Human Cell (2015) 28:81–90
DOI 10.1007/s13577-014-0106-y

RESEARCH ARTICLE

Dental pulp stem cells suppress the proliferation of lymphocytes via transforming growth factor-β1

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Received: 10 November 2014 / Accepted: 24 December 2014 / Published online: 21 January 2015

Abstract Dental pulp stem cells (DPSCs) possess self-renewal capability, multi-lineage differentiation potential, and can generate a dentin-pulp-like tissue in vivo, which is promising for tooth regeneration. To enlarge the cells resource of DPSCs and explore the feasibility of DPSCs-mediated immune therapy, it is prerequisite to investigate the immunological properties of DPSCs and the underlying mechanisms. Human DPSCs and peripheral blood mononuclear cells were isolated and cultured. Then we used lymphocytes proliferation assays, cytokines detection, Transwell cultures, neutralization experiments, and flow cytometry to examine the in vitro immune characteristics of DPSCs. We found that DPSCs failed to stimulate allogeneic T cells proliferation and suppressed T cells proliferation, B cells proliferation, and mixed lymphocyte reaction. In addition, DPSCs could up-regulate IL-10, down-regulate the production of IL-2, IL-17, and IFN-γ, and did not affect the production of IL-6. Monoclonal antibody against transforming growth factor-β1 restored the T cells proliferation inhibited by DPSCs. Moreover, the population of regulatory T cells increased significantly and T-helper 17 cells decreased significantly in peripheral blood mononuclear cells co-cultured with DPSCs. These data confirmed that DPSCs are low immunogenic, could inhibit the proliferation of lymphocytes, regulate the production of cytokines in vitro, and the secretion of transforming growth factor-β1 may be involved in this event.

Keywords Dental pulp stem cells · Immunogenicity · Immunosuppression · Regulatory T cells · Regenerative medicine

Abbreviations

BMSCs	Bone marrow mesenchymal stem cells
DPSCs	Dental pulp stem cells
ELISA	Enzyme-linked immunosorbent assays
IDO	Indoleamine 2,3-dioxygenase
IFN-γ	Interferon γ
iNOS	Inducible nitric oxide synthase
L-NAME	*N*-nitro-L-arginine methyl ester
MLR	Mixed lymphocyte reaction
MSCs	Mesenchymal stem cells
1-MT	1-Methyl-L-tryptophan
PBMCs	Peripheral blood mononuclear cells
PDLSCs	Periodontal ligament stem cells
PGE2	Prostaglandin E2
PHA	Phytohemagglutinin
SI	Stimulation index
TGF-β1	Transforming growth factor-β1
Th17	T-helper 17
Tregs	Regulatory T cells

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Introduction

Mesenchymal stem cells (MSCs) are probably present in most of adult tissues, including bone marrow, adipose

tissue, synovial tissue, amniotic fluid, placental tissue, palatine tonsil, and dental pulp [1, 2]. As a unique population of MSCs in dental pulp, dental pulp stem cells (DPSCs) possess self-renewal capability, multi-lineage differentiation capacity, and clonogenic efficiency, and can generate a dentin-pulp-like tissue in vivo when DPSCs were transplanted into immunocompromised mice, thus making them ideal cell source for tooth regeneration [3, 4]. In addition, owing to their ease of access, no donor side effects, higher proliferation rate, and prospective multipotent properties, DPSCs can also be used to treat systemic diseases, including diabetes, Parkinson disease, myocardial infarction, etc. [5–7].

Our previous study showed that allogeneic swine DPSCs could successfully regenerate bio-root in miniature pig, then a porcelain crown was made on the bio-root and eventually restored tooth function. Moreover, no immunological responses to the bio-roots were observed, indicating DPSCs may be low immunogenic and/or have immunoinhibitory properties [8]. In this study, we studied the immunological characteristics of DPSCs and the underlying mechanisms.

Materials and methods

Cell culture

All protocols for the handling of human tissue were approved by the Research Ethical Committee of Weifang Medical University, China.

Dental pulp tissues were obtained from normal human impacted third molars of patients (18–30 years old) at the Department of Stomatology, Yidu Central Hospital, Weifang Medical University after the patients gave their informed consent. DPSCs were isolated and cultured from single-colony clusters as described in previous reports [3, 4]. Briefly, the pulp tissue was gently separated from the crown and root, minced and digested in a solution of 3 mg/ml collagenase type I (Worthington, Freeheld, NJ, USA) and 4 mg/ml dispase (Roche, Mannheim, Germany) for 30 min at 37 °C, passed through a 70-μm strainer (Falcon; BD Labware, Franklin Lake, NJ, USA) to obtain a single-cell suspension, and seeded into 25 cm^2 culture flasks (Costar, Cambridge, MA, USA) containing alpha-modification of Eagle's medium (GIBCO; Invitrogen, Carlsbad, CA, USA) supplemented with 15 % fetal bovine serum (GIBCO), 100 μmol/l ascorbic acid 2-phosphate (WAKO, Tokyo, Japan), 2 mmol/l glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin (Invitrogen). Cultures were incubated at 37 °C in a humidified atmosphere supplemented with 5 % CO_2. Medium was changed twice a week. Upon reaching 80 % confluence, DPSCs were detached with 0.25 % trypsin/1 mM EDTA (Sigma-Aldrich, St. Louis, MI, USA), replated and cultured for further study. The cells used in this study were at 3–4 passages.

