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Original Research Article

Depletion of androgen receptor (AR) in mesenchymal stem cells (MSCs) inhibits induction of CD4+CD25+FOX3+ regulatory T (Treg) cells via androgen TGF-β interaction

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ABSTRACT

MSCs produce CD4(+)CD25(+)FOX3(+) regulatory T (Treg) cells from activated peripheral blood mononuclear cells (PBMC), T-CD4+ and T-CD8+ cells *in vitro* and *in vivo*. Here we investigated whether the deficiency of androgen/AR in MSCs influence Treg induction from total PBMC, splenocytes, CD4+CD25-through AR/TGF-β interaction. Eight to 12-week-old wild type and general androgen receptor knockout (ARKO) mice were used. MSCs were collected, characterized and function of Treg cells was studied. Our result showed that depletion of AR suppressed the immunosuppressive effect of MSCs, and demonstrated that WT-MSC-induced Treg cell expansion was partially impaired by blocking androgen receptor signal. Furthermore, the levels of TGF-β were lower in the T cell coculture with ARKO-MSC compared to WT-MSC. Exposure of ARKO-MSC cells to exogenous active TGF-β partially restored the induction of Treg cell expansion by ARKO-MSC cells. Our data suggest that ARKO-MSC hampers Treg cell expansion and function via androgen/AR and TGF-β signal pathways interaction. To the best of our knowledge, this study is the first investigating the

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CD4+FOXP3+ T cells
Casodex

interaction of MSCs from ARKO mice and WT Tregs in an allogeneic co-culture model. Together, these results might provide great insight into treatment of inflammatory and autoimmune diseases.

Introduction

MSCs are heterogeneous, multipotent cells that constitute a small segment of the stromal cell population in the bone marrow and are characterized by their capability to differentiate into cells of mesodermal lineage making them an important cell source that replenish damaged tissues (Pittenger et al., 1999). Clinical studies (Le Blanc et al., 2004), *in vivo* (Bartholomew et al., 2002) and *in vitro* models (Di Nicola et al., 2002) clearly demonstrated the MSCs immune regulatory function. Interestingly, MSCs turn isolated T-CD4 cells, mouse splenocytes and IL-2-activated human peripheral blood mononuclear cells (PBMC) to CD4+CD25+, CD4+CTLA4+, CD4+CD25+ CTLA4+ T cells and, CD4+CD25+high Foxp3+ T regulatory (Treg) cells *in vitro* and *in vivo* (Aggarwal and Pittenger, 2005; Maccario et al., 2005). This Il-2 immune function might be hampered by reducing its production by caffeine that leads to downregulation of antibody production in a mouse model (Pohanka, 2015). Autologous MSC administration also exhibited an increase in the number of CD4 + CD25+Foxp3+ cells in patients with kidney transplantation in addition to a subsequent improvement in their renal function (Perico et al., 2011).

The aforementioned observations hint to the anti-inflammatory effects of MSCs through the induction of a regulatory T cell phenotype that qualify them to be largely used in several clinical trials to treat inflammatory diseases such as liver cirrhosis, sepsis, ostoegenesis perfecta and myocardial infarction (Terai et al., 2006). However, the function of activated MSCs is different in both genders. For example, when MSCs exposed to stress challenges, male MSCs secrete less cytokines than female BM-MSCs (Markel et al., 2007). On another note, Huang et al. (2013) showed that targeting AR in the MSCs improves their self-renewal and migration potentials and increases paracrine effects to perform anti-inflammatory and anti-fibrotic actions to improve liver repair in a couple of liver cirrhosis mouse models induced by CCl4 or TAA. Similarly, targeting AR in thymic epithelial AR may offer a new therapeutic approach by improving the efficacy of grafting of bone marrow transplantation (BMT) by enhancing T-cell reconstitution (Lai et al., 2013). Likewise, deletion of the AR gene significantly hindered maturation of neutrophils and decreased the proliferative capacity of their precursors (Chuang et al., 2009). These observations might hint to a potential effect of androgen/AR on the immunomodulatory function of MSCs through interaction with Treg that is not previously fully investigated to the best of our knowledge. Therefore, in this study we investigated the capacity of ARKO-MSCs to produce functional CD4+ CD25+Foxp3+ Treg cells.

