Mesenchymal Stem Cells Derived from Human Inflamed Dental Pulp Exhibit Impaired Immunomodulatory Capacity *In Vitro*

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ABSTRACT

Introduction: Dental pulp stem cells (DPSC) are very attractive in regenerative medicine. In this study, we focused on the characterization of the functional properties of mesenchymal stem cells derived from DPSCs. Currently, it is unknown whether inflammatory conditions present in an inflamed dental pulp tissue could alter the immunomodulatory properties of DPSCs. This study aimed to evaluate the immunomodulatory capacity *in vitro* of DPSCs derived from healthy and inflamed dental pulp. **Methods:** DPSCs from 10 healthy and inflamed dental pulps (irreversible pulpitis) were characterized according to the minimal criteria of the International Society for Cell Therapy, proliferation, differential potential, and colony-forming units. Furthermore, the immunomodulatory capacity of DPSCs was tested on the proliferation of T lymphocytes by flow cytometry and the *in vitro* enzyme activity of indoleamine 2, 3-dioxygenase. **Results:** There were no significant differences in the DPSC characteristics and properties such as immunophenotype, tridifferentiation, colony-forming units, and proliferation of the DPSCs derived from normal and inflamed pulp tissue. Furthermore, there were significant differences in the immunomodulatory capacity of DPSCs obtained from human healthy dental pulp and with the diagnosis of irreversible pulpitis. **Conclusions:** Our results showed that DPSCs isolated from inflamed dental pulp showed typical characteristics of MSCs and diminished immunosuppressive capacity *in vitro* in comparison with MSCs derived from healthy dental pulp. Further investigation *in vivo* is needed to clarify the mechanism of this diminished immunosuppressive capacity. *(J Endod 2020;46:1091–1098.)*

KEY WORDS

Healthy dental pulp; inflamed dental pulp; mesenchymal stem cells; pulpitis

SIGNIFICANCE

In this study, we demonstrate that dental pulp stem cells isolated from inflamed dental pulp showed a diminished immunosuppressive capacity *in vitro* in comparison with stem cells derived from healthy dental pulp.

Mesenchymal stem cells (MSCs) are multipotent adult stem cells that can be easily isolated from different tissues[1,2]. MSCs derived from oral tissues have emerged as an interesting therapeutic strategy for the treatment of human diseases because they are easily obtained and because of their functional and immunomodulatory properties[2–4]. MSCs derived from dental pulp stem cells (DPSCs) were first isolated in the year 2000 from the healthy pulp of extracted third molars free of caries[5,6]. DPSCs present a high rate of colony formation and can differentiate into odontoblasts, adipocytes, chondrocytes, osteoblasts, and neurons[7]. DPSCs exhibit a higher rate of proliferation compared with MSCs derived from bone marrow[5,8,9], and they are clonogenic cells capable of regenerating tissues such as blood vessels[8,10,11]. DPSCs also inhibit the proliferation of stimulated T lymphocytes, as similar cases in proinflammatory or autoimmune diseases[10,12]. Third molars have a large amount of pulp tissue and therefore many DPSCs[13]; however, these teeth may not be available at the same time when the MSCs are needed[1,6,7]. Therefore, this need provides the opportunity for searching for other sources of MSCs[14], such as dental tissues that have been considered waste, as in the case of dental inflamed pulp[15]. Teeth with a diagnosis of irreversible pulpitis have an inflamed dental pulp characterized by vasodilation, increased vascular permeability, activation of cells of the immune system, and increased neuronal activity[16]. In permanent teeth with irreversible pulpitis, treatment is the complete removal of the vital dental pulp (pulpectomy)[2,17].

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Some studies reported viable DPSCs in inflamed dental pulp[18,19]. Cell proliferation, stem cell marker expression, and odontogenic and osteogenic differentiation were assayed, and they concluded that inflamed dental pulp might contain putative MSCs[16,20]. The functionality and chemotactic property of DPSCs can be reflected in the immunosuppressive properties that were previously reported[21–23]. Although further studies are required to investigate the efficacy and safety of inflamed DPSC therapies in human clinical trials, diminished immunosuppressive capacity may be a therapeutic strategy to prevent or treat transplant rejection or an autoimmune disease. Under this context, this study aimed to evaluate the functional properties of DPSCs derived from healthy and inflamed human dental pulps.

MATERIALS AND METHODS

Subjects

This protocol got the approval (no. 03/2017) from the Ethical Committee of the Metropolitan Health Service, Santiago, Chile. Dental pulp was obtained from donors 23–44 years old who visited the university's dental clinic. After signing an informed consent form, healthy pulp was collected from 5 third molars free of caries, and inflamed pulp was obtained from 5 teeth with irreversible pulpitis. The diagnostic criteria were based on the American Association of Endodontics classification [1]. Fresh tissues in cold media were sent to the central laboratory for processing (Supplemental Table S1 is available online at www.jendodon.com). Experimental groups were defined as healthy groups (DPSC-H) and inflamed groups (DPSC-I).

