



Mesenchymal stem cells from periapical lesions modulate cytokine production by local immune cells

Mezenhimske matične ćelije iz periapeksnih lezija moduliraju produkciju citokina od strane lokalnih imunskih ćelija

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Abstract

Background/Aim. Mesenchymal stem cells (MSCs) have been shown to suppress immune and inflammatory reactions. However, it is not known whether MSCs from inflammatory tissues, such as periapical lesions (PLs) have similar effects. This question was addressed in this study in which the aim was to examine the capacity of PL-MSCs for modulating cytokine production by local immune cells. **Methods.** PL-MSCs were isolated from asymptomatic (as) and symptomatic (sy) PLs. Their phenotype was analyzed by flow cytometry by detecting MSC surface markers. Anti-inflammatory and immunomodulatory properties of PL-MSCs were examined by measuring cytokine production in direct co-culture experiments with mononuclear cells (MNCs) isolated from asPLs and syPLs, respectively. The levels of cytokines in supernatants were determined by specific ELISA kits. **Results.** Both PL-MSCs lines were characterized by typical MSC phenotype, with the predominance of CD29, CD44, CD90, CD105 and CD166.

However, the lines, independently of their similar phenotype had the same modulatory effect on cytokine production, but the response of asPL-MNCs and syPL-MNCs was different, in spite of similar composition of these MNCs. Both MSC lines inhibited the production of inflammatory cytokines, such as interleukin-10 (IL-10) and tumor necrosis factor-0 (TNF-0). However, IL-8 was only down-regulated in the co-culture of these MSC lines with syPL-MNCs. The PL-MSCs also modulated the production of immunoregulatory cytokines. Transforming growth factor-0 (TGF-0) was up-regulated by both as- and syPL-MNCs but IL-10 was up-regulated only by asPL-MNCs. **Conclusion.** Our results showed that PL-MSCs contribute to the restriction of local inflammatory and immune responses, but this effect is probably less efficient during the exacerbation of PL inflammation.

Key words: periapical diseases; stem cells; periapical abscess; inflammation; cytokines; phenotype; flow cytometry.

Apstrakt

Uvod/Cilj. Pokazano je da mezenhimske matične ćelije (engl. *mesenchymal stem cells* – MSC) suprimiraju imunske i inflamacijske reakcije. Međutim, nije poznato da li MSC iz inflamacijom zahvaćenih tkiva, kao što su periapeksne lezije (engl. *periapical lesions* – PLs) ispoljavaju slične efekte. Upravo je ovo pitanje razmatrano u našem radu čiji cilj je bio da se ispita sposobnost PL-MSC da moduliraju produkciju citokina od strane imunskih ćelija lokalno. **Metode.** MSC su izolovane iz asimptomatskih (engl. *asymptomatic* – as) i simptomatskih (engl. *symptomatic* – sy) PL zuba. Njihov fenotip je određen metodom protočne citofluorimetri-

je na osnovu detekcije površinskih markera specifičnih za MSC. Antiinflamacijska i imunomodulacijska svojstva PL-MSC ispitivana su merenjem produkcije citokina u kokulturi sa mononuklearnim ćelijama (engl. *mononuclear cells* – MNCs) izolovanim iz asPLs i syPLs. Nivo produkovanih citokina u supernatantima odredivan je ELISA metodom. **Rezultati.** Obe PL-MSC linije karakterisao je fenotip tipičan za druge tipove MSC u kome je dominirala ekspresija CD29, CD44, CD90, CD105 i CD166 markera. Izolovane PL-MSC linije su, nezavisno od fenotipske sličnosti, ispoljile isti modulacijski efekat na produkciju citokina, ali je odgovor asPL-MNCs i syPL-MNCs bio različit, uprkos sličnom sastavu MNCs u oba tipa lezija. Oba tipa MSC li-

nija inhibirala su produkciju proinflamacijskih citokina kao što su interleukin 1 β (IL-1 β) i faktor nekroze tumora α (TNF- α). Međutim, produkcija IL-8 bila je snižena jedino u kokulturi sa syPL-MNCs. PL-MSC linije su takode modularale produkciju imunoregulatorijskih citokina. Produkcija transformišućeg faktora rasta β (TGF- β) bila je povećana u kokulturama sa oba tipa MNC, i asPL-MNCs i syPL-MNCs, a nivo IL-10 bio je povećan jedino u kokulturi sa asPL-MNCs. **Zaključak.** Naši rezultati pokazuju da PL-

MSC doprinose smanjenju lokalne inflamacije i imunskog odgovora, ali je ovaj efekat verovatno manje efikasan u toku egzacerbacije inflamacije u PLs.