Human peripheral blood mononuclear cells (PBMCs), periodontal ligament stem cells (PDLSCs), and bone marrow mesenchymal stem cells (BMSCs) from healthy donors were obtained and cultured as previous reports, respectively [9–11]. For each experiment, DPSCs, PDLSCs, and BMSCs were used at the same passage.

Characterization of DPSCs

To characterize DPSCs, the expression profiles of STRO-1, CD90, or CD146, three surface molecules of DPSCs, were analyzed by fluorescein-activated cell sorter Calibur flow cytometry (BD Immunocytometry Systems, San Jose, CA, USA) as previously reported [8]. Multi-lineage differentiation assays toward osteogenic and adipogenic pathways were performed as previous reports [3, 4]. Four weeks later, von Kossa staining and Oil red O staining were used to identify calcification of the extracellular matrix and lipid-laden fat cells, respectively.

Lymphocytes proliferation assays

To determine immunogenicity of DPSCs, DPSCs (5.0×10^4) were used as stimulator cells and were co-cultured with an equal number of allogeneic PBMCs (responder cells). PBMCs cultured alone and co-cultures of two allogeneic PBMCs were as controls. All of the stimulator cells were treated with 25 μg/ml mitomycin C (Sigma-Aldrich) before co-culture for immune assays. Five days later, Cell Counting Kit-8 kits (Dojindo Laboratories, Japan) were used to examine the proliferation of T cells. The absorbance at 450 nm was measured with a microplate reader.

A mitogen proliferative assay was used to assess the effect of DPSCs on T cells proliferation. PBMCs (5.0×10^4) stimulated by 0.5 μg/ml phytohemagglutinin (PHA; Sigma-Aldrich) were mixed at various stimulator-responder ratios with allogeneic DPSCs; 1.0×10^4, 5.0×10^4, and 2.5×10^5 DPSCs were added. T cells were harvested on day 5 and the proliferation was measured as described above.

To study the effects of DPSCs on a two-way mixed lymphocyte reaction (MLR), DPSCs from the third person (third party) were added at the beginning of the experiments. PBMCs from two individuals were incubated with various numbers of DPSCs from third party. The proliferation of responder cells was assessed after 5 days as described above.

B cells isolation and culture were the same as previous report [12]. In the presence of a complex of stimuli,

including the CpG synthetic oligonucleotide (2.5 μg/ml; Oligonucleotide, Sangon Biotech, Shanghai, China), recombinant CD40L (100 ng/ml; R&D, Minneapolis, MN, USA), goat anti-human immunoglobulin antibodies (2 μg/ml; Immunotech, Jackson, West Grove, PA), IL-2(50 U/ml; R&D), IL-4 (10 ng/ml; R&D), and IL-10 (10 ng/ml; R&D), the proliferation of B cells co-cultured with DPSCs was tested at the cell ratios as mentioned above.

To compare the immunomodulatory capabilities of DPSCs with those of PDLSCs and BMSCs, two kinds of MSCs possessing profound immunomodulatory abilities, the T cells proliferation stimulated by 0.5 μg/ml PHA, in the presence of DPSCs, PDLSCs, and BMSCs, at various stem cells/PBMCs ratios (0.02:1, 0.2:1, 1:1, 2:1, 5:1, 10:1), were examined as described above.

Cytokine examination

Co-cultures containing PBMCs (5.0×10^4), DPSCs (5.0×10^4), and PHA (0.5 μg/ml) were established. Five days later, enzyme-linked immunosorbent assays (ELISA) were used to quantify IL-2, IL-6, IL-10, IL-17, and interferon γ (IFN-γ) (all from R&D) in the supernatants.

Real-time RT-PCR analysis

For quantitative RT-PCR, 2 μg aliquots of RNA from PHA-stimulated PBMCs for 3 days were synthesized according to the manufacturer's protocol (Invitrogen). Real-time PCR was performed with the SYBR Green PCR kit (Qiagen, Germany) using Icycler iQMulti-color, Real-time PCR Detection System. The primers set for PCR included: IFN-γ (sense, 5-gcagccaacctaagcaagat-3; antisense, 5-caaaccg gcagtaactggat-3), and β-actin (sense, 5-gatcattgctcctcctg agc-3; antisense, 5-gtcatagtccgcctagaagcat-3). β-actin was used as an internal control. PCR processes were performed as described previously [13].

Transwell cultures

Transwell chambers with a 0.4 μm pore size membrane (Costar, Cambridge, MA, USA) were used to physically separate the PBMCs from the DPSCs. PBMCs (5.0×10^4) were seeded with PHA (0.5 μg/ml) in the upper chamber, and DPSCs (5.0×10^4) were placed in the bottom chamber. After 5 days of co-culture, T cells were harvested, and the proliferation was measured.

DPSCs conditioned medium was generated as previous report [14]. DPSCs (5.0×10^4) were pre-treated with or without 25 ng/ml IFN-γ (Sigma-Aldrich) for 3 days, then the supernatants were collected and filtered through a 0.22 μm filter (Millipore, Bedford, MA, USA). PHA-stimulated PBMCs were cultured with 50 % regular medium plus 50 % DPSCs conditioned medium. On day 5 of culture, the proliferation of the PBMCs was measured as described.

