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Umbilical Cord-Derived Mesenchymal Stromal Cells Modulate Monocyte Function to Suppress T Cell Proliferation

Antony J. Cutler,* Vasanti Limbani,* John Girdlestone,*† and Cristina V. Navarrete*†

Mesenchymal stromal cells (MSCs) may be derived from a variety of tissues, with human umbilical cord (UC) providing an abundant and noninvasive source. Human UC-MSCs share similar in vitro immunosuppressive properties as MSCs obtained from bone marrow and cord blood. However, the mechanisms and cellular interactions used by MSCs to control immune responses remain to be fully elucidated. In this paper, we report that suppression of mitogen-induced T cell proliferation by human UC-, bone marrow-, and cord blood-MSCs required monocytes. Removal of monocytes but not B cells from human adult PBMCs (PBMCNs) reduced the immunosuppressive effects of MSCs on T cell proliferation. There was rapid modulation of a number of cell surface molecules on monocytes when PBMCs or alloantigen-activated PBMCNs were cultured with UC-MSCs. Indomethacin treatment significantly inhibited the ability of UC-MSCs to suppress T cell proliferation, indicating an important role for PGE2. Monocytes purified from UC-MSC coculture had significantly reduced accessory cell and allostimulatory function when tested in subsequent T cell proliferation assays, an effect mediated in part by UC-MSC PGE2 production and enhanced by PBMCNC alloactivation. Therefore, we identify monocytes as an essential intermediary through which UC-MSCs mediate their suppressive effects on T cell proliferation.

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Mesenchymal stromal cells (MSCs) have a profound immunosuppressive capability in vitro (1) and are currently being assessed as a novel cellular therapeutic anti-inflammatory agent in numerous clinical trials. MSCs are being considered as an adjunct to conventional therapy or a stand-alone therapy to treat a range of autoimmune diseases, acute and chronic kidney rejection posttransplant, and acute and chronic graft-versus-host disease (GVHD) and to aid tissue repair/wound healing (2, 3). Expanded MSCs have been shown to have no toxicity or adverse events when infused (4), and the most promising use of MSCs to date is in the treatment of patients with steroid-resistant GVHD following hematopoietic stem cell transplantation (5). Most clinical trials currently use MSCs derived from bone marrow (BM); however, MSCs may also be derived from umbilical cord blood (UCB), adipose tissue, placenta, dental pulp, and umbilical cord (UC) (reviewed in Ref. 6).

The mechanisms by which MSCs may ameliorate GVHD are unclear, but experimental evidence suggests that MSCs act on many of the leukocyte subsets involved directly or indirectly in the generation of an antihost immune response posthematopoietic stem cell transplantation. MSCs reduce the proliferative capacity of naive and memory T cells, B cells and effector function of γδ T cells, NK cells, and neutrophils. MSCs also block and modulate the in vitro maturation and function of monocyte-derived dendritic cells (moDCs) (reviewed in Ref. 2). MSCs secrete a plethora of growth factors, cytokines, and immunomodulatory mediators. PGE2, NO, HLA-G, IDO, IL-10, TGF-β, hepatocyte growth factor, IL-6, and insulin-like growth factor-1 have all been implicated in MSC-induced immunosuppression (reviewed in Ref. 7).

Acute GVHD is initiated by host-derived dendritic cells (DCs), activated by the inflammatory environment induced by conditioning regimens. Donor T cells recognize mismatched MHC molecules on the host tissues and cells including DCs, become activated, proliferate, and differentiate into mature effector cells to drive pathogenic responses (8). Following myeloid ablative conditioning or reduced intensity conditioning of the patient, host DCs are rapidly replaced by donor-derived DCs in the blood (9) and the skin (10). Donor-derived DCs may then indirectly present host-derived Ag and exacerbate the graft-versus-host response (11). Because monocytes are able to develop into DCs under inflammatory conditions (12), it is conceivable that suppression of GVHD by MSCs is related to their ability to influence the maturation and function of DCs (13, 14).

We and others have determined that UC-derived MSCs suppress T cell proliferation (15, 16). In this paper, we extend these observations and explore the cellular interactions used by MSCs for optimal suppression of T cell proliferation. We show that monocytes are required for the optimal suppression of T cell proliferation induced by TCR cross-linking by UCB-, BM-, and UC-MSCs. Within 24 h of coculture and prior to their differentiation into DCs, monocyte phenotype and function are modulated by interaction with MSCs. Accessory function and the allostimulatory capacity of monocytes are downregulated by MSCs via a mechanism that is in part driven by UC-MSC-derived PGE2. Thus, MSCs appear to act indirectly on T cells via monocytes, which act as an essential intermediary cell allowing optimal suppression of T cell proliferation by MSCs.