Ključne reči: periapikalne bolesti; ćelije, matične; apsces, periapikalni; zapaljenje; citokini; fenotip; citometrija, protočna.

Introduction

Dental and periodontal tissues are a significant reservoir of mesenchymal stem cells (MSCs), which have been extensively investigated as a potent tool for the regenerative medicine^{1, 2}. This hypothesis is based on relatively easy availability of these cells, their rapid propagation in culture, possibility to differentiate in different types of cells (osteoblasts, odontoblasts, chondroblasts, fibroblasts, adipocytes, neuronal cells) and other cells of mesenchymal origin^{3, 4}. In addition, MSCs possess anti-inflammatory and immunosuppressive properties, making them usable for the treatment of chronic inflammatory and autoimmune diseases^{5, 6}.

Recently, MSCs have been isolated from inflamed dental and periodontal structures such as dental pulp⁷, and periodontal ligament⁸. We have also established several MSCs lines from periodontal lesions and showed that these cells had many phenotypic similarities with MSCs from healthy tissue, such as dental pulp and dental follicle^{9, 10}. However, it remained unclear whether periapical lesions (PL)-MSCs have similar functions as their counterpart from healthy periapical dental tissue, bearing in mind that PLs are chronic granulomatous pathoses¹¹. It is well-known that PLs are triggered by bacterial infection from necrotic dental pulp and that these are characterized histologically by the presence of inflammatory infiltrate, mainly composed of mononuclear cells (MNCs)^{12, 13}. In clinically asymptomatic (as) PLs, a balance between proinflammatory/osteodestructive and anti-inflammatory/osteoprotective processes is established¹²⁻¹⁴. The inflammatory processes are frequently exacerbated by the new wave of bacterial invasion, which is followed by pain, swelling and other clinical symptoms of infection. Histologically, such symptomatic (sy) PLs are characterized by the influx of neutrophil granulocytes within already established granulomatous tissue^{12, 13, 15}.

Based on our previous results, showing that dental MSCs suppress the immune response mediated by dendritic cells (DC), but that they may stimulate immune reactions under certain conditions¹⁶, we wondered is there a difference between the capacity of PL-MSCs established from syPLs and asPLs, respectively, to influence the local production of cytokines. We hypothesized that different inflammatory cell substrate in syPLs and asPLs¹⁵ is associated with different cytokine production. Therefore, to standardize the experimental conditions, we isolated MNCs from clinically different PLs and established an *in vitro* model by co-culturing these cells with asPL-MSCs or syPL-MSCs. We showed for

the first time, that PL-MSCs, independently of its origin, are able to down-regulate the production of pro-inflammatory cytokines and up-regulated immunomodulatory cytokines by PL-MNCs. However, their immunomodulatory activity was lower during exacerbation of PL inflammation.

Methods

Donors and periapical tissues

A total of 10 PLs, 5 sy PLs and 5 as PLs, were collected from patients at the Department for Oral Surgery, Clinic for Stomatology, Military Medical Academy (MMA), Belgrade, during tooth extraction or apical surgery. Sy PLs and asPLs were classified according to the presence or absence of clinical symptoms¹⁵. The donors (range, aged 22–56 years) were without systemic diseases and had radiographic evidence of PLs. The donors have not been treated with antibiotics for 2 month before the surgery. No distinctions between samples were made regarding the etiology or the tooth type. One of specimens from asPLs and one from syPLs were used for the establishment of MSCs lines and the rest was used for preparation of inflammatory cells (ICs). The study was approved by the Ethical Committee of MMA, and informed consents were obtained. After collection, the tissue was placed into medium consisting of RPMI 1640 (Sigma, Munich, Germany) and antibiotics (gentamycine/penicillin/ streptomycin, 1% each) and transported to the laboratory.