For the immunomodulatory effect to occur, direct MSC/T cell and indirect contact through soluble suppressive factors produced by MSCs, such as TGF-β1, PGE2 and IL-10 are required for converting CD4+ T cells to Foxp3 and CD25High expression cells (English et al., 2009). Interestingly, lethal autoimmune syndrome appears after constitutive ablation of TGF-β receptor (TR) through thymic development in mice. Hence, TGF-β may serve as an essential factor for peripheral tolerance by supporting maintenance and function of regulatory T (Treg) cells. Similarly, former reports demonstrated that TGF-β is essential for the production of functionally active Tregs (Wan and Flavell, 2007). On another hand, earlier studies have shown that TGF-β promotes proliferation of MSCs (Stewart et al., 2010) and is important for Treg expansion (Nguyen et al., 2011). Androgen also upregulates the expression of TGF-β in the bone marrow compartment (Olsen et al., 2001). It has been also demonstrated that androgen regulated transcription of TGF-β through direct binding of androgen/AR to the potential ARE within TGF-β promoter region (Jones et al., 2009). Hence, in this report we investigated whether TGF-β has a role in androgen mediated immunomodulatory effect of MSCs.

ARKO male mice and WT male littermates were used in this study to collect bone marrow MSCs (BM-MSCs). We focused on the importance of AR integrity in the immunomodulation properties of MSCs via investigating ARKO-MSC/T-cell interactions. The study showed that depletion of AR decreased the immunomodulatory effects of BM-MSCs on Tregs. It also demonstrated that AR is important for MSCs to expand Treg cells. Moreover, ARKO-MSCs could not efficiently recruit Tregs from total PBMC, total CD4+ cells, splenocytes, and from CD4 +CD25− T cells with the lowest expansion level being observed in CD4+CD25− T cells. Furthermore, after coculture with ARKO-MSCs, Treg cells were less suppressive compared to those cocultured with WT-MSC. These results suggested the importance of AR in MSCs for the generation of CD4+CD25 +FOX3+ regulatory T cells. In addition, the current study showed that TGF-β level in ARKO-MSC coculture was lower compared with that in WT-MSCs/Treg coculture and hence pointed to that TGF-β might mediate the effect of loss of function of MSCs on Treg conducted by androgen deficiency. Consequently transplantation of corrected MSCs might be considered to improve some autoimmune diseases and enable us to better fight several irreparable diseases in the future.

Materials and methods

Mice

We utilized cre-Lox approach to produce WT and ARKO mice. We generated the floxed AR/AR mice according to our previously published manuscript (Yeh et al., 2002). All animal experiments were approved by the University Committee on Animal Resources of the University of Rochester.

Isolation of MSCs

MSCs were isolated from eight- to ten-week-old ARKO and WT male mice. Bone marrow cells were collected by as previously described (Peister et al., 2004). Briefly, tibias and femurs were dissected from adult mice at 8–10 weeks old. After bones were cut, the marrows were flushed out with 5 ml DMEM by using a needle and syringe, and resuspended in DMEM plus 15% FBS. The cell suspension ($1 \times 106/cm^2$) was plated in a modified minimum essential Eagle's medium (MEM)© (α-MEM, Gibco, Auckland, NZ) supplemented with 20% fetal bovine serum (FBS) (Hyclone, Thermo Fisher Scientific, Brebières, France), 2 mM glutamine and 100 U/mL penicillin with 100 mg/mL streptomycin (Gibco, Auckland, NZ) (α-20). At sub-confluence, cells were replated at a density of 20,000 cells/cm^2 and, after the second passage, MSCs were isolated using EasySep™ Mouse Mesenchymal Stem/Progenitor Cell Enrichment Kit (Stem Cell Technologies) by negative selection method by excluding cells of hematopoietic and endothelial lineages (CD45, TER119 antibodies linked to magnetic beads) according to manufacturer's instructions. MSCs were characterized for expression of hematopoietic and mesenchymal cell antigens by fluorescence-activated cell sorting (FACS) analysis as previously described in Huang et al. (2013).

Characterization of MSCs was conducted according to the methods described in our group earlier paper (Huang et al., 2013).

Briefly, CFU-f assay for self-renewal was conducted by culturing 2×10^6 mononucleated cells onto 6-well plates for 21 days then fixed with methanol and stained with Methylene blue for 15 min and counted. More than 20 cells were counted as positive CFU-f. Regarding MTT assay, 5000 cells/well were plated onto 24-well plates. At different time points, MTT solution was added to cells to react for 30 min, medium removed, and DMSO added to dissolve the MTT salt. The absorbance values were measured at 575–650 nm. Concerning Ki67 staining, MSCs were seeded on the 4-well chamber slides and cultured to confluence and then fixed with methanol. After fixation, washing and blocking they were incubated with anti-Ki67 then incubated with biotinylated secondary antibody (Vector Laboratories) and ABC solution (Vector Laboratories). Cells were stained by AEC (DAKO, Carpentaria, CA), followed by Mayor's hematoxylin counterstaining. For BrdU labeling, MSCs were seeded on the 4-well chamber slides and cultured to confluence. BrdU labeling reagent (Invitrogen) was added to the cultured cells at 1:100 dilution in the culture medium for 24 h. After labeling, staining was performed according to the manual instruction (Invitrogen, BrdU staining kit).