Isolation and Culture *In Vitro* of DPSCs

DPSCs derived from healthy (DPSC-H) and inflamed (DPSC-I) dental pulp tissues were cultured as previously described[20]. Briefly, DPSCs were grown in complete culture medium with MEM Eagle-Alpha-modified medium (Invitrogen, Carlsbad, CA), 10% fetal bovine serum (HyClone, Logan, UT), and 1% antibiotic (penicillin-streptomycin, Invitrogen) at 37°C and 5% CO_2.

Trilineage Differentiation

For osteogenic differentiation, 30,000 cells/cm^2 were seeded in a 9.5-cm^2 plate with osteogenic differentiation medium (StemPro, Thermo Fisher Scientific, Waltham, MA). The medium was changed every 4 days, and on day 21, cells were stained with von Kossa stain (Abcam, Cambridge, UK). For chondrogenic differentiation, 30,000 cells were cultured with chondrogenic differentiation medium (StemPro). The medium was changed every 4 days, and on day 14, cells were stained with Safranin O stain (Sigma-Aldrich, St Louis, MO). For adipogenic differentiation, 30,000 cells/cm^2 were seeded with adipogenic differentiation medium (StemPro); the medium was changed every 4 days. On day 21, cells were stained with Oil Red O (Sigma-Aldrich). Cells were monitored on a CKX41 inverted optical microscope (Olympus, Tokyo, Japan), and images were captured with a camera and processed with Micrometrics SE Premium software (New York City, NY).

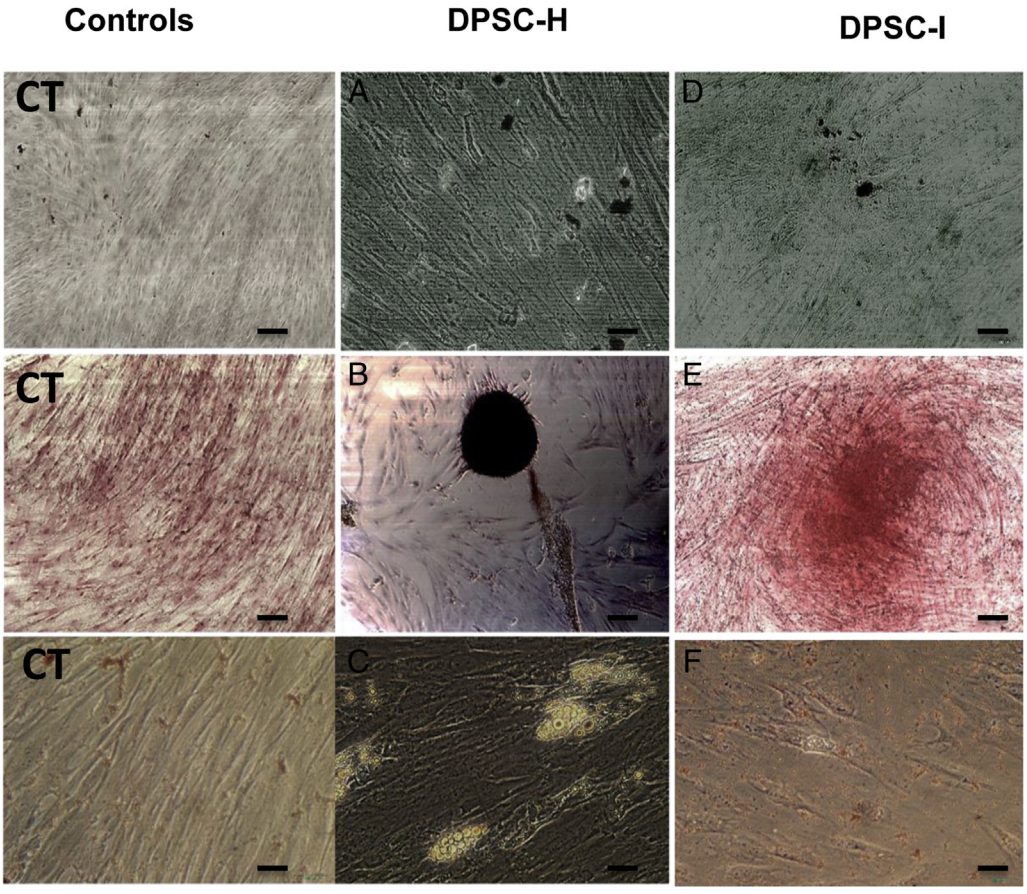

FIGURE 1 – DPSC trilineage differentiation. DPSC-H and DPSC-I were cultured in the absence (controls) or presence of differentiation media culture as described in the Materials and Methods section. DPSC controls (CT), (*A–C*) DPSC-H, and (*D–F*) DPSC-I for osteogenic, chondrogenic, and adipogenic differentiation, respectively. Magnification 10×, bar = 100 μm.

DPSC Immunophenotypic Profile

Phenotyping was assayed at cell passage 4 using the following antibodies: CD73, CD90, CD105, CD11b, CD19, CD34, CD45, and HLA-DR. Sample analysis was performed using the BD FACSCanto II (BD Biosciences, Franklin Lakes, NJ) with BD FACSDiva software. Values were expressed as percentages with standard deviations (Supplemental Table S1).