Establishment of PL-MSC lines

PLs were excised by the curettage of firmly attached periodontal tissue from dental radices with a scalpel at the time of teeth extraction. Briefly, PLs tissues were digested in a Minimum Essential Medium (MEM, α -modification; Sigma, Munich, Germany) solution with a type I collagenase (1mg/mL; Sigma) and DNAase (25mg/mL; Sigma) for 1 h in 0.5% CO₂ atmosphere in an incubator with 5% CO₂. The cells were cultured in standard medium composed of α -MEM supplemented with 10% foetal calf serum (FCS; Sigma), 2-mercaptoetanol (2-ME) (Sigma), L-aspartat-2-phosphate (Sigma) and penicillin and streptomycin antibiotics 1% each (Galenika, Belgrade, Serbia). Passaging was performed by using equal volumes of 0.02% trypsin (Sigma) and 0.02% of Na-ethylenediaminetetraacetic acid (EDTA) dispersed in phosphate-buffered saline (PBS) solution. PL-MSCs used in the experiments were from the 4th passage.

Preparation of mononuclear cells

Inflammatory cells were isolated from asPLs and syPLs by a classic enzyme (collagenase/DNAase) digestion procedure, previously optimized at the Institute for Medical Research, MMA¹⁷. The total number of cells per lesion was in the range between 8.6×10^5 – 2.2×10^6 . MNCs were prepared from PL-ICs by using LymphoPrep gradient (Nycomed, Oslo, Norway) and counted. Cytospins of both PL-ICs and PL-MNCs were stained with May-Grünwald Giemsa (MGG) and analysed by light microscopy. Identification of cell subsets on cytospins was made by clear morphological criteria. The term „other cells“, was used for morphologically unidentified cells, including some blast-like cells and apoptotic cells. On each cytospin at least, a total of 500 cells were counted and particular cell subsets were determined. The results were presented as percentages of cell subsets relative to a total number of nucleated cells¹⁷.

Phenotypic characterization of PL-MSC lines

For characterization of PL-MSC lines, the flow cytometry was used. The cells were stained with the following monoclonal antibodies (mAbs) conjugated with fluorescein isothiocyanate (FITC): anti-CD29, -CD105, -CD44, -CD19, -CD14, -CD45 (Immunotools, Friesoythe, Germany), -CD146, -CD46, -CD166, -CD90 (Serotec, Kidlington, UK). The indirect labelling was performed using anti-STRO-1 (Millipore/Chemicon) mAb, followed by secondary anti-mouse IgG1-FITC mAb (Serotec). During the staining procedure, the adequate isotype controls were set and the samples were acquired with a flow cytometer (Partec, CyFlow[®] Cube 6, Germany), as previously described⁹.

Co-culture experiments

The effect of as- or syPL-MSCs on the production of cytokines by as- and syPL-MNCs were evaluated in the co-culture by using direct cell-to-cell contacts. PL-MNCs (1×10^5 cells/well) were cultivated with PL-MSCs (1×10^4 /well) in triplicates, using 96-well plates for 48 h in the presence of phorbol myristate acetate (PMA; 20 ng/mL; Sigma) and Ca^{2+} ionophore (A23187, 1 μM ; Sigma) during the last 8 h. Separate cultures of corresponding PL-MNCs and PL-MSCs, treated identically, were used as control. The levels of cytokines, produced in the PL-MSCs/PL-MNCs co-cultures, were compared with the sum of cytokines produced from separate PL-MNCs and PL-MSCs cultures. Each PL-MSC line was co-cultivated with four different as- or syPL-MNCs to evaluate cytokines production.

Cytokine detection

Concentrations of interleukin 1 β (IL-1 β), tumor necrosis factor α (TNF- α), IL-10 and transforming growth factor β 1 (TGF- β 1) referred as TGF- β , from the culture supernatants were determined using the commercial ELISA kits (R&D Systems), following instructions of the manufacturer.