Identification of AR expression in WT-MSCs by Western blot analysis assay

It was conducted based on the method portrayed in our group previous paper (Huang et al., 2013).

Collection of peripheral blood mononuclear cells (PBMC), spleen and bone marrow cells

Blood samples: Half to 1 mL was collected by submandibular bleeding and drawn into heparinized tubes and centrifuged on Ficoll-Hypaque gradients (GE Healthcare Bioscience). PBMC were recovered, washed in AIM-V medium (Invitrogen), counted in a Trypan blue dye and immediately used for experiments. Mice were then euthanized by CO_2 asphyxiation and the spleens were weighed. The spleens were teased apart and disaggregated on top of a cell strainer in a cell culture dish containing 5 ml of cold media (FACS wash) and stored on ice until labeling with antibodies for flow cytometric analysis of CD4+ and FOXP3+. Bone marrow cells were obtained from the tibias and femurs of each animal by flushing the marrow cavity with RPMI 1640 medium (Life Technologies, Inc., Grand Island, NY) using a syringe equipped with a 26-gauge needle. Single cell suspensions were prepared by homogenizing the tissues between the frosted ends of microscope slides or by using a ground glass homogenizer. The cell suspensions were transferred to a 50 ml conical tube, centrifuged 10 min, resuspended in 5 ml RBC lysis buffer, and incubated for 3 min at room temperature. The cells were then centrifuged and washed twice with 15 ml FACS wash buffer, $350 \times g$ 5 min and resuspended at 5×10^6 ml^{-1}.

Separation of immune cell

Immune cells in different organ compartments were isolated with EasySep selection kit according to the manufacturer's (Stem Cell Technologies) suggested procedures and sorted with FACSAria.

MSC/T-cell cocultures

WT- and ARKO-MSCs were cocultured with CD4+ T cells for 5 days (ratio 1:5) in phenol red-free RPMI-1640 medium (GIBCO BRL) supplemented with 10% serum depleted Human Processed Serum (HPS), 1% penicillin/streptomycin, and 2 mM L-glutamine. In some experiments, ARKO-MSCs were cocultured with CD4+ T cells in the presence or absence of exogenous acid-activated TGFβ1 (0.02 nM) (R&D Biosystems, Minneapolis, MN).

Suppression assays

Peripheral blood collected-WT-CD4+ cells were stimulated with 1 μg/mL of plate-coated anti-CD3 plus 2.5 μg/mL of soluble anti-CD28 for 72 h. Then they were cocultured with WT-MSCs or ARKO-MSCs for six days to generate 'conditioned' Treg subpopulations. Allogenic T cell including CD4+CD25− T cells (4×10^4/well) and antigen presenting cells (APC) were collected from peritoneal wash (CD11b+) (2×10^4/wells). Different concentrations of 'conditioned' Treg cells (CD4+CD25+) were evaluated for their ability to suppress allogenic T cell proliferation when plated together in 96 wells plate. T cell proliferation was evaluated by thymidine incorporation pulse labeling with [3H] thymidine (1 μCi per well) for 16 h.

TGF-β ELISA

One million ARKO- or WT-MSCs were seeded with CD4+ in triplicate in the wells of a 12-well plate in triplicate in 1 ml serum-free RPMI for 24 h with different concentrations of AR inhibitor (Casodex). From each experiment, 100 ml culture