Materials and Methods

Cell lines and PBMCs

Human UC-derived and cord blood (CB)-derived MSCs, generated as previously described (16, 17), were maintained in culture in low glucose-DMEM

Abbreviations used in this paper: BM, bone marrow; CB, cord blood; DC, dendritic cell; DC-SIGN, dendritic cell-specific intercellular adhesion molecule-3–grabbing non-integrin; GVHD, graft-versus-host disease; moDC, monocyte-derived dendritic cell; MSC, mesenchymal stromal cell; UC, umbilical cord; UCB, umbilical cord blood.
supplemented with 10% FBS, penicillin, streptomycin, and l-glutamine (Invitrogen, Carlsbad, CA). Human BM-MSCs were purchased from Lonza, Basel, Switzerland and maintained in culture in Mesencult medium with 10% supplements (StemCell Technologies, Grenoble, France). Upon reaching confluence cells were released from the plate using trypsin-EDTA (Sigma-Aldrich, St. Louis, MO), counted, and replated. UC-MSCs were used from passages 3 to 15 with similar results obtained throughout. Human adult PBMCs (PBMCNs) of buffy coat samples chosen at random were provided by National Health Service Blood and Transplant (Colindale, UK). PBMCNs were prepared by density centrifugation over Lymphoprep (Axis-Shield, Oslo, Norway), and HLA typing was performed by the Histocompatibility and Immunogenetics Department, National Health Service Blood and Transplant, Colindale Center. UC-MSCs were obtained from third-party donors and HLA mismatched with the PBMCNs used in these experiments.

Flow cytometry

Cells in PBS, 0.05% sodium azide, 2.5% FBS and 1% Fc block (Miltenyi Biotec, Bergisch Gladbach, Germany) were stained with CD14 specific Ab (ImmunoTools, Friesoythe, Germany) to identify monocytes and counter-stained using a selection of CD73+, CD123+, CD206+, DC-specific intercellular adhesion molecule-3-grabbing nonintegrin (DC-SIGN)−, and HLA-DR−specific Abs (all BD Biosciences, Oxford, U.K.). Isotype controls and unstained cells were used for all fluorochromes (BD Biosciences). Stained cells were then washed and resuspended in PBS, and data were acquired using a FACSort flow cytometer and CellQuest software (BD Biosciences). Data were analyzed using WinMDI software (The Scripps Research Institute, La Jolla, CA).

Cell purification

T + NK cells, monocytes, and B cells were removed from PBMCNs, using anti-CD2, CD14, or CD19 microbeads (Miltenyi Biotec), respectively. Untouched CD4+ T cells and monocytes were isolated using the CD4+ T cell isolation kit II or monocyte isolation kit II, respectively (Miltenyi Biotec). All cells were purified using an autoMACS, according to the manufacturer’s instructions (Miltenyi Biotec).

Monocyte conditioning and purification

Monocytes were purified from nonactivated or alloantigen-activated PBMCN-conditioning cultures as follows. Nonactivated PBMCN cultures (5 × 10^4 PBMCN ± 2 × 10^4 UC-MSCs) and alloantigen-activated PBMCN cultures (2.5 × 10^5 CD4+ T cell and 2.5 × 10^5 CD2-depleted allogeneic PBMCNs ± 2 × 10^4 UC-MSCs) were cultured in RPMI 1640 medium (Invitrogen) supplemented with 10% human AB serum (Lonza, Basel, Switzerland) and penicillin/streptomycin for 24 h. After 24 h, PBMCNs or alloantigen-activated PBMCN MSC cocultures were harvested and initially centrifuged over a 28% Percoll (Sigma-Aldrich) density gradient to enrich for MSCs. All cultures were further centrifuged over a 35% Percoll density gradient to enrich for monocytes. The resulting interface was harvested and washed and untouched CD4+ T cells isolated using the monocyte isolation kit II (Miltenyi Biotec). All cell sorts were carried out using an autoMACS.

