play an important role in interactions between immature Langerhans cells and skin tissue [18,37,38]. Upon maturation, Langerhans cells downregulate the anchor protein E-cadherin in order to migrate to the local lymph node [38]. The influence of MSC on E-cadherin was therefore examined. In co-culture experiments, MSC prevented downregulation of E-cadherin mRNA expression in TNF-α stimulated DC, as measured by semi-quantitative and quantitative real time PCR (Fig. 5A and B). This was reflected in E-cadherin protein expression, as detected by immunoblot, which was similar to that of immature DC and greater than that observed in mature DC (Fig. 5C). Taken together these data indicate that MSC influence DC maturation to prevent loss of tissue anchor proteins and limit acquisition of responsiveness to lymph node derived signals.

4. Discussion

This study demonstrates that MSC interfered with the three cardinal aspects of DC maturation. DC matured with TNF-α and cultured in the presence of MSC failed to show upregulation of the maturation marker CD86 and did not increase MHC class II surface expression. Furthermore, MSC suppressed the capacity of DC to support antigen specific proliferation by T cells and interfered with the display of specific peptide:MHC complex by DC as measured using the Eα presentation system. In addition this study demonstrated for the first time that MSC preserve DC expression of tissue anchoring proteins, prevent
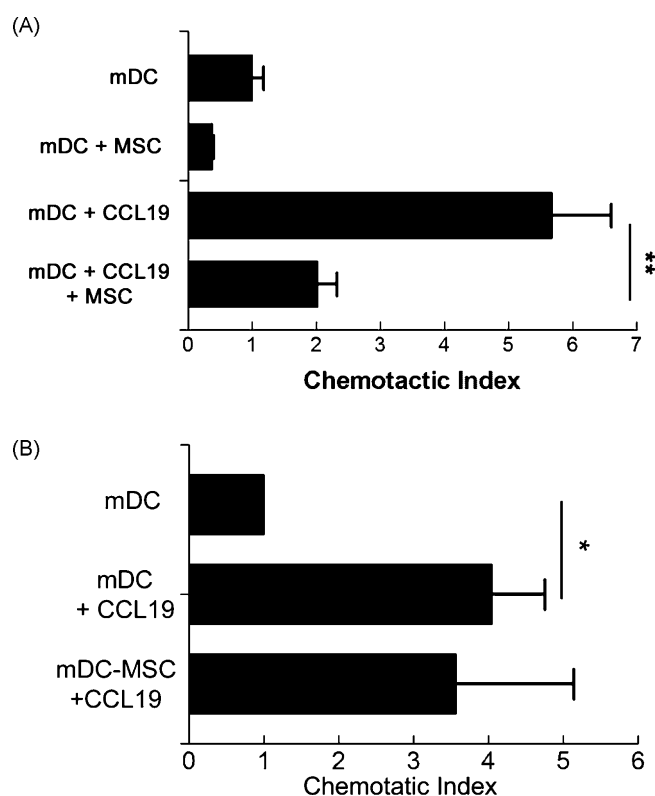

Fig. 4. Murine MSC interfered with DC migration toward CCL19. The ability of DC matured with TNF-α in the presence or absence of MSC for 48 h to migrate toward CCL19 was examined (A). TNF-α stimulated DC co-cultured in the presence of MSC displayed a significantly reduced chemotactic response to CCL19 compared to mature DC alone (**, $p < 0.01$). Chemotactic ability was not permanently compromised (B). DC previously co-cultured with MSC, removed from MSC co-cultures and re-stimulated with TNF-α for 48 h migrated towards CCL19. Data are expressed as chemotactic index calculated against the basal level of mature DC migration to medium alone. Results are mean (±S.E.) of counts determined from three random fields of view in duplicate chambers ($n = 2$), data shown is representative from a minimum of three independent experiments.
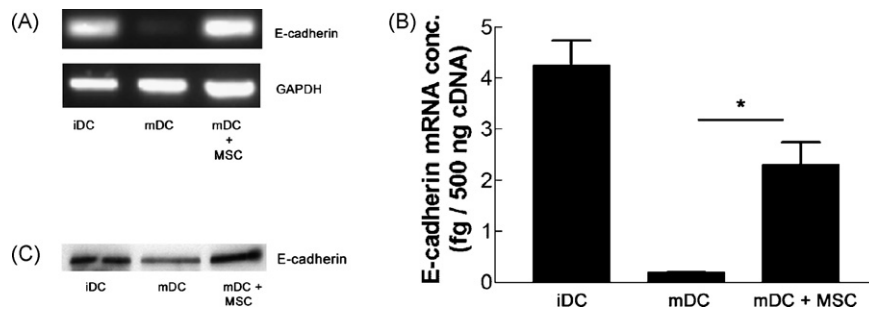
Fig. 5. Murine MSC prevented E-cadherin downregulation by TNF-α stimulated DC. (A) E-cadherin mRNA was measured by semi quantitative PCR of immature DC, mature DC or mature DC cultured in the presence of MSC at 24 h. (B) Quantitative real time RT-PCR also showed that E-cadherin mRNA expression was reduced in mature DC (*, $p < 0.05$), but not by mature DC cultured in the presence of MSC. (C) E-cadherin protein expression in mature DC cultured in the presence of MSC detected by immunoblot was similar to that of immature DC. Data from quantitative PCR is presented as mean (±S.E.) of triplicate data ($n = 3$). All data are representative of a minimum of three experiments.