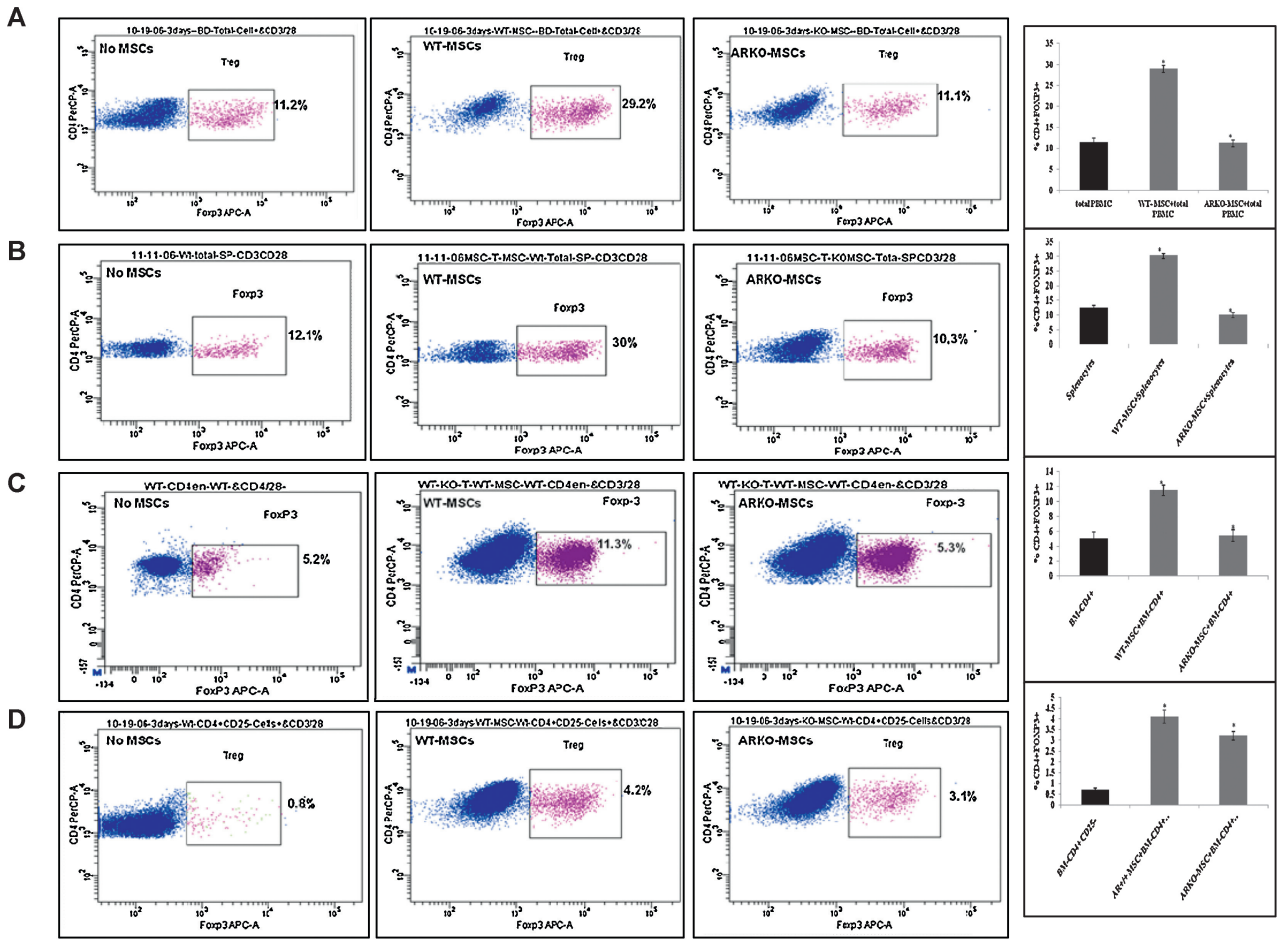

Fig. 1 – ARKO-MSC were unable to induce CD4+FOXP3+ T cells expansion from gated CD4+ cells from (A) total PBMC, (B) splenocytes, (C) bone marrow CD4+ cells and (D) bone marrow CD4+CD25−. MSCs, mesenchymal stem cells; Treg, regulatory T cells; BM, bone marrow Treg phenotype was determined according to the percentage of CD4+CD25+ cells that express Foxp3 by flow cytometry ($n = 3$, *statistically significant); iTreg, induced Treg.

supernatants were stored at −20 °C until tested for ELISA (eBioscience Inc, San Diego, CA, USA) as per manufacturer's guidelines.

MSC purification from WTCD4+ in the cocultures

MSCs were washed three times with a PBS/0.05 mM ethylenediamine tetraacetic acid (EDTA) buffer to detach lymphocytes from MSCs. MSCs were then trypsinized and resuspended in α-20 and cultured for two hours. Then, MSCs were washed three times with a PBS/0.05 mM EDTA to eliminate possible rule out contamination with lymphocytes. The percentage of MSCs was 98%.

Statistical analysis

All data were presented as the means of at least three experiments ±1 standard deviation (SD). The data were analyzed using the paired Student's t test at the significance level $2\alpha = 0.05$. Spearman correlations were performed to estimate relationships between percentages of T cells positive for a given marker or expression levels of these markers.

Results

In a previous work we generated ARKO (C57BL6/FVB) mice bearing a deletion of the AR DNA-binding domain using conditional Cre-lox recombination-based strategy (Altuwaijri et al., 2009). In one of our former reports, we isolated murine bone marrow-derived MSCs by CD45+ microbeads isolation kit and characterized them (Huang et al., 2013).