Cell proliferation

Alloantigen stimulation. This was performed using the MLR assay as follows: freshly isolated CD4+ T cells (2.5 × 10^5/ml in PBS) were incubated with 1.25 μM CFSE (Sigma-Aldrich) at room temperature. After 5 min, 10×25 volume of PBS was added, and the cells were incubated for an additional 10 min. CFSE-labeled cells were washed twice in PBS and recounted prior to culture. CFSE-labeled CD4+ T cells (5 × 10^5) were incubated with allogeneic highly purified monocytes from conditioning cultures (5 × 10^6) at a ratio of 10:1. After 7 d of culture, proliferating and activated T cells were identified using CD3 Ab (ImmunoTools), CD25 (Serotec, Kidlington, U.K.), and CFSE dye dilution. Monocytes were excluded from the analysis using CD14 Ab (ImmunoTools). Stained cells were then washed, resuspended, and acquired using the FACSort flow cytometer as described previously.

PHA and bead stimulations. PBMCNs or purified cell populations plated at 5 × 10^4 cells/well in RPMI 1640 medium + 10% AB serum (Lonza) in U-bottomed 96-well plates (Nunc, Naperville, IL) were stimulated with anti-CD2-, -CD3-, -CD28, T cell activation beads (1.75 × 10^6; Miltenyi Biotec), or PHA (1 μg/ml; Sigma-Aldrich) with or without MSCs (2 × 10^4 cells/well). Some assays were carried out in the presence or absence of indomethacin (5–10 μg/ml; Sigma-Aldrich). Trinitated [3H]thymidine (37 kBq/well; Amersham Biosciences, Little Chalfont, Buckinghamshire, U.K.) was added after 2 d of culture, and the cells were harvested 16–18 h later. Thymidine incorporation was measured in a beta counter (PerkinElmer, Wellesley, MA).

Inhibition and measurement of UC-MSC PGE2 production

UC-MSCs were incubated with 5–10 μM indomethacin (Sigma-Aldrich) or an equivalent volume of carrier (DMSO) (Sigma-Aldrich) in low glucose-DMEM + 10% FBS and penicillin/streptomycin for 24 h. Indomethacin-treated or control UC-MSCs were subsequently harvested, washed, and cultured with nonactivated or alloantigen-activated PBMCNs at a ratio of 1:2.5 in RPMI 1640 medium-10% AB serum for 24 h. PGE2 levels were measured in tissue culture supernatant by ELISA following the manufacturer’s instructions (Assay Designs, Enzo Life Sciences, Farmingdale, NY).

Statistical analysis

Analysis of statistics was carried out using SPSS (version 15) software by using unpaired or paired student’s t tests where appropriate. All data are presented as mean ± SD, and p < 0.05 was considered significant.

Results

Monocytes are required for optimal suppression of T cell proliferation by MSCs

We have previously shown that UC-MSCs reduce the proliferation of T cells in vitro (16) and therefore sought to examine and define the cellular interactions required for MSCs to exert their suppressive effect. Addition of monocytes has previously been shown to restore the ability of BM-MSCs to suppress proliferation of purified CD4+ T cells in response to superantigen (18). We confirmed that addition of monocytes to cocultures of CD4+ T cells

**FIGURE 1.** MSCs require the presence of monocytes to suppress T cell proliferation. A. PBMCNs (black bar), CD19-depleted PBMCNs (gray hatched bar) or CD14-depleted PBMCNs (gray bar) from four independent blood donors were incubated with activation beads in the presence or absence of UC-MSCs. Proliferation assessed by [3H] uptake after 72 h of culture. The degree of suppression of T cell proliferation induced by UC-MSCs is expressed as a percentage relative to T cell proliferation in the presence of nonactivated PBMNCs (gray bar) or CD14-depleted PBMNCs (gray bar) from four independent donors and HLA mismatched with the PBMNCs used in these experiments. The degree of suppression of T cell proliferation induced by UC-MSCs is expressed as a percentage relative to T cell proliferation in the absence of UC-MSCs. Data are shown as mean values ± SD. Statistical significance of differences were determined using paired Student t test. ****p = 0.0001. B–D. PBMCNs (black bar) or CD14-depleted PBMCNs (gray bar) (n = 4) were incubated with activation beads with or without MSCs from UCB (B), BM (C), or UC (D) (n = 2 MSC lines per source) and proliferation assessed by [3H] uptake after 72 h of culture. The degree of suppression of T cell proliferation induced by MSCs expressed as a percentage relative to T cell proliferation in the absence of MSCs. The data are representative of two independent experiments and shown as mean values ± SD. Statistical significance of differences were determined using paired Student t test.
and MSCs reconstituted the optimal suppressive action of MSCs on T cell proliferation (data not shown) and extended these observations to demonstrate that depletion of monocytes from PBMCs completely abolished the capacity of UC-MSCs to suppress T cell proliferation induced by direct activation of T cells by anti-CD2, -CD3, and -CD28 stimulation. Depletion of B cells had no effect on the capacity of MSCs to suppress T cell proliferation (Fig. 1A). Importantly, MSCs derived from UCB (Fig. 1B) and BM (Fig. 1C) in addition to those derived from UC (Fig. 1D) all required monocytes to suppress proliferation of T cells in vitro.