Neutralization assays

Next, neutralization assays were performed to investigate the possible factors responsible for the immunosuppression mediated by DPSCs [15]. The co-cultures containing PBMCs (5.0×10^4), DPSCs (5.0×10^4), and PHA (0.5 μg/ml) were established. The following reagents were added to the co-cultures, respectively: neutralizing monoclonal antibody for human transforming growth factor-β1 (TGF-β1), or an isotype-matched monoclonal antibody (10 μg/ml; Novus Biologicals), and chemical inhibitors specific for indoleamine 2,3-dioxygenase (IDO; inhibitor, 1-methyl-L-tryptophan (1-MT), 500 μM; Sigma-Aldrich), prostaglandin E2 (PGE2; inhibitor, indomethacin, 20 μM; Sigma-Aldrich), inducible nitric oxide synthase (iNOS; inhibitor, *N*-nitro-L-arginine methyl ester (L-NAME), 1 mM; Sigma-Aldrich), and T cells proliferation was measured following treatment.

Measurement of TGF-β1

To further identify the role of TGF-β1 responsible for the effect of DPSCs on T cell proliferation, ELISA was used to quantify the TGF-β1 (R&D) in collected supernatants from cultures of either DPSCs alone (5.0×10^4; 1–5 days after seeding), DPSCs + PBMCs (5.0×10^4; 1–5 days after seeding), or co-culture of DPSCs (5.0×10^4), PBMCs (5.0×10^4), and PHA (0.5 μg/ml) as previous report [16]. The measurement was performed according to the protocols of the manufacturers. Optical density at 450 nm was read on a Microplate Spectrophotometer (Molecules Devices, Sunnyvale, CA, USA).

Detection of regulatory T cells and T-helper 17 cells

Flow cytometry was used to determine the percentage of CD4+CD25+Foxp3+ regulatory T cells (Tregs) and CD4+IFNγ-IL17+ T-helper 17 (Th17) cells. For Tregs [17, 18], at 2 days post co-culture of PBMCs (5.0×10^4), DPSCs (5.0×10^4), and PHA (0.5 μg/ml) as previous reports, PBMCs were harvested and stained with PerCP-conjugated anti-CD4 antibody (BD Biosciences, San Diego, CA, USA) and FITC-conjugated anti-CD25(BD Bioscience) antibody for 30 min in the dark at 4 °C. Next, PBMCs were fixed, permeabilized and stained with PE-conjugated anti-Foxp3 antibody (BD Bioscience) with the Foxp3 Staining Buffer Set (eBioscience, San Diego, USA). To detect Th17 cells, PBMCs were stimulated for 4 h with phorbol myristate acetate (5 ng/ml; Sigma-Aldrich) and ionomycin (500 ng/ml; Sigma-Aldrich) in the presence of

GolgiStop (BD Bioscience), a protein transport inhibitor. Then, PBMCs were incubated with APC-conjugated anti-CD4 antibody (BD Bioscience) for 30 min at 4 °C in the dark. After fixation and permeabilization, cells were stained with anti-IFN-γ and anti-IL-17 antibodies.

Statistical analysis

All data are expressed as mean ± SD from at least three independent experiments. The stimulation index (SI) values were calculated as follows: SI = (proliferation of stimulated lymphocytes with or without MSCs)/(proliferation of unstimulated lymphocytes alone). Statistical significance was assessed by the Student's *t* test, and a *P* value less than 0.05 was considered statistically significant.

Results

Characterization of DPSCs

DPSCs were positive for STRO-1, CD146, CD90, and the positive percentage of these surface molecules were 12.6 ± 2.8, 79.8 ± 6.6, and 96.8 ± 2.2 %, respectively, as assessed by flow cytometric analysis (Fig. 1a–c). In addition, DPSCs could advance into osteogenic and adipogenic differentiation under inductive medium (Fig. 1d, e), in accordance with our previous results [8].

DPSCs inhibit lymphocytes proliferation

When DPSCs pre-treated with mitomycin C were co-cultured with allogeneic normal PBMCs at a ratio of 1:1, DPSCs failed to stimulate allogeneic T cells proliferation (Fig. 2a), indicating that DPSCs displayed low immunogenicity. Then, PBMCs were stimulated by a T cells mitogen (PHA) or MLR in the presence of DPSCs at indicated cell ratios, and cell proliferation was determined. As demonstrated in Fig. 2, PBMCs proliferation triggered by PHA (Fig. 2b) or by MLR (Fig. 2c) was significantly inhibited by DPSCs. Interestingly, DPSCs also suppressed the B cells proliferation induced by a complex of stimuli (Fig. 2d), just as the effect of PDLSCs on B cells proliferation [12]. Next, we commenced to compare the inhibitory function of DPSCs with PDLSCs and BMSCs, two well-characterized MSCs possessing immune suppressive capabilities. The results showed that all tested cell types significantly inhibited T cells proliferation at different cell ratios. Strikingly, the suppressive capability of DPSCs is comparable to that of PDLSCs, and is stronger than BMSCs (Fig. 2e). Taken together, these results indicate that DPSCs suppress allogeneic T cells and B cells proliferation, and their capability is higher than BMSCs.

DPSCs alter the cytokines production

PBMCs were stimulated with PHA in the presence of DPSCs (the ratio of PBMCs to DPSCs was 1:1), and the presence of IL-2, IL-6, IL-10, IL-17, and IFN-γ in the supernatants was determined by ELISA. As shown in Fig. 3, the production of IL-2, IL-17, and IFN-γ was significantly inhibited in the presence of DPSCs, whereas the concentration of IL-10 was significantly elevated, and the production of IL-6 was unchanged.