ARKO-MSCs could not induce functional CD4+CD25+Foxp3+ T cells during differentiation of CD4+ cells

To assess whether ARKO-MSCs are able to activate and induce generation of Treg cells, we compared the frequency of CD4+CD25+Foxp3+ cells in total PBMC (Fig. 1A), splenocytes (Fig. 1B), CD4+ cells (Fig. 1C) and CD4+CD25− cells (Fig. 1D) with no coculture or when cocultured with either WT-MSCs or ARKO-MSCs. We found that total PBMC, splenocytes and bone marrow CD4+ cells and CD4+CD25− cells cocultured with WT-MSCs contained an average of 29.2%, 30%, 11.3% and 4.2% CD4+CD25+Foxp3+ T lymphocytes, respectively, whereas this

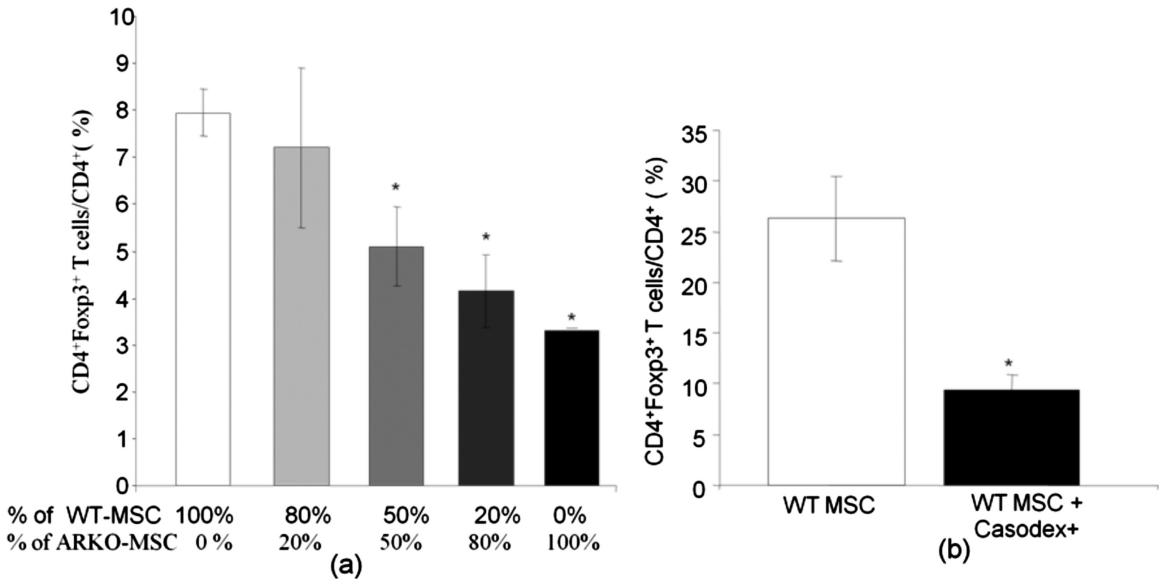

Fig. 2 – WT-MSC induced Treg cell expansion was partially AR dependent. (A) WT-MSC induced a dose dependant CD4 +FOXP3+ T cells expansion when cocultured with PBMC CD4+ and (B) AR inhibitor (Casodex) suppressed Treg cell expansion induced by WT-MSC. Cocultured with PBMC CD4+ T cells in the presence of AR inhibitor (10 nM Casodex) (*n* = 3, *statistically significant).

percentage appeared decreased in ARKO-MSCs cocultures (11.1%, 10.3%, 5.3% and 3.1%, respectively). Thus we hypothesized that lack of AR suppresses MSCs ability to generate induced CD4+CD25+Foxp3+ T cells.

ARKO-MSCs conditioned Tregs could not suppress allogenic T cell proliferation

In a functional test, we assessed the suppressive capacity of conditioned Tregs cells obtained after ARKO-MSC incubation with peripheral blood CD4+ T cells on the allogenic T cell population. CD4+ T cells were previously stimulated with 1 μg/mL of plate-coated anti-CD3 plus 2.5 μg/mL of soluble anti-CD28 for 72 h. By using different ratios of ARKO-MSCs:WT-MSCs (0:1, 0.2:0.8, 0.5:0.5, 0.8:0.2 or 1:0), we showed a dose dependent reduction in the capacity of the conditioned Treg cells to inhibit the proliferation of activated allogenic T cell (Fig. 2A). WT MSC and ARKOMSC were proportionally mixed and CD4+ T cells were cultured with the mixed population. CD4+FOXP3+ T cells were detected and analyzed by FACS. Our data indicated that ARKO-MSCs showed an inverse dose dependant CD4+FOXP3+ T cells expansion (Fig. 2A). Therefore, the average percentage of CD4+FOXP3+ T cells declined from 7.9% to 3% with increasing ratio of ARKO-MSCs from 0% to 100%, respectively.

WT-MSCs induce Treg cell induction was partially AR dependent

In order to determine whether androgen/AR signaling pathway is the only mechanism that mediates MSCs effect on Treg cells induction, we used Casodex that was previously used in former reports (Lin et al., 2013). CD4+ T cells from peripheral blood were cocultured with WT-MSC without or with 10 nM Casodex. Casodex repressed the average percentage of generated CD4+FOXP3+ T cells in WT-MSCs/CD4+ coculture to 33%. The study showed that Casodex partially suppressed Treg cell expansion induced by WT-MSC (Fig. 2B). This indicates the direct partial role of androgen/AR signaling pathway in MSCs effect on the Treg induction.