Colony-forming Unit Assay

To assess colony-forming unit (CFU) assay, 150 DPSCs in passages 2–3 were seeded in 6-well plates with 2.5 mL complete culture media and were incubated at 37°C and 5% of CO_2 for 14 days. The media was removed, and CFU was fixed with 500 μL alcohol:acetone (Lablisan #1014; Laboratorios Lisan, Santiago, Chile) for 2 minutes and stained with violet crystal (Lablisan #1010; Laboratorios Lisan). Plates seeded in biological triplicates were photographed with a digital camera (Lumix DMC-FZ100; Panasonic, Osaka, Japan) in a fixed position and without zoom. The number of colonies was counted, except those <2 mm or CFU slightly stained. The efficiency percentage of colony formation unit (% CFU) was calculated according to the following formula:

$$\% \text{ CFU} = (\text{N}^\circ. \text{ of total CFU} \times 100)/150$$

where 150 is the number of cells seeded at the beginning of the experiment.

Cell Proliferation Assay

Cell proliferation was assayed using the WST-1 kit (Roche, Basel, Switzerland) according to the supplier's instructions. Briefly, 1500 DPSCs were seeded in a 96-well plate, and cell proliferation was tested at 24, 48, 72, 96, and 120 hours in duplicate. Absorbance at 570 nm was measured in a microplate reader (RT 2100C; Rayto, Shenzhen, China).

T-lymphocyte Proliferation Assay

Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood healthy donors by density gradient centrifugation using Ficoll (1.077 g/mL, Sigma-Aldrich). PBMCs were labeled with fluorescent probe CellTrace

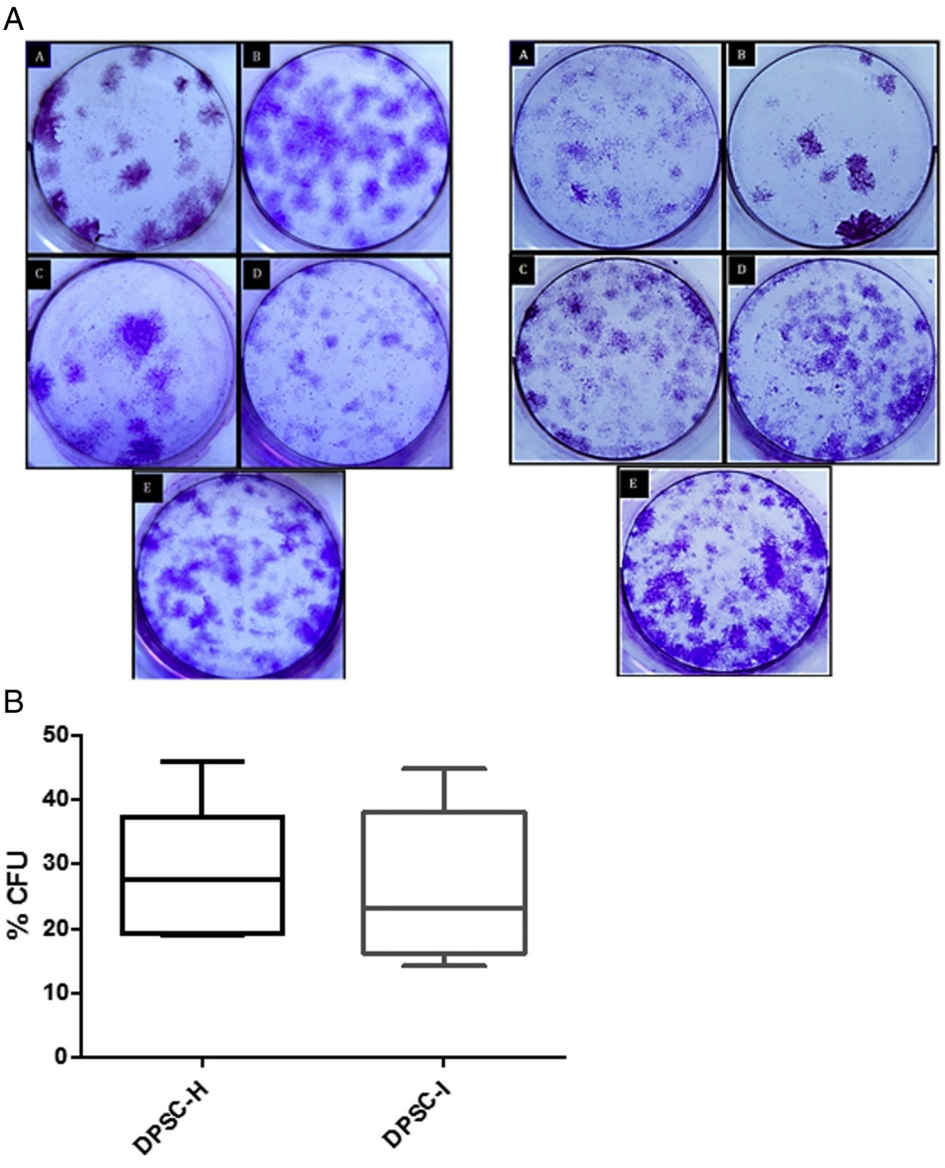

FIGURE 2 – CFUs and % efficiency CFU of DPSC-H and DPSC-I. To assess CFU assay, DPSCs were cultured in complete media for 14 days as described in the Material and Methods section. (*A*) A CFU plate stained with violet crystal corresponds to each of the 5 samples from both experimental groups, the healthy groups (DPSC-H), and inflamed groups (DPSC-I). (*B*) The boxplot-type graph represents the data distribution and interquartile range of the % CFU. No significant differences were observed.