Coculture of PBMCNs and UC-MSCs induces rapid phenotypic changes in the monocyte population

BM-MSCs alter the phenotype of mDCs differentiated in vitro with GM-CSF and IL-4 (13, 14, 19) and that of mature macrophages (20). We therefore followed the phenotype of monocytes cocultured for 24 h and 7 d in vitro with third party UC-MSCs in nonactivated (PBMCN ± UC-MSC) or alloantigen activated (CD4+ T cell and CD2-depleted APCs in a 1:1 ratio) UC-MSCs for 24 h or 7 d. The characteristics and surface phenotype of cells gated on CD14 expression were analyzed. Statistical significance of differences in geometric mean channel fluorescence was determined using paired Student t test. *p < 0.05; **p < 0.01; and ***p < 0.001. The data are representative of four independent experiments and shown as mean values ± SD.

FIGURE 2. UC-MSCs induce phenotypic and physical changes in the monocyte population. Flow cytometric analysis of monocyte phenotype in culture. Non-activated PBMCN (A, B) or alloantigen activated (CD4+ T cells: CD2-depleted APCs in a 1:1 ratio) (C) were cultured with (n, n = 4) or without (N, n = 6) UC-MSCs for 24 h or 7 d. The characteristics and surface phenotype of cells gated on CD14 expression were analyzed. Statistical significance of differences in geometric mean channel fluorescence was determined using paired Student t test. *p < 0.05; **p < 0.01; and ***p < 0.001. The data are representative of four independent experiments and shown as mean values ± SD.

MSC coculture reduces the accessory and allostimulatory function of monocytes

Monocytes are a major component of the cellular influx into an inflammatory environment (21). They are also able to differentiate into inflammatory DCS or macrophages with both pro- and anti-inflammatory properties dependent on environmental stimuli (22–24). To investigate whether monocyte function as well as phenotype was modulated following UC-MSC exposure, we analyzed the capacity of monocytes to support T cell proliferation in response
to PHA stimulation, because highly purified CD4+ T cells only respond to PHA in the presence of accessory cells such as monocytes (25). Monocytes (>85% CD14+ with <0.5% UC-MSC contamination) purified from nonactivated PBMCs following 24 h of coculture with UC-MSCs had profoundly reduced accessory cell function in subsequent PHA stimulation assays when compared with those cultured in medium alone (Fig. 3A). Monocytes purified from highly pure CD14+ and UC-MSC coculture also had reduced accessory function compared with those cultured in medium alone (Fig. 3B), indicating that other mononuclear cell populations are not required for the MSC effect on monocytes. UC-MSC–modulated monocytes were further assessed for their capacity to induce proliferation of allogeneic CD4+ T cells. CD14+ cells purified from nonactivated PBMC and UC-MSC coculture had a reduced ability to induce proliferation of allogeneic T cells (Fig. 3C). In addition, monocytes purified from highly pure CD14+ and UC-MSC coculture exhibited reduced allostimulatory capacity in two of three experiments (data not shown). The data suggest that UC-MSCs may interact directly with monocytes to rapidly impair their accessory functions without any requirement for a third-party leukocyte subset.