TGF-β1 plays a crucial role in DPSCs-mediated T cells suppression

After stimulated with PHA for 3 days, PBMCs showed an up-regulation in the expression of IFN-γ (Fig. 4a). To

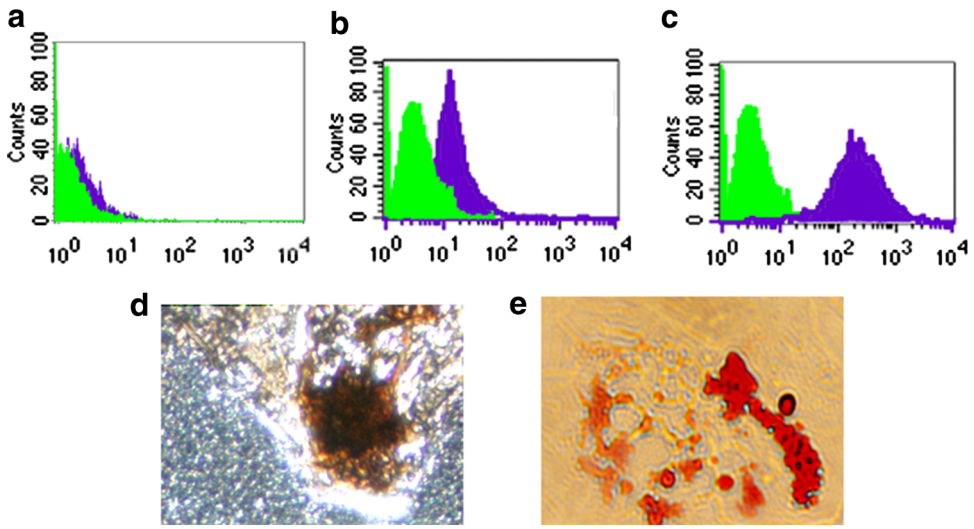

Fig. 1 PDLSCs expressed STRO-1 (**a**), CD146 (**b**), and CD90 (**c**), three cell surface molecules of mesenchymal stem cells. PDLSCs were found to possess the potential to form nodular structure stained black by von Kossa staining (**d**) and to develop into Oil Red O-positive lipid-laden fat cells (**e**) under osteogenic and adipogenic inductive medium