Violet (CTV) (5 μmol/L, C34557; Thermo Fisher Scientific, Waltham, MA). PBMCs were stimulated with phytohemagglutinin (PHA, 10 μg/mL, L-8754 [Sigma-Aldrich]) and cultured in the presence or absence of DPSCs in a 1:10 and 1:100 ratio (DPSCs:PBMCs) in Roswell Park Memorial Institute medium containing 10% fetal bovine serum, 2 mmol/L L-glutamine, 50 μmol/L β-mercaptoethanol, and 1% penicillin-streptomycin at 37°C in 5% CO_2. On the fifth day, cells were stained with CD3-FITC (Beckman Coulter, Brea, CA) and acquired on the flow cytometer FACSCanto II. The percentage of proliferative CD3+ T cells was assessed by cell division based on CTV assay according to the manufacturer's instructions. T-cell proliferation in the absence or presence of DPSCs at different ratios is shown in Supplemental Figure S1 (available online at www.jendodon.com).

Measurement of Indoleamine 2, 3-Dioxygenase Activity

The cell medium was previously supplemented with tryptophan (Sigma-Aldrich) for indoleamine production; 2000 cells/cm^2 in 96-well plate were incubated with 10 μg/μL tryptophan at a final concentration of 1 μg/μL for 48 hours at 37°C and 5% CO_2 because negative controls were considered DPSC-H/I without tryptophan. A calibration curve was built with L-kynurenine (Sigma-Aldrich). One hundred sixty microliters of supernatant with 10 μL 30% trichloroacetic acid (Merck, Kenilworth, NJ) diluted in sterile distilled water were incubated for 30 minutes at 50°C (hydrolysis of N-formylkynurenine in kynurenine) and was centrifuged at 2000 rpm for 10 minutes. One hundred microliters were transferred to 96-well plates (SPL; Life Sciences, Gyeonggi-do, Korea). One hundred microliters of Ehrlich solution was added (Sigma-Aldrich) at 12 mg/mL (diluted in acetic acid), and the absorbance was read at 450 nm in an enzyme-linked immunosorbent assay 2100C plate reader (Rayto).

Statistical Analysis

The percentage of CFU data was expressed as distribution and interquartile range of the median. WST-1 data were represented as means of optical density units with standard deviation. Two groups were compared with the Mann-Whitney test and analysis of variance with a confidence interval of 95%. For the immunosuppressive assay, a Kruskal-Wallis test was performed for comparisons between the experimental groups. For all analyses, was used GraphPad Prism7 (GraphPad Software, San Diego, CA). Data are presented as the mean ± standard deviation. $P < .05$ was considered statistically significant.

RESULTS

DPSC Culture

DPSC-H emerged from the explant on day 10, and DPSC-I appeared in the culture on day 14. Cells from both groups showed typical MSC fibroblastlike morphology and plastic adherence (Fig. 1, controls).

Differentiation Assays

Mineralized areas according to calcium deposits were detected for osteogenic lineage. In the chondrogenic phenotype the mucopolysaccharide matrix was shown, and lipid vacuoles were found in the adipogenic DPSC-H/I cell cultures (Fig. 1*A*–*F*).

Immunophenotypic Profile

DPSC-H expressed CD73 (99.8% ± 0.1%), CD90 (93.1% ± 10.8%), CD105 (90.7% ± 13.6%), CD11b (0.3% ± 0.3%), CD19 (0.4% ± 0.3%), CD34 (1.2% ± 1.1%), CD45 (0.3% ± 0.3%), and HLA-DR (1.3% ± 1.2%). DPSC-I showed expression for CD73 (99.8% ± 0.1%), CD90 (93.1% ± 10.8%), CD105 (90.7% ± 13.6%), CD11b (0.3% ± 0.3%), CD19 (0.4% ± 0.3%), CD34 (1.1% ± 1.1%), CD45 (0.3% ± 0.3%), and HLA-DR (1.3% ± 1.1%). The flow cytometry analysis results are provided in Supplemental Table S2 (available online at www.jendodon.com).

CFU Assay

A great variability of shape, size, and distribution of CFU was observed without a defined pattern in both experimental groups. The percentage of CFU did not show statistically significant differences between DPSC-H and DPSC-I (Fig. 2*A* and *B*).

Cellular Proliferation Assay WST-1

DPSC-H and DPSC-I showed very similar cell proliferation between 24 and 72 hours. However, after 96 hours, the DPSC-H group showed greater proliferation, and the DPSC-I group proliferated less but were not statistically significant. Mann-Whitney analysis did not show significant differences between the groups over time (Fig. 3).