Inhibition of PGE2 production releases T cells from UC-MSC–induced suppression

PGE2 has previously been identified as one of many MSC-derived soluble factors proposed to modulate immune function (2). PGE2 was constitutively produced by UC-MSCs and increased levels were detected where nonactivated or alloantigen-activated PBMCs were cocultured with UC-MSCs (Fig. 4A). As MSC-derived PGE2 may modulate moDC maturation (26) and suppress T cell proliferation (27), we therefore assessed whether UC-MSCs mediated their immunosuppressive effects via PGE2. In agreement with a previous report (15, 16), we found that UC-MSCs were unable to inhibit PHA-induced proliferation of PBMC in the presence of indomethacin (a nonspecific cyclooxygenase-1/2 inhibitor) (Fig. 4B).

UC-MSCs reduce monocyte allostimulatory capacity—an effect partially mediated by UC-MSC PGE2 production and enhanced by activation

PGE2 has complex and often divergent actions on different cells of the immune system (28). We therefore addressed whether UC-MSC–derived PGE2 had a role in modulating monocyte allostimulatory function. UC-MSCs were pretreated with indomethacin for 24 h prior to coculture with nonactivated PBMCs or alloantigen-activated PBMCs. Treatment abolished constitutive PGE2 production by UC-MSCs but did not affect PGE2 production in the subsequent culture (data not shown). Indomethacin treatment of UC-MSCs significantly reduced their ability to modulate monocyte allostimulatory function compared with those purified from UC-MSCs treated with carrier (DMSO) alone. Indeed, monocytes purified from nonactivated PBMC or alloantigen-activated PBMC coculture with indomethacin-pretreated UC-MSCs were able to stimulate allogeneic T cells to approximately a 2- and 4-fold greater extent, respectively, than monocytes purified from nonactivated PBMCs or alloantigen-activated PBMCs cultured with control UC-MSCs (Fig. 5A, 5B).

Inflammation has been proposed to “license” MSCs to suppress immune function (29). We therefore compared the allostimulatory

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**FIGURE 3.** Monocytes from PBMC/UC-MSC coculture have reduced accessory cell and allostimulatory function. A, CD4+ T cells were cultured in medium alone (gray bar), with PHA (1 μg/ml) (hatched bar), with PHA and CD14+ cells purified from a 24-h UC-MSC/nonactivated PBMC coculture (black bar), or with PHA and CD14+ cells purified from nonactivated PBMC culture alone (white bar). Proliferation was measured by [3H] uptake after 72 h of culture. The data shown are representative of five independent experiments and shown as mean values ± SD. B, CD4+ T cells were cultured in medium alone (gray bar), with PHA (hatched bar), with PHA and CD14+ cells purified from CD14+ cell/UC-MSC coculture after 24 h (black bar), or with PHA and CD14+ cells purified from CD14+ cells in medium alone (white bar). Proliferation was measured by [3H] uptake after 72 h of culture. The data shown are representative of two independent experiments and shown as mean values ± SD. C, CD14+ cells were purified from nonactivated PBMCs cultured in medium alone (white bar, n = 2) or with UC-MSC (black bar, n = 2) for 24 h and cultured with allogeneic CFSE-labeled CD4+ T cells. CD4+ T cell proliferation was assessed at 7 d by CFSE dye dilution and upregulation of CD25. The data are presented as proliferation induced by CD14+ cells relative to controls where proliferation is a reference at 100% and is shown as mean values ± SD.

**FIGURE 4.** PGE2 mediates UC-MSC suppression of T cell proliferation. A, PGE2 was measured in the supernatant of PBMCs (5 × 10^4) (spotted bar, n = 2), MLR (2.5 × 10^5) CD4+ T cells + 2.5 × 10^6 CD2-depleted APCs (white bar, n = 3), PBMC + UC-MSC (2 × 10^5) (black spotted bar, n = 2), MLR + UC-MSC (black bar, n = 2), or UC-MSC alone (gray bar) cultures after 24 h. B, A total of 5 × 10^5 PBMCs were cultured with PHA (1 μg/ml) (white bar), with PHA + 2 × 10^6 UC-MSC + DMSO (black bar) or with PHA + 2 × 10^6 UC-MSC + 5 μM indomethacin (hatched bar). Proliferation was measured by [3H] uptake after 72 h of culture. The data shown are representative of five independent experiments and shown as mean values ± SD.
We therefore assessed whether PGE2 acted alone or in concert with UC-MSC–derived PGE2 act on the monocyte population to modulate their function and suppress T cell proliferation.