TGF-β1 is down-regulated in the cocultures of ARKO-MSCs and CD4+ cells

Many reports have revealed that soluble factors including TGF-β1 is directly involved in Treg cell induction (Bacchetta et al., 2005). We tested whether androgen/AR signaling pathway influence TGF-β secretion in MSCs and consequently perturb Treg induction. To verify this hypothesis, WT-MSC or ARKO-MSCs and CD4+ T cells were cocultured as described previously. Then we quantified the production of TGF-β in MSCs/CD4+ coculture by ELISA in the culture supernatant. The results showed that the expression of TGF-β in ARKO-MSCs/CD4+ coculture was remarkably decreased by 62% (Fig. 3A). We next tested whether AR blocker Casodex will be able to hinder TGF-β protein expression. TGF-β protein expression was negatively correlated with increasing dose of Casodex so the expression declined from 400 pg/ml to 10 pg/ml with administration of 0 to 10 μM of Casodex, respectively (Fig. 3B). These data indicated that androgen/AR signaling manipulated secretion of TGF-β from MSCs/CD4+ coculture and may thus influence induction of Treg cells.

TGFβ partially reconstitutes the MSCs wild-type function

The aforementioned results refer to TGFβ might have a central role in regulating MSCs function and Treg cell induction so we investigated whether the addition of