T-cell Immunosuppression Assay

DPSC-H significantly suppressed the proliferation of CD3 T lymphocytes activated with PHA ($P < .05$, Fig. 4*A*). This immunosuppressive effect is conserved at 1:10 and 1:100 ratios ($P < .05$, Fig. 4*B*). DPSC-I was not able to suppress the proliferation of the T lymphocytes at both ratios as done by DPSC-H, suggesting a functional alteration in their immunomodulatory capacity (Fig. 4*B*).

Indoleamine 2, 3-Dioxygenase Activity

Our results confirm the immunosuppressive effect of DPSC-H, which showed higher indoleamine 2, 3-dioxygenase (IDO) activity compared with DPSC-I (Fig. 4*C*, $P < .05$).

DISCUSSION

Dental pulp tissue was collected from young donors (23–44 years old) because of the ideal conditions for MSC culture expansion[16,19]. The immunophenotypic profile for DPSC-H/I

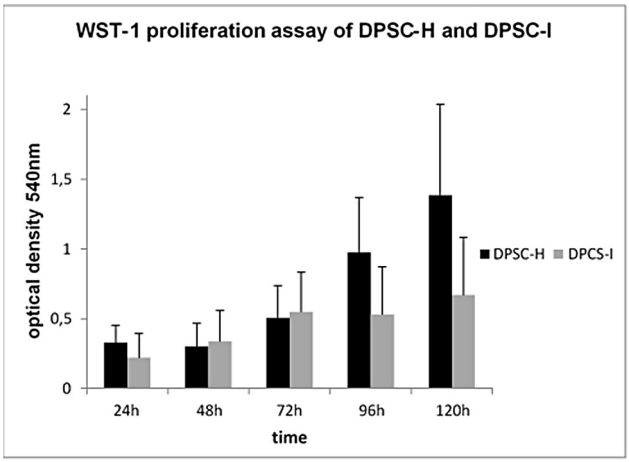

FIGURE 3 – WST-1 proliferation assay of DPSC-H and DPSC-I assessed at 24, 48, 72, 96, and 120 hours. Five samples of each experimental group were seeded in 96-well plates and cultured at different times to evaluate its proliferation at 570 nm of absorbance. Data were expressed as means with standard deviation.