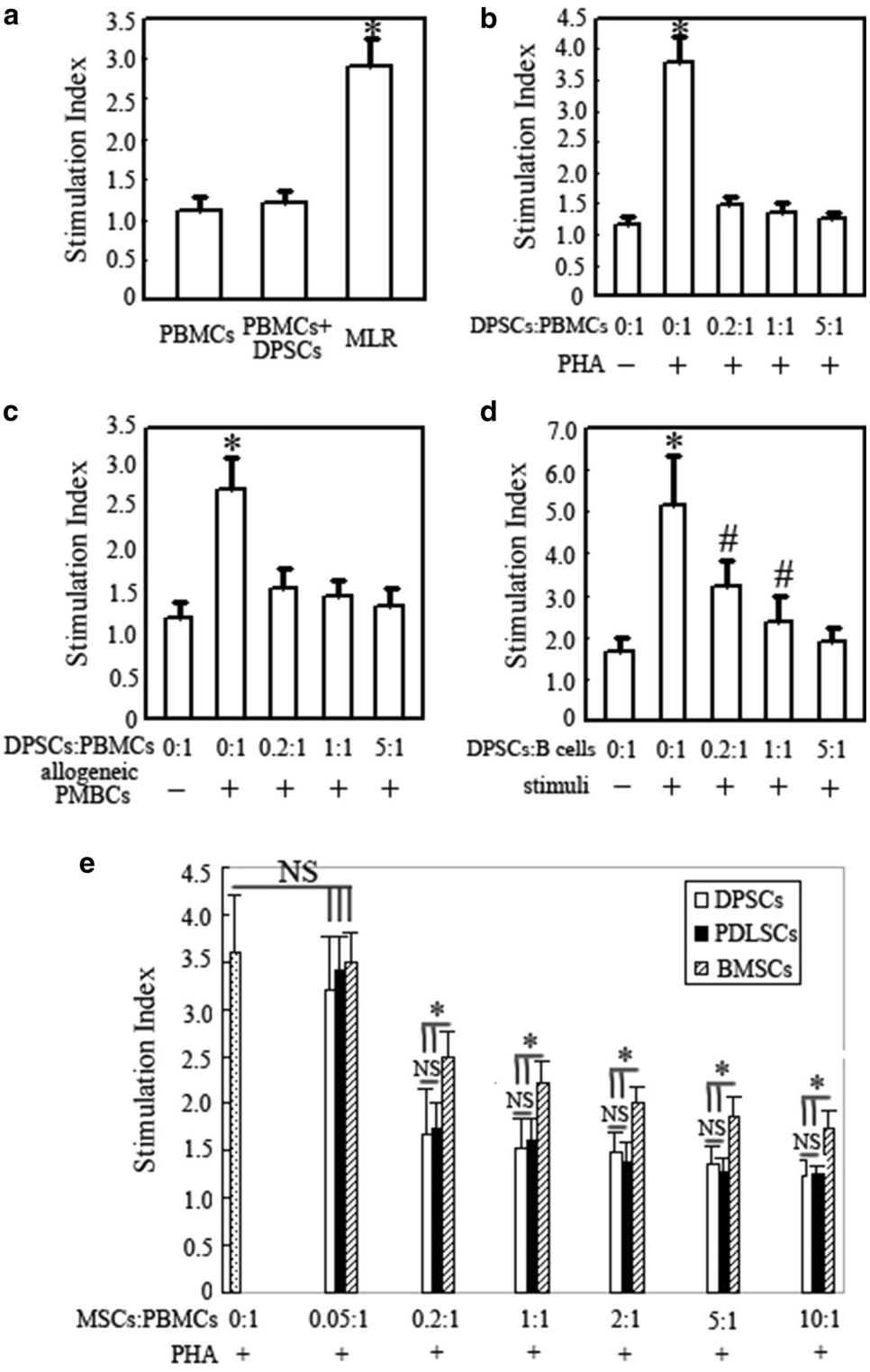

Fig. 2 DPSCs do not elicit allogeneic PBMCs proliferation and suppress lymphocytes proliferation. **a** Co-culture of PBMCs with DPSCs showed that DPSCs failed to stimulate allogeneic T cells proliferation. T cells proliferation stimulated by PHA (**b**) and a two-way mixed lymphocyte reaction (**c**) was inhibited by DPSCs. **d** DPSCs suppressed the B cells proliferation. **e** The suppressive capability of DPSCs is comparable to that of PDLSCs, and is stronger than BMSCs. Data represent the mean ± SD. * $P < 0.01$, compared with other groups; # $P < 0.05$, compared with the group of B cells alone. *NS* not significant

examine whether cell–cell contact was required for inhibition, Transwell culture system was used to separate PHA-treated PBMCs from DPSCs. We found that T cells proliferation was equally inhibited by cell–cell contact culture and the Transwell culture, suggesting that inhibition was independent of cell–cell contact and might involve soluble factors (Fig. 4b). In addition, the conditioned medium derived from DPSCs did not inhibit

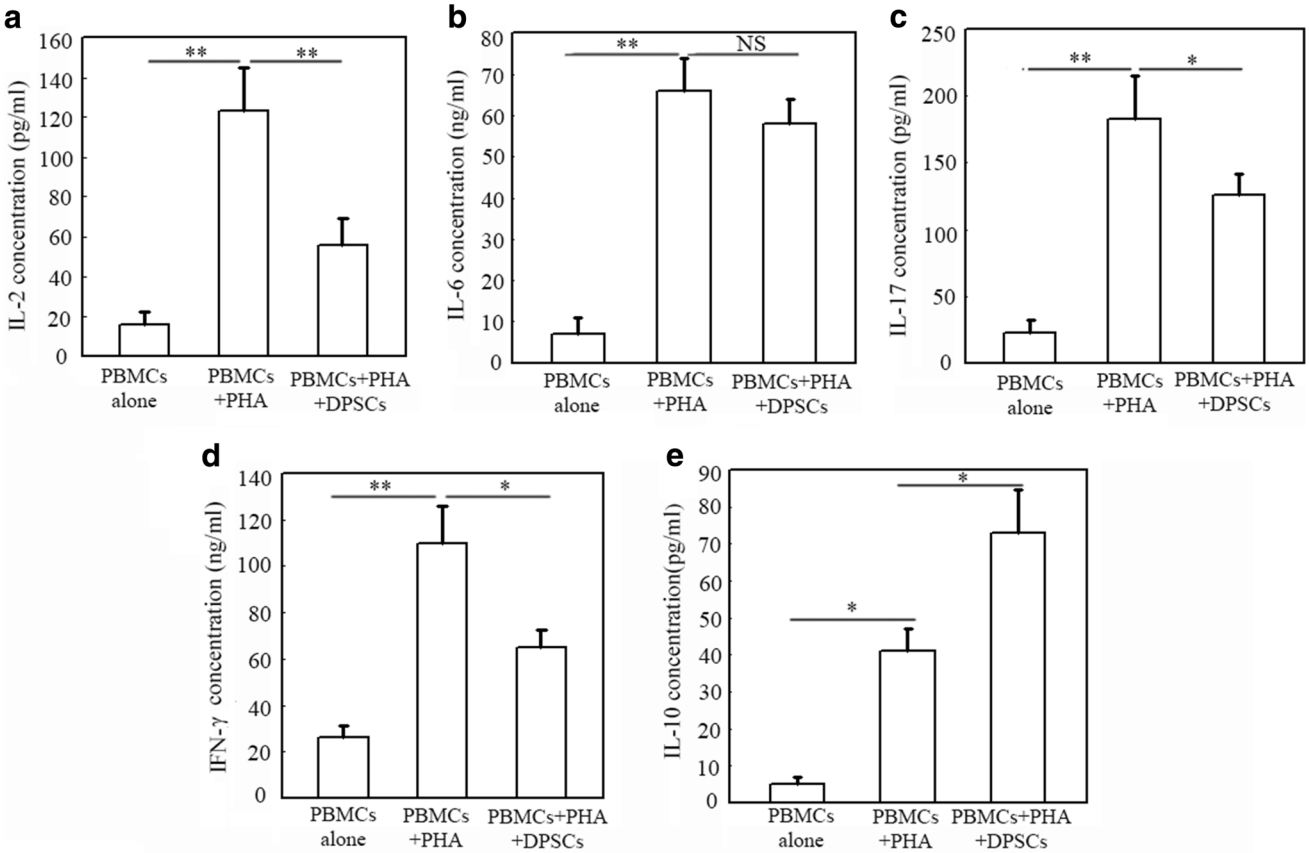

Fig. 3 The change of cytokines production. DPSCs significantly inhibited the production of IL-2 (**a**), IL-17 (**c**), IFN-γ (**d**), promoted the concentration of IL-10 (**e**), and did not affect the production of IL-6 (**b**). Data represent the mean ± SD. ** $P < 0.01$. * $P < 0.05$. *NS* not significant