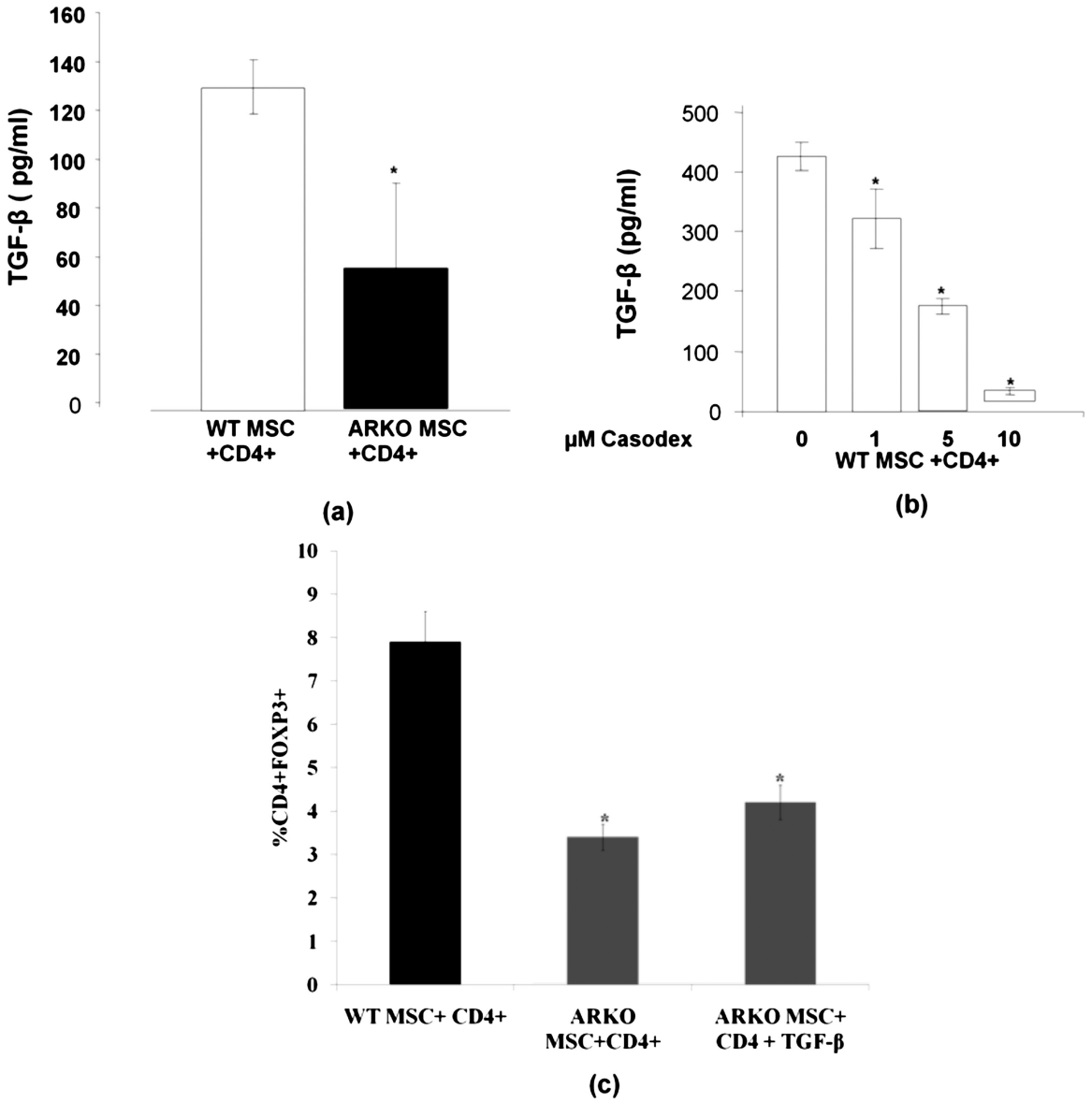

Fig. 3 – TGF-β1 production from supernatants of coculture of PBMC CD4+ cells with MSCs and effect of TGF-β1 treatment. (A) TGF-β1 production in the supernatants of MSCs co-cultured with CD4+ cells was quantified and (B) Casodex, the androgen blocker, blocked TGF-β production in WT MSCs/CD4+ coculture in a dose response manner. (C) Exogenous administration of active TGF-β (0.02 nM) rescued induction of CD4+FOXP3 from AR MSCs/CD4+ coculture. All the values represent means ± SED of three independent experiments. MSCs, mesenchymal stem cells; TGF-β1, transforming growth factor β1 (*n* = 3, * statistically significant).

exogenous active TGFβ could reverse the defect of ARKO-MSCs in inducing Treg. The addition of exogenous active TGFβ induced an increase in Treg cell induction in ARKO-MSCs/CD4+ cells coculture (Fig. 3C). However, the increase was modest, approximately 1.2 times, compared with non-TGFβ treated ARKO-MSCs/CD4+ cells coculture. These results hinted toward exogenous active TGFβ was not completely sufficient to overcome the defect in ability of ARKO-MSCs to induce Treg. Hence, these data suggest that induction of Treg might be mediated partially by TGFβ in addition to other mediators.

Discussion

MSCs have been largely used in the clinical trials due to their ability to suppress the proliferation of T-cells. They can differentiate into endothelial progenitor cells which then migrate to injured sites for the repair (Cai et al., 2011). However, conflicting results have been previously declared following MSC injection in *in vivo* models demonstrating either no beneficial outcome or an increase in Treg population in different animal models of autoimmune diseases (Gonzalez

et al., 2009; Gonzalez-Rey et al., 2010). MSCs are known to depend on diverse mechanisms to exert their immunomodulatory properties including their ability to produce functional Treg cells. Several reports demonstrated that Treg cells are generated from direct MSC/T cell interactions (Aggarwal and Pittenger, 2005; Maccario et al., 2005). In line with that, MSCs when cocultured with PBMC for three days lead to generation of CD4+CD25+Foxp3+ cells. The generated cells were functional and diminished the proliferation of alloantigen activated T cells (Prevosto et al., 2007). Thus, it is highly sought to better understand how the adult stem cells can impede with hyperactivation of the host immune responses and alter the autoimmune process. This regulation might be conducted also via secreted soluble cytokines and small molecular weight mediators as in case of allogeneic MSCs that inhibit B cell proliferation by inducing cell cycle arrest in the G0/G1 phase and by the production of soluble factors (Tarlinton, 1994, 2006; Tabera et al., 2008). MSCs secretion of TGF-β and PGE2 induce Treg cells from CD4+ T lymphocytes (English et al., 2009). Therefore, there is a strong agreement that contact and soluble factors interaction between T cells and MSCs is necessary for Treg cell generation to perform an immunosuppressive effect (Djouad et al., 2003). Here, we noticed, and proved, the effect of MSCs on Treg expansion and progression. Our reports clearly revealed that MSCs when cultured with CD4+ population induced Treg generation. This result is in concordance with other reports that showed that MSCs encouraged the induction of CD4+CD25+FoxP3+ regulatory T lymphocytes (Treg) (Tasso et al., 2012).

On another note, MSCs response to the stress challenge, such as oxidative stress, is different in both sexes (Crisostomo et al., 2006, 2007). Interestingly, administration of nilutamide, anti-androgen, could motivate the growth of Embryonic Stem Cells (ESCs), while testosterone could not (Chang et al., 2006). Furthermore, ESCs were highly activated toward cardiomyocytes differentiation with high doses of androgen (Goldman-Johnson et al., 2008). However, AR effect has not at all been verified for the potential of MSCs in Treg induction. Therefore, our investigation tries to elucidate the role of AR in an attempt to improve MSCs clinical applications. The results of this study are the first to illustrate that depletion of AR reduces the capacity of MSCs to provoke functional CD4+CD25+Foxp3+ regulatory cells, when cultured with activated CD4+ T cells. This reduction in Treg expansion might be conversely beneficial since bone marrow Treg cells may enhance cancer bone metastasis and participate in bone deposition in prostate cancer patients with bone metastasis (Zhao et al., 2012).