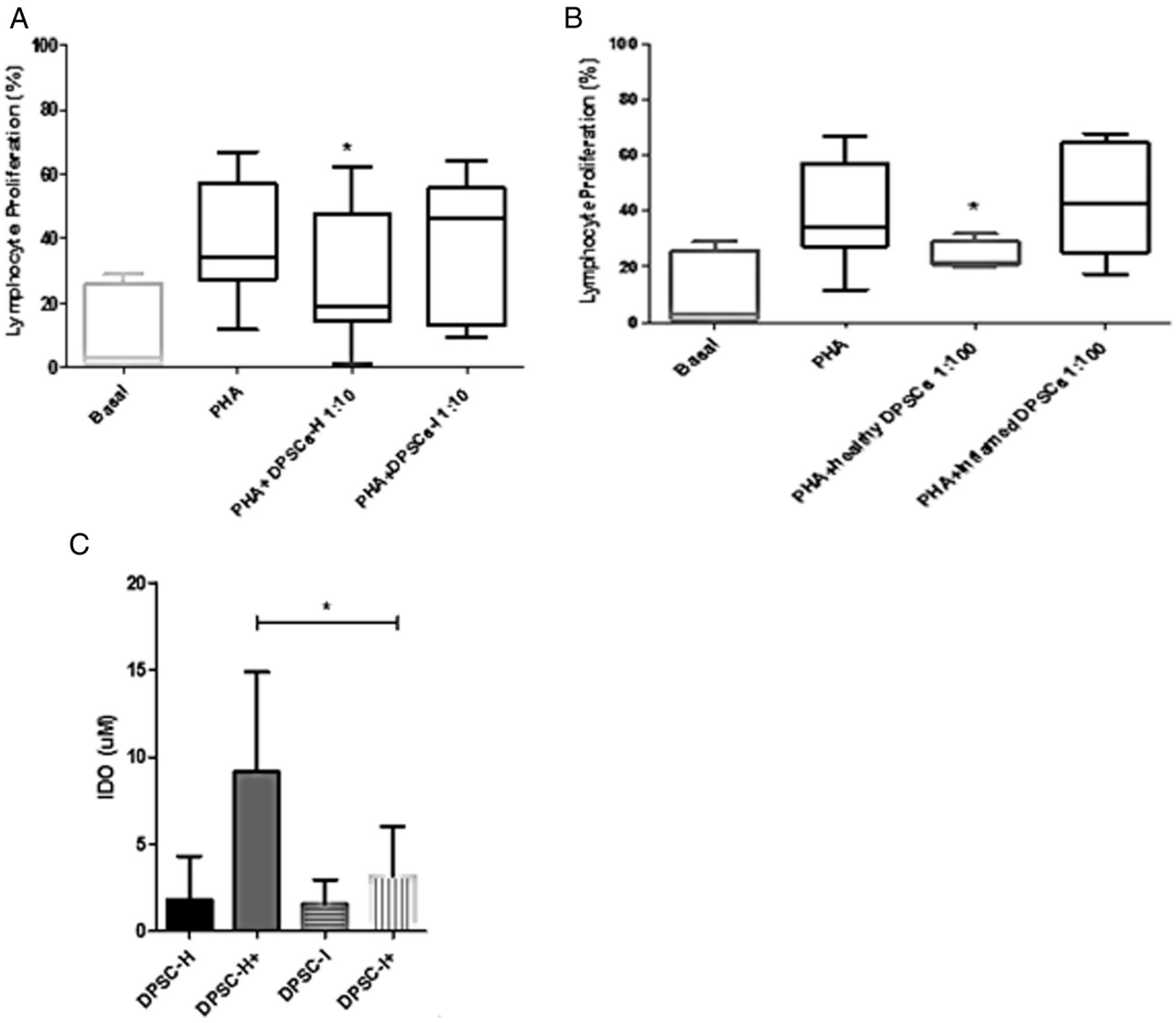

FIGURE 4 – The immunomodulatory capacity of DPSCH-/I. The lymphocyte proliferation assay at different ratios of DPSC: PBMC and IDO activity. (*A*) DPSC:PBMC in a ratio of 1:10 and (*B*) 1:100 ratio. PBMCs were labeled with CTV and stimulated with PHA and cultured in the absence or presence of DPSCs at 1:10 or 1:100 ratios (DPSCs:PBMCs) for 5 days as described in the Material and Methods section. T-cell proliferation was evaluated by flow cytometry, gating on CD3+ cells. Data are expressed as median ± interquartile range and 95% confidence interval. A Mann-Whitney test was performed, $*P < .05$; $N = 5$ and $n = 3$. (*C*) IDO activity was measured in the supernatant of the DPCS cultures in the absence or presence of tryptophan using an L-kynurenine curve as described in the Materials and Methods section. DPSC-H and DPSC-I corresponded to the DPSC groups without stimulation with tryptophan (negative controls), and the DPSC-H+ and DPSC-I+ corresponded to those stimulated with tryptophan. Data were expressed as median ± interquartile range and 95% confidence intervals. A Mann-Whitney test was performed, $*P < .05$; $N = 5$ and $n = 3$.

showed a similar phenotype to those described in bone marrow MSCs and according to the International Society for Cell Therapy criteria with a high proliferative capacity. The obtained results of tridifferentiation for DPSC-H/I agreed with those previously described[18,19]. DPSC-H/I acquired a star-shaped characteristic of osteoblasts because of the von Kossa staining and the precipitation of silver nitrate[24]. Cells in adipogenic differentiation showed morphologic changes more evident in DPSC-H than DPSC-I with slight potential for adipogenic differentiation according to previous studies[18,19,24]. These differences could be explained by the cellular origin; dental pulp influences the differentiation of tissues of ectodermal origin (odontogenic, chondrogenic, etc), unlike the adipose tissue origin that is in the mesoderm, which would make the differentiation of DPSCs to adipocyte cells more difficult[19,24]. DPSC-H/I in chondrogenic lineage showed morphologic characteristics of the cell mass matrix of proteoglycans[21,25-27]. The percentage of CFU showed very similar behavior for DPSC-H/I. At 96 hours, DPSC-H showed increased proliferation; however, these differences were not statistically significant. Similar results were reported by Pereira et al[18] and with MSCs derived from periodontal ligament with values of 15%–35% for % CFU[28]. Cell proliferation assay WST-1 revealed that DPSC-H/I had a very similar proliferative capacity between 24 and 72 hours of culture, but DPSC-I proliferated less after 96 hours compared with DPSC-H. The inflammatory state could have activated and differentiated the DPSCs to other cell types such as odontoblasts or fibroblasts to repair the damaged tissues. Also, inflammatory processes for irreversible pulpitis generated damage caused by the increase in