**Discussion**

MSCs have been shown to modulate the actions of a diverse range of immune cells and a number of soluble factors derived from, or induced by MSCs have been proposed to mediate the anti-inflammatory or suppressive effects of MSCs (2). Given the increasing use of MSCs as a cellular therapy product in many clinical trials it is of critical importance to elucidate the cellular interactions required for MSCs to suppress immune responses and to understand whether MSCs act directly on effector cells or via a third party. In this study, we demonstrate that MSCs from diverse sources are dependent on monocytes to suppress T cell proliferation induced by TCR cross-linking and that UC-MSCs act via an indomethacin-sensitive agent PGE2. MSCs derived from UC tissue rapidly alter monocyte phenotype, affect accessory cell function, and fundamentally reduce their ability to activate and induce the proliferation of allogeneic T cells. The modulatory effect of UC-MSCs on monocytes was enhanced in an activated environment and was in part dependent on constitutive UC-MSC PGE2 production. Importantly, the modulatory effect of UC-MSC–derived PGE2 was effective only during initial contact between UC-MSCs and monocytes contained within nonactivated PBMCs or alloantigen-activated BM-MSCs.

Our data clearly implicate monocytes as a key intermediary in UC-MSC–induced suppression of T cell proliferation. This may be a key interaction in vivo as peripheral blood and splenic monocytes are rapidly recruited to inflammatory sites (30, 31) and are able to differentiate into both inflammatory DCs and macrophages (21, 22). Once differentiated, both of these mononuclear phagocyte cell populations play pivotal roles in promoting immune responses and resolving inflammation depending on the environmental cues (22, 32). BM-MSCs have been shown to alter or block the maturation of monocytes into DCs (13, 14, 19, 26).

However, in contrast to our studies, monocytes and BM-MSCs in earlier reports were cultured in the presence of GM-CSF and IL-4 over 5–6 d in vitro to induce DC differentiation. Our data suggest that monocyte function is directly and stably modulated by UC-MSCs within 24 h of coculture (Figs. 3, 5) and that MSCs from disparate sources all act through monocytes to reduce T cell proliferation (Fig. 1B–D). Furthermore, the allostimulatory capacity of highly purified CD14+ monocytes was greatly reduced following culture with UC-MSCs (data not shown). The data reinforce the notion that MSCs from a variety of sources primarily and directly act through monocytes to reduce T cell proliferation.

In agreement, BM-MSCs have previously been shown to interact with monocytes to modulate immune responses through soluble mediators (33) and to reduce monocyte accessory function in superantigen-stimulated cultures (18). It is of note that BM-MSCs may in addition reduce mature DC function (14) and alter macrophage phenotype and function (20).

The phenotypic changes observed in the monocyte population suggest that UC-MSCs induce macrophages rather than a DC subset. We found no evidence for induction of a CD14lo/CD1c, CD11a, or CD83+ DC population or modulation of CD80 or CD86 on the CD14 positive population (data not shown). Although originally described as a DC-specific marker, DC-SIGN may be expressed by macrophages (34). CD123 (IL-3Rα) expression is associated with a plasmacytoid DC phenotype and was maintained following culture with UC-MSCs; however, plasmacytoid DCs do not coexpress CD14 (35). Indeed, CD14 expression was significantly increased initially in the presence of UC-MSCs but then declined over time dependent on the activation status of the cultures.
(Fig. 2). In agreement, monocytes or CD14+ precursors cultured with BM-MSC in DC differentiation conditions maintained expression of CD14 (13, 14) and were suggested to exhibit a macrophage-like morphology (13). IL-6 may play a key role in driving monocyte differentiation into macrophages (36). IL-6 is produced by UC-MSCs (data not shown) and in concert with M-CSF may (13, 14) or may not (26) mediate BM-MSC modulation of moDC differentiation. However, in our hands, neutralization of IL-6 and M-CSF had no effect on the ability of UC-MSCs to modulate monocyte phenotype (data not shown). Finally, expression of CD73, a component of the immunosuppressive adenosine pathway, is upregulated in the presence of UC-MSC (Fig. 2B, 2C) and PGE2 (data not shown) and is induced on mature activated macrophages (37).