PBMCs proliferation, whereas the conditioned medium obtained from DPSCs pre-treated with IFN-γ for 3 days could partially inhibit PBMCs proliferation (Fig. 4b). To determine the factors taking part in DPSCs-mediated immunosuppression, monoclonal antibody against TGF-β1, or an isotype-matched monoclonal antibody, and indomethacin (which inhibits PGE2), L-NAME (which inhibits iNOS), or 1-MT (which inhibits IDO), were added to co-cultures of DPSCs and PBMCs plus PHA and T cells proliferation was assessed. Only anti-TGF-β1 antibody restored the T cells proliferation that was inhibited by DPSCs. In contrast, the isotype-matched antibody and the inhibitors of iNOS, IDO, and PGE2 failed to restore T cells proliferation (Fig. 4c). Moreover, we used ELISA to quantify TGF-β1 in the culture supernatants of DPSCs, DPSCs + PBMCs, and DPSCs + PBMCs + PHA. Kinetic studies revealed that the concentrations of TGF-β1 in the culture of DPSCs alone and DPSCs + PBMCs (1–5 days after seeding) were relatively stable. However, TGF-β1 in the supernatants of DPSCs co-cultured with PHA-treated PBMCs was significantly elevated (Fig. 5).

DPSCs enhance Tregs and reduce Th17 cells

We co-cultured DPSCs with PHA-stimulated PBMCs for 48 h. PHA-stimulated PBMCs were then collected and analyzed for CD4, CD25, and Foxp3 expression. The population of Tregs increased significantly in co-cultures with DPSCs compared with PHA-stimulated PBMCs (Fig. 6a). Next, we examined CD4+IFNγ-IL17+ Th17 cells in PBMCs stimulated with PMA and ionomycin and co-cultured with DPSCs. We found that the percentage of Th17 cells was significantly decreased in PBMCs co-cultured with DPSCs (Fig. 6b). Thus, the ratio of Tregs/Th17 cells was elevated when DPSCs added (Fig. 6c).

Discussion

In addition to their self-renewal and multilineage differentiation abilities, BMSCs also have been demonstrated that they had superior immunomodulatory capacity in vitro and in vivo. BMSCs were able to suppress the activation and proliferation of almost all subsets of immune cells,