volume and compression within the crown together with the presence of smaller blood vessels, which triggered the appearance of extensive lumens of blood or lymphatic vessels[29,30]. Osteocalcin increased in pulpal repair factors [31] and fulfills the function of increasing the pulp repair factors and stimulating the differentiation of DPSCs to odontoblasts that generate dentin. The immunosuppressive effect of MSCs would produce an anti-inflammatory effect on the pulp and gingival tissue because of the local immune response mediated mainly by T lymphocytes[30]. In a clinic evaluation, MSCs derived from healthy pulp were able to repair a mandibular bone defect[30]. Regarding the immunomodulation assays, DPSC-H at a ratio of 1:10 showed diminished T-lymphocyte proliferation, but no significant differences were found in DPSC-I. Other authors compared the immunomodulatory properties in different doses (eg, in a 1:100 ratio where the results were dose dependent, 1:10 tended to act as immunosuppressive, and 1:100 ratios tended to immunostimulate the proliferation of T lymphocytes)[29,32]. The inhibitory or stimulatory effect of the proliferation of CD3 T lymphocytes depends on the ratio used, which agrees with our results that showed that in the 1:10 ratio (DPSC-H:TCD3) DPSC-H tend to decrease the proliferation of CD3 T lymphocytes, but these data showed no significant differences. It has been established that DPSC-H inhibits the proliferation of T lymphocytes stimulated with PHA with this effect being even greater than the immunomodulatory effect of bone marrow MSCs[30]. The differences found could be attributed to the fact that the pulp donors correspond to a different group of PMBC donors. Regarding this point, we suggest collecting relevant clinical information considering age, sex, and associated diseases. No similar studies were found regarding the immunosuppression of DPSC-I. In our study, the immunomodulatory capacity in the ratio of 1:100 (DPSC-H or DPSC-I: TCD3) showed a difference in the proliferation of T lymphocytes. Our results showed that the immunosuppressive properties of DPSCH/I at the ratio of 1:10 were very similar, but at the ratio of 1:100, there is a significant difference in the immunosuppressive effect. The local environment of the dental pulp (cytokines such as interleukin [IL]-6 and IL-17) can influence the functional characteristics of MSCs. Sun et al[33] showed that bone marrow MSCs in patients with systemic lupus erythematosus showed decreased secretion of cytokines (IL-6, IL-7, IL-11, and macrophage colony-stimulating factor). Also, Nie et al[34] showed that MSCs from patients with systemic lupus erythematosus had a defective phenotype and extended the suppression on the proliferation of allogeneic T lymphocytes, concluding that the ability of MSCs to interact with the immune system is altered according to the local environment. IDO enzymatic activity of MSCs is the principal mechanism of T-cell suppression. IDO is correlated to the immunosuppressive capacity of MSCs by the depletion of the monocyte fraction from PBMCs[35,34]. The explanation involved in the immunosuppressive effect in the 1:100 ratio can be related to a low IDO activity of DPSC-I and could be correlated to a loss of its immunosuppressive capacity, which was revealed with an increment of the MSC:PBMC ratio. We want to highlight a study by Yazid et al[35] in which it is suggested that dental stem cells showed a similar immunosuppressive capacity related to cell dose. Compared with cells from the inflamed deciduous pulp, healthy pulp stem cells effectively suppress mitogen-induced T-cell proliferation in a dose-dependent manner. It is essential to clarify that the dental pulp samples analyzed in this study were not grouped by age and sex. We suggest that these variables be considered for future investigation.

IDO catalyzes the degradation of tryptophan in the metabolic pathway to kynurenine, and immunosuppression would occur by the depression of tryptophan. Our results showed a higher IDO activity in DPSC-H, which is correlated with an increment of its immunosuppressive capacity, compared with DPSC-I, which has low IDO activity and loss of its immunosuppressive capacity. Gathering all of our results, there are no significant differences in the DPSC-H/I characteristics and properties such as immunophenotype, tridifferentiation, CFU, and proliferation. However, there are differences in the immunomodulation capacity of DPSC-H/I. In clinical practice, these immunosuppressive differences between DPSCs derived from healthy and inflamed dental pulp will have to be considered for potential use in regeneration.

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Carolina Inostroza Silva and Ana María Vega-Letter contributed equally to this study.

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The authors deny any conflicts of interest related to this study.

SUPPLEMENTARY MATERIAL

Supplementary material associated with this article can be found in the online version at www.jendodon.com (10.1016/j.joen.2020.05.003).