In humans, androgen replacement therapy reduces autoantibody levels in hypogonadal men with lupus (Olsen and Kovacs, 1995). A potential mechanism entails the production of downregulatory mediators such as TGF-β. Interestingly, in male mice with experimental autoimmune encephalomyelitis (EAE), augmented production of TGF-β in intact males was observed during induction of protective tolerance, indicating a hormone-dependent cytokine response (Bebo et al., 1999). There is a growing body of evidence that TGF-β1 and IL-10 cytokines mediate the generation of Treg cells (Hori et al., 2003; Sakaguchi, 2004). In addition, it has been reported that MSCs can produce TGF-β1, IL-10 and PGE2 (English et al., 2009). Hence, we assessed the concentration of TGF-β1 in the supernatants of WT-MSCs and ARKO-MSCs co-cultured with CD4+ cells. In addition to Treg generation, we found that TGF-β1 production was significantly reduced in supernatants collected from the co-culture when ARKO-MSCs were used. This observation comes in line with former studies showing that TGF-β1 may play a central role in the induction of Treg cells by MSCs (English et al., 2009; Ghannam et al., 2010).

TGF-β expression is very likely to be mediated through AR signaling pathway. This hypothesis was tested by treating the WT-MSCs/CD+ coculture with AR-blocking agent (Casodex). When Casodex was added to WT-MSCs culture, a remarkable decline of TGF-β was observed with increasing Casodex concentration. Therefore, it indicates that MSCs expression of AR is required for generation of MSCs-derived mediators, including TGF-β. Interestingly, Robledo et al. (1996) found receptors for TGF-β in bone marrow stromal cells. The ability to partially rescue the wild-type functionality of ARKO-MSCs with exogenous active TGFβ1 suggested that there is no inherent defect in their capacity to pull together the proteomic machinery crucial for the induction of Treg, or in their ability to respond to active TGF-β. Former studies have indicated that TGF-β1 and IL-10 are vital for Treg cells generation (Hori et al., 2003; Sakaguchi, 2004). Former investigations demonstrated that androgens upregulated expression of TGF-β in the thymus (Olsen et al., 1993). In addition, androgen suppressive effect on developing B cells was found to be mediated through androgen receptors in bone marrow stromal cells through TGF-β as a potential mediator (Olsen and Kovacs, 2001). Despite the fact that molecular mechanisms by which androgens boost up TGF-β expression are not completely understood, a functional link is possible given the similarities in effects of TGF-β and androgens on developing B cells. For example, studies suggest that immature B cells are very responsive to androgen (Ellis et al., 2001) and TGF-β (Keller et al., 1990). This effect on B cell development is conducted by downregulation of stromal cell-derived IL-7 in response to DHT or TGF-β (Lee et al., 1989; Tang et al., 1997). More interestingly, both TGF-β (Lee et al., 1987; Lebman and Edmiston, 1999) and DHT (Olsen et al., 1994, 1998) exerted apoptosis and inhibition of cellular proliferation in B cell development and thymus, respectively. All in all, androgens and TGF-β share many exciting facets of B cell development. Very importantly, our study hints to similar observations but in Treg cells through interaction with MSCs. However, several questions remain unanswered. For example, we have not determined the mechanism(s) through which androgen regulates TGF-β expression in MSCs.

Conclusion

To summarize, the present study shows for the first time that depletion of AR in MSCs inhibited its ability to induce functional Treg cells during the differentiation process of CD4+ cells. Generating Treg cells was accompanied by an increase of TGF-β production in the supernatant of the WT-MSCs/CD4+ co-cultures and resulted in the inhibition of the proliferation of allogenic T cells. However, ARKO-MSCs/CD4+ cocultures that had a lower expression of AR and a lower TGF-β production could not induce functional Treg cells. Interestingly, blocking AR receptor similarly reduced TGF-β expression in

the MSCs/CD4+ co-cultures. Finally, treatment of ARKO-MSCs/CD4+ cocultures with active TGF-β partially rescued the defect of ARKO-MSCs in Treg induction. Hence, this study probably revealed TGF-β as a downstream mediator of Treg activity. In aggregate, these data may hint to underlying mechanisms of many diseases including common hypogonadism in the HIV-infected men by suggesting the investigation of TGF-β-mediated AR pathway (Haynes et al., 2000; Rochira and Guaraldi, 2014). Further identification of the molecular and cellular pathways that are engaged in the effects of androgens on Treg proliferation may reveal fundamental mechanisms and propose new MSCs therapeutic approaches to autoimmune diseases.

Conflict of interest

The authors hereby declare that there is no conflict of interest.

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