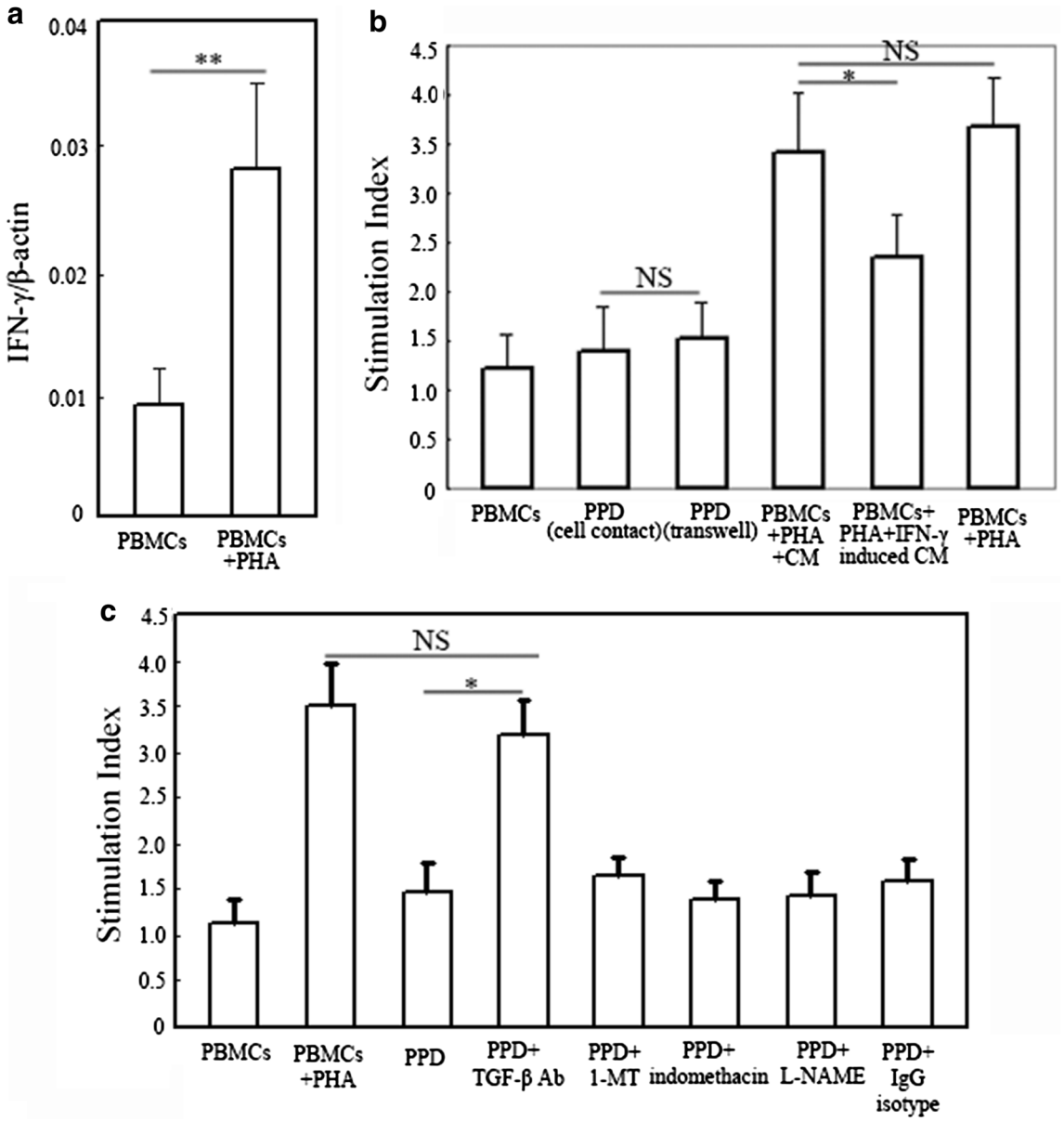

Fig. 4 TGF-β1 is involved in DPSCs-mediated T cells suppression. **a** After stimulated with PHA for 3 days, PBMCs showed an up-regulation in the expression of IFN-γ. **b** T cells proliferation was significantly inhibited when co-cultured with DPSCs in the Transwell culture system. **c** Monoclonal antibody against TGF-β1 restored PBMCs proliferation that had been inhibited by DPSCs, whereas indomethacin, L-NAME, 1-MT, and isotype-matched antibody, failed to restore PBMCs proliferation. Data represent the mean ± SD. ** $P < 0.01$. * $P < 0.05$. *NS* not significant, *PPD* co-culture of PBMCs + PHA + DPSCs, *CM* DPSCs conditioned medium

including T cells, B cells, natural killer cells, macrophages, dendritic cells, etc. [19]. This unique feature made BMSCs one of the most promising cell types in treating a variety of immune and inflammation-related diseases, such as graft-versus-host disease [20], and autoimmune diseases [21].

DPSCs also have been shown to possess immunoregulatory properties [22–24], but the underlying mechanism is not fully elucidated. In the current study, DPSCs failed to elicit PBMCs proliferation, which may imply that DPSCs are low immunogenic. In addition, we found that DPSCs inhibit MLR, the proliferation of PBMCs stimulated with PHA, and also the proliferation of B cells in the presence of a complex of stimuli, just as PDLSCs, another dental tissue-obtained MSCs displaying low immunogenicity and profound immunosuppressive ability, including inhibition of T cells proliferation [13, 16] and B cells proliferation [12]. Moreover, when compared with PDLSCs and BMSCs, DPSCs possess the equivalent capacity as PDLSCs of inhibiting the proliferation of PBMCs induced by mitogen, and own stronger inhibitory activity than

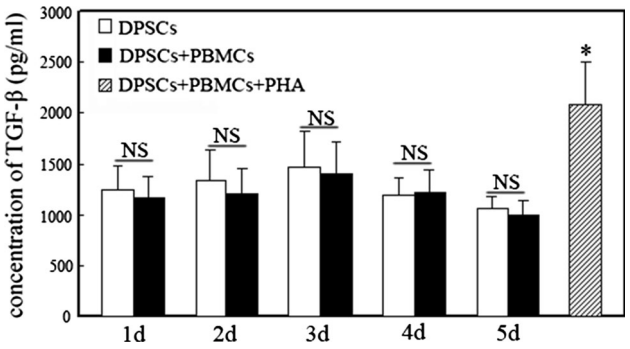

Fig. 5 Kinetic examination of TGF-β1 concentrations. The concentration of TGF-β1 significantly increased in the co-culture of DPSCs and PHA-induced PBMCs compared to DPSCs alone and DPSCs + PBMCs. Data represent the mean ± SD. * $P < 0.05$. *NS* not significant

BMSCs at the indicated ratios of stem cells/PBMCs. Interestingly, our data are in accordance with the first report on the immunosuppressive activity of DPSCs, which shows that the addition of DPSCs or BMSCs resulted in 91 and 75 % inhibition of T cell response, respectively, assessed by a ^{3}H-thymidine assay [22].

Cytokines play critical roles in the cellular immune responses as well as in humoral immune responses. The immunosuppressive properties of MSCs are associated with the production of cytokines, and MSCs, including BMSCs, palatine tonsil-derived MSCs and limbal MSCs, have been shown to alter the cytokines production of T cells [1, 2, 21, 25–27]. Increased secretion of proinflammatory cytokines, including IL-2 and IFN-γ, was involved in a series of immune disorder-related diseases, wherein inhibition of these proinflammatory cytokines has been shown to be beneficial in the treatment of the immune disorders [21, 25]. In addition, IL-17, an important inflammatory cytokine produced by Th17 cells, has been linked to the pathogenesis of a variety of autoimmune diseases, such as systemic lupus erythema and systemic sclerosis [21, 26]. Moreover, IL-10 was displayed to have well-documented anti-inflammation properties, such as in the regulation of T cells, promotion of suppressive phenotype of T cells, and antagonize the action of proinflammatory cytokines [25, 28]. Furthermore, when BMSCs were systemically infused into the patients or animal models of autoimmune diseases, IL-10 was up-regulated and IL-17 was down-regulated [29]. Our experiments also demonstrated that DPSCs reduced the production of IL-2, IL-17, IFN-γ, whereas the production of IL-10 was significantly elevated in the presence of DPSCs, thus may further inhibit the maturation and migration of antigen presenting cells, induce suppressor T cells formation, alleviated the inflammatory status [26, 28, 29]. At the same time, IL-6 concentration was unchanged after DPSCs treatment, notwithstanding IL-6 was shown to be inhibited by limbal MSCs [27]. This discrepancy may be attributed, at least in part, to the different origin of MSCs.