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SUPPLEMENTARY MATERIAL

SUPPLEMENTAL TABLE S1 - Donor and Dental Tissue Information

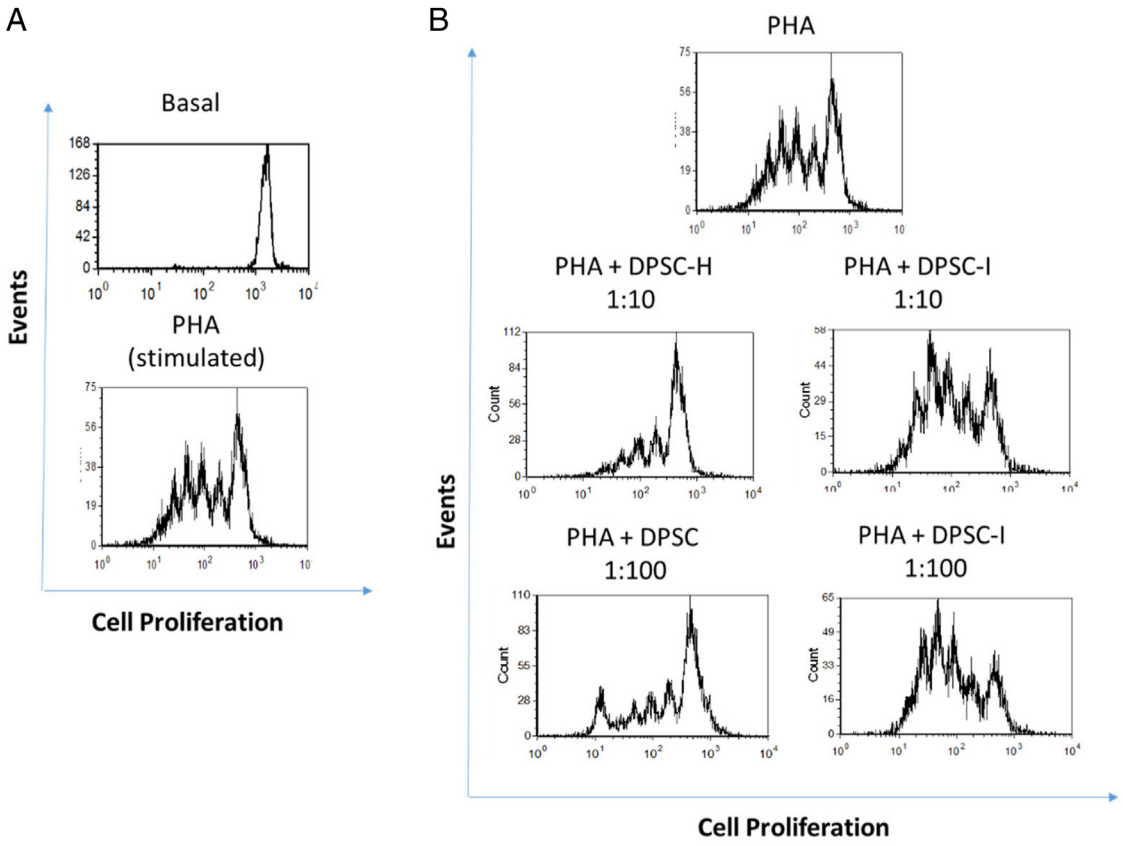
Sample number	Sex	Age (y)	Tooth (no. or type)	Dental pulp diagnoses	MSC	Clinical observations
1	Female	23	Third molar	Healthy pulp	DPSC-H	Dental extraction for orthodontic treatment
2	Female	26	Third molar	Healthy pulp	DPSC-H	Dental extraction for orthodontic treatment
3	Male	26	Third molar	Healthy pulp	DPSC-H	Dental extraction for orthodontic treatment
4	Male	35	Third molar	Healthy pulp	DPSC-H	Dental extraction for orthodontic treatment
5	Female	30	Third molar	Healthy pulp	DPSC-H	Dental extraction for orthodontic treatment
6	Male	33	2.1	Irreversible pulpitis	DPSC-I	Presence of deep caries; moderate to severe pain, which persists for more than 3 seconds after the stimuli is retired
7	Female	44	1.5	Irreversible pulpitis	DPSC-I	Presence of faulty restoration lingering thermal pain
8	Male	38	1.5	Irreversible pulpitis	DPSC-I	Presence of faulty restoration, lingering thermal pain, without pulpal necrosis or abscess
9	Female	23	1.6	Irreversible pulpitis	DPSC-I	Presence of deep caries; moderate to severe pain, which persists for more than 3 seconds after the stimuli is retired
10	Male	28	2.1	Irreversible pulpitis	DPSC-I	Presence of deep caries; moderate to severe pain, which persists for more than 3 seconds after the stimuli is retired

DPSC-H, dental pulp stem cells derived from healthy tissue; DPSC-I, dental pulp stem cells derived from inflamed tissue.

SUPPLEMENTAL TABLE S2 - Immunophenotypes of Dental Pulp Stem Cells

Samples code	CD73 (%)	CD90 (%)	CD105 (%)	CD11b (%)	CD19 (%)	CD34 (%)	CD45 (%)	HLA-DR (%)
1 DPSC-H	99.6	73.9	67.0	0.1	0.1	0.1	0.1	0.1
2 DPSC-H	99.9	96.6	98.3	0.2	0.3	0.6	0.2	0.8
3 DPSC-H	99.8	97.3	97.6	0.3	0.5	2.4	0.3	2.8
4 DPSC-H	100	99.4	99.2	0.1	0.2	0.4	0.2	0.6
5 DPSC-H	99.8	98.3	91.4	0.8	0.8	2.1	0.9	2.3
1 DPSC-I	**99.6**	**73.9**	**67.0**	**0.1**	**0.1**	**0.1**	**0.1**	**0.1**
2 DPSC-I	99.9	96,6	98.3	0,2	0,3	0,6	0,2	0,7
3 DPSC-I	99.8	97.3	97.6	0.3	0.5	2.4	0.3	2.7
4 DPSC-I	100	99.4	99.2	0.1	0.2	0.4	0.2	0.6
5 DPSC-I	99.8	98.3	91.4	0.8	0.8	2.2	0.9	2.3

DPSC-H, dental pulp stem cells derived from healthy tissue; DPSC-I, dental pulp stem cells derived from inflamed tissue.
Flow cytometry analysis of human dental pulp stem cells from 10 donors (marked as DPSC-H and DPSC-I) showed that dental pulp stem cells were positive for the expression of CD73, CD90, and CD105 and lacked the expression of the negative markers HLA-DR, CD45, CD34, CD19, and CD11b.



SUPPLEMENTAL FIGURE S1 – T-lymphocyte proliferation in the absence or presence of DPSCs derived from healthy and inflamed pulp at different ratios. (*A*) The proliferation of a peak in the histogram of basal T lymphocytes was observed with the staining performed with CTV that was intercalated between the cells that proliferate and generated each peak that was evidenced by flow cytometry. The lower image shows the cellular proliferation of PHA-stimulated T lymphocytes. (*B*) The superior image establishes the cellular proliferation of the T lymphocytes activated with PHA as stimulation control and thus evaluated the following proliferations (lower images) that corresponded to the T lymphocytes activated with PHA in contact with healthy and inflamed DPSCs at different ratios of 1:10 and 1:100 (DPSCs:PBMCs).