CCR7 chemokine receptor expression and reduce the migration capacity of DC to lymph node derived chemokines.

Modulation or prevention of DC maturation is a well-described observation but remains poorly characterised. It is known that immature or so-called "semi-mature" DC have immunosuppressive or regulatory characteristics. This has been best characterised in infectious disease models. For example, Influenza virus hemagglutinin (HA) and a virulence factor from *Bordetella pertussis* filamentous hemagglutinin (FHA) have been shown to modulate DC maturation marker expression [19,20] reduce IL-12 production [20] and induce IL-10 induction [19]. The present study using TNF-α driven maturation, studies by Nauta et al. [22] using CD40L and especially those of Djouad et al. [39] using LPS clearly demonstrate that MSC influence DC to maintain or adopt a similar phenotype. This is consistent with previous studies that have demonstrated that MSC constitutively express TGF-β1, and HGF [7,8,40] and with our previous demonstration that IFN-γ enhanced MSC immunomodulation [7,12]. The present study suggests that MSC maintain DC with an immature or "semi-mature" suppressor phenotype [38] which contributes to the immunosuppressive capacity of MSC.

The mechanisms of MSC modulation of DC maturation have yet to be elucidated fully. However, studies by Djouad et al. [39] have demonstrated a role for IL-6 in MSC modulation of maturation marker expression. A possible role for IL-6 had also been alluded to by Nauta et al. [22] in 2006. Similar neutralisation studies in our system confirmed that MSC derived IL-6, but not HGF or TGF-β1 prevented upregulation of DC maturation marker expression (data not shown). These data concerning MSC modulation of DC are in contrast to MSC modulation of allo-antigen driven responses measured in MLR which are mediated by MSC derived PGE-2 and in some circumstances by indoleamine 2,3, dioxygenase expression [7,12,41–43]. Thus, MSC modulate diverse aspects of the adaptive immune response through multiple different mechanisms.

In addition to modulation of DC maturation markers, MSC interfered with DC antigen presentation ability as demonstrated here using an antigen specific T cell proliferation assay and specific peptide:MHC II antigen display. Although MSC significantly interfered with DC presentation of ovalbumin to DO11.10 CD4+ T cells, the inhibition of antigen presentation was only

partial. There are a number of possible explanations for this. It may be that a monoclonal DO11.10 CD4+ T cell response to ovalbumin presented by DC is less susceptible to suppression than polyclonal or wildtype populations, or that multiple mechanisms contribute to overall suppression and reduction and antigen display is only one contributory factor. In studies by Jiang et al. focusing on monocyte to DC differentiation, human allogeneic MSC were shown to hamper antigen presentation by DC in a keyhole-limpet hemocyanin (KLH) antigen specific T cell model. Allogeneic MSC downregulated both antigen presentation and IL-12 expression by DC [21]. Although the DO11.10 system used here might be less susceptible to immunosuppression by MSC, the use of a well-defined antigen and transgenic T cell model avoided the potentially confounding effect of allogenicity. Furthermore, the use of whole OVA protein, as opposed to specific peptide, indicated that full antigen processing is occurring rather than "bystander" presentation of peptide in a T cell to T cell manner. Nevertheless T cell proliferation is a complex interaction and may not be a straightforward marker of APC activity, therefore the Eα antigen display system was employed to confirm that Ag-peptide display at the DC surface was reduced by MSC co-culture. These findings and those of Jiang et al. [21] lead to the conclusion that MSC reduce the APC function of DC and that this is not related to allogenicity, but involves modulation of DC antigen presentation machinery.

DC ability to migrate to the local lymph nodes and the subsequent presentation of antigen to T cells play an essential role in initiation of adaptive immunity [44]. In tissues, mature DC must become responsive to lymph node derived signals, but must also downregulate tissue anchoring proteins such as E-cadherin [18,37,38] that would otherwise veto DC migration and antigen presentation to naïve T cells. This study provided the first indication that this process is modulated by MSC, such that DC exposed to TNF-α failed to downregulate E-cadherin. Likewise, the expression of the chemokine receptor CCR7 by mature DC plays an important role in DC migration to lymph node derived chemokines such as CCL19. MSC significantly reduced both CCR7 surface expression by DC and the functional migration to CCL19. Together, these data show that MSC interfered with key aspects of DC migration. The implications of these findings are that sites of repair where MSC are present (or tissues targeted

in regenerative medicine) should either display an accumulation of immature DC, or reduce DC recruitment to draining lymph nodes. We are currently examining that question, but intriguingly it suggests that MSC may have an additional application as an immunosuppressive therapy in autoimmune disease.

The ability of MSC to suppress alloreactivity has now been characterised by a number of groups [7–10], however, the need for MSC to possess this activity has been perplexing. The influence of MSC on DC shown here may partially resolve this problem. Firstly, both immature DC and MSC are normally resident in the bone marrow. The capacity for MSC to veto DC function would help impede premature maturation of DC, prior to DC exit from the bone marrow. Secondly, the main role of adult MSC is in tissue repair. This process is de facto part of the resolution phase that follows physiological or microbial insult. Therefore, whilst DC maturation needs to be promoted during inflammatory events, MSC mediated resolution would favour a non-inflammatory environment and preservation of DC immaturity. The ability of MSC to modulate the three major aspects of DC maturation shown herein is consistent with that hypothesis.

Acknowledgements

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