Macrophage and monocyte populations are heterogeneous with respect to the activatory and tissue environment guiding their phenotype and function (23). UC-MSC upregulated expression of CD206 on CD14+ cells (Fig. 2B, 2C) a molecule frequently associated with an alternatively activated macrophage phenotype (38). CD206 was also induced on mature macrophages by BM-MSCs (20). Alternatively activated macrophage defined by their induction by IL-4/13 signaling may induce tissue remodeling, regulate immune responses (24) and inhibit T cell proliferation in vitro (39). Indeed, DC-SIGN expression another molecule induced by UC-MSCs (Fig. 2) may also be induced by IL-4 (40). However, IL-4 was not detected following UC-MSC and PBMC cocultures (data not shown), and therefore from our data, we would not classify the induced macrophage population as alternatively activated.

PGE2 can exert opposing effects on the immune system (28). Depending on the context, PGE2 may either be pro- (41) or anti-inflammatory (42). BM-MSC–derived PGE2 has been proposed to block DC differentiation (26) and induce monocyte IL-10 production (43). We and others (15) have shown that PGE2 production may play a key role in UC-MSC–induced suppression of T cell proliferation (Fig. 4). PGE2 is constitutively produced by BM-MSCs (27) and monocytes (44). Therefore to study the role of UC-MSC–derived PGE2 in modulating monocyte function, UC-MSCs were pretreated with indomethacin, thus reversibly blocking constitutive UC-MSC PGE2 production (data not shown), prior to culture with immune cells. However, comparable amounts of PGE2 were detected in subsequent cultures with nonactivated, alloantigen-activated PBMCs or purified CD14+ cells whether UC-MSCs were pretreated with indomethacin or not (data not shown). The data indicating, as previously described (27), a strong induction of de novo cyclooxygenase activity when MSCs are cultured with leukocytes. Despite this observation, monocytes purified from coculture with indomethacin-treated UC-MSC–stimulated allogeneic CD4+ T cells to proliferate to a significant degree above those from control UC-MSC cultures (Fig. 5). PGE2 treatment of PBMCs reproduced some but not all of the phenotypic changes induced by UC-MSCs on monocytes (data not shown).

However, treatment of PBMCs with PGE2 in isolation in some instances enhanced the allostimulatory capacity of monocytes (Fig. 5C). Therefore, the absence of PGE2 production by UC-MSCs at the initial stages of priming may therefore allow other mediators to “re- wire” monocytes to be either nonresponsive to PGE2 or alter their response to PGE2.

BM-MSCs have shown promise in the treatment of GVHD but only in patients with active disease (5, 45). MSCs share with monocytes the ability to home to sites of inflammation (46) and may enhance recruitment of macrophages to sites of injury (47). However, depending upon the stimulus, the suppressive action of MSCs may be enhanced or diminished by inflammation. Triggering through TLRs inhibited MSC-suppressive function (48), whereas IFN-γ enhanced the suppressive ability of BM-MSCs (49, 50). The allostimulatory capacity of monocytes purified from a MLR was significantly reduced compared with those purified from PBMCs following coculture with UC-MSC (Fig. 5A, 5B). Our data support the idea that MSC suppression is enhanced in an allogeneic environment. As discussed, IFN-γ, PGE2, and IL-10 all may play a role in MSC-induced suppression. However, in our studies, induction of PGE2 and IL-10 secretion were equivalent in either nonactivated PBMC or alloantigen-activated PBMC coculture with UC-MSCs, and neutralizing IL-10 in these cultures did not affect the ability of UC-MSCs to modulate monocyte allostimulatory function (data not shown). Secretion of IFN-γ, TNF-α, IL-6, and IL-1β were all induced following PBMC culture with UC-MSCs but were not enhanced by stimulation (data not shown). Therefore, as-yet unidentified factors are induced during UC-MSC and MLR coculture, which are able to further reduce monocyte allostimulatory capacity and to enhance the suppressive capacity of UC-MSCs.

In conclusion, our data demonstrate that UC-MSCs require monocytes to suppress mitogen-stimulated T cell proliferation. Monocyte phenotype and accessory and allostimulatory functions were stably and profoundly modulated following interaction with UC-MSCs. UC-MSC PGE2 production, an indomethacin-sensitive agent, appeared to play a key role in the suppression of T cell proliferation and in part the modulation of monocyte function. Importantly, the influence of UC-MSC–derived PGE2 on monocyte function was limited to the initial phase of contact. Finally, PGE2 treatment of PBMCs alone could not reproduce a similar modulation of monocyte function. Therefore UC-MSC–derived PGE2 acts at the initial contact between MSC and monocyte and in concert with other factors to modulate monocyte function and suppress T cell proliferation.

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Disclosures
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