The immunosuppressive mechanisms of MSCs remain to be clarified, and mediation by soluble factors is strongly suggested [2], while some studies indicated the cell–cell contact is more important [30, 31]. We observed that the suppression of PBMCs was preserved when DPSCs were separated from the PBMCs by a semipermeable membrane, suggesting that DPSCs inhibited T cell proliferation by soluble factors, which appear to be released by DPSCs. Then, it is necessary and of great value to define the critical soluble factors involved in the immunosuppressive function of DPSCs. Because it has been reported that MSCs suppress PBMCs proliferation through the production of TGF-β1, PGE2, NO, IDO [25, 32–34], we thus characterized these candidate inhibitory molecules. Our data showed that neither 1-MT and indomethacin nor L-NAME restored the PBMCs proliferation suppressed by DPSCs. Conversely, anti-TGF-β1 antibody restored the PBMCs proliferation, comparable to the PHA-stimulated PBMCs proliferation, suggesting that TGF-β1 appears to be the key

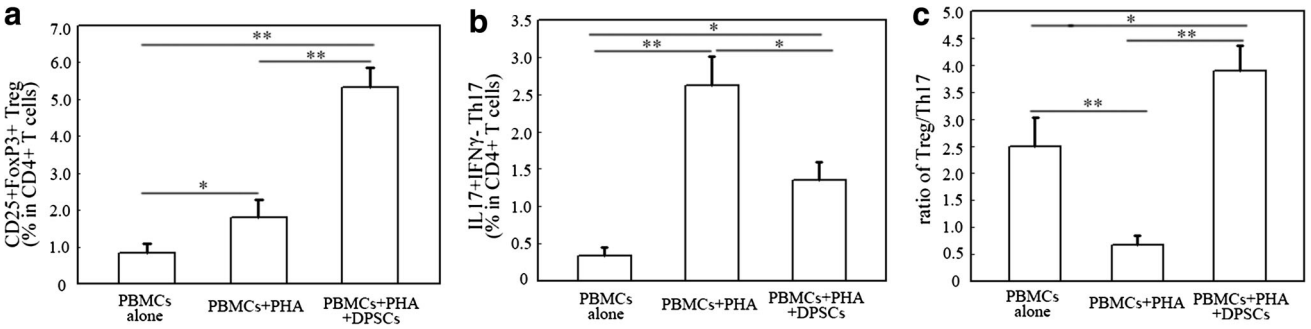

Fig. 6 DPSCs enhanced Tregs and reduced Th17 cells. **a** The population of Tregs increased significantly in co-cultures with DPSCs compared with PHA-stimulated PBMCs. **b** The percentage of Th17 cells was significantly decreased in PBMCs co-cultured with DPSCs. **c** The ratio of Tregs/Th17 cells was elevated by DPSCs. Data represent the mean ± SD. ** $P < 0.01$. * $P < 0.05$

suppressive factor responsible for the DPSCs-induced PBMCs proliferation suppression. However, it is also important to investigate the change of DPSCs-mediated immunomodulation after the expression of TGF-β1 is down-regulated in PBMCs in future's study. Furthermore, the conditioned medium derived from DPSCs alone did not show the inhibitory ability on the PBMCs proliferation, whereas conditioned medium from DPSCs pre-treated with IFN-γ for 3 days suppressed the PBMCs proliferation, indicating that IFN-γ may be the activation factor derived from T cells to make DPSCs exert their immunosuppressive functions.

Our results showed that after co-culture with DPSCs, Tregs were elevated and CD4+IFNγ-IL17+ Th17 cells were reduced, thus up-regulating the ratio of Tregs/Th17 cells. As a subpopulation of T cells, Tregs are capable of suppressing various immune responses and thereby regulating immune homeostasis and tolerance to antigens [35–37]. Th17 cells take part in the etiopathogenesis of various inflammatory and autoimmune diseases [38]. Tregs have been shown to prevent pathogenic autoimmunity by suppressing proliferation and production of proinflammatory cytokines in the effector immune cells, such as Th17 cells [21, 39]. In addition, it is well known that the generation of Tregs is mainly induced by the presence of TGF-β1 [40–42]. In our study, in parallel to the up-regulation of TGF-β1 after co-culture with DPSCs and anti-TGF-β1 antibody-mediated restoration of the PBMCs proliferation inhibited by DPSCs, elevated TGF-β1 lead to the up-regulation of Tregs, and ultimately, to immune tolerance [26].

Our research demonstrated that DPSCs were low immunogenic, could inhibit the proliferation of PBMCs and regulate the production of cytokines, indicating that DPSCs may be used in allogeneic individuals or serve as a cell source for immune therapy.

Acknowledgments This work was supported by Grants from the National Natural Science Foundation of China (No. 81070799 to G.D., and No. 81222011 to Y.L.), the Excellent Young Researchers Foundation of Shandong Province (No. BS2010SW033 to G.D.), and Science and Technology Activities of Beijing Overseas Students Preferred Foundation (to Y.L.).

Conflict of interest The authors declare that they have no conflict of interest.

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