The standard curves were set up based on known concentrations of the cytokines.

Statistical analysis

Results are presented as mean \pm SD. Mann-Whitney test was used to investigate the differences between the experimental and corresponding control samples. Values at $p < 0.05$ or less were considered to be statistically significant.

Results

Flow cytometric analysis of PL-MSC lines, established from asPLs and syPLs, showed very similar phenotypic properties (Table 1). Most cells expressed CD90, CD44, CD105, CD166 and CD29. A half of the cells expressed CD46 and CD146, whereas STRO-1 was detected on a subset of MSC (9.3% of asPL-MSC and 13.2% of syPL-MSC, respectively).

Table 1
Phenotypic characteristics of 2 mesenchymal stem cell (MSC) lines established from human periapical lesions (PL)

Markers	Expression (%)	
	asPL-MSC	syPL-MSC
CD90	99.8	97.4
CD44	99.4	95.9
CD29	98.8	97.4
CD166	93.6	94.2
CD105	98.3	96.1
CD46	57.0	50.3
CD146	50.4	42.4
STRO-1	9.3	13.2

PL-MSCs were prepared for the flow cytometry analysis, as described in the methods section. The labeled cells were gated according to the forward scatter/side scatter parameters and the percentage of positive cells for the indicated markers were calculated based on the isotype control. Results are presented as % of positive cells. Standard deviation between duplicates was not higher than 2%.

as – asymptomatic; sy – symptomatic.

To study how these MSC lines modulate local cytokine production we established an *in vitro* model by co-cultivating PL-MSC lines and MNCs prepared from either as- or syPL-ICs. As presented in Table 2, syPLs differed from asPLs by higher proportion of granulocytes and lower percentages of lymphocytes. After removal of granulocytes by density gradient, no significant differences in the cellular composition of MNCs between asPLs and syPLs were observed (Table 3). The MNC subsets composed predominantly of lymphocytes, followed by almost equal proportion of plasma cells and macrophages (M \emptyset), whereas the percentage of DC was the lowest.

The analysis of cytokines from the co-cultures showed that all examined cytokines were produced by PL-MNCs, but very little or none by MSC lines. MSC lines, independently of their origin, exerted the same modulatory effect on cytokine production by PL-MNCs. However, the response of asPL-MNCs and syPL-MNCs was different.

Table 2

Cellular composition of a total of inflammatory cells (ICs) isolated from human periapical lesions (PL)		
Cell subsets	asPL-ICs (%)	syPL-ICs (%)
GR	14.2 ± 5.3	47.6 ± 8.7***
LY	46.5 ± 10.8	22.5 ± 6.9**
PC	19.3 ± 8.1	10.4 ± 3.2
MØ	15.9 ± 3.4	11.3 ± 3.1
DC	2.6 ± 1.3	2.2 ± 0.6
Other cells	3.1 ± 3.1	6.0 ± 3.3

Inflammatory cells were isolated from asymptomatic and symptomatic periapical lesions (both n = 4), as described in the section methods. Cytospins were stained with May-Grünwald Giemsa and analysed by light microscopy. The percentages of cell subsets were determined based on morphological criteria after calculation of 500 cells on each cytospin. Values are given as mean ± standard deviation (SD) (n = 4). ** p < 0.01, *** p < 0.001 compared to corresponding controls. GR – granulocytes; LY – lymphocytes; PC – plasma cells; MØ – macrophages; DC – dendritic cells; as – asymptomatic; sy – symptomatic.

Table 3

Cellular composition of mononuclear cells (MNCs) isolated from human periapical lesions (PL)		
Cell subsets	asPL-MNCs (%)	syPL-MNCs (%)
GR	3.1 ± 1.3	1.7 ± 1.2
LY	43.3 ± 8.1	48.8 ± 9.3
PC	21.4 ± 9.1	21.2 ± 6.7
MØ	23.8 ± 3.9	21.2 ± 3.5
DC	4.5 ± 0.8	3.5 ± 1.6
Other cells	4.0 ± 2.7	3.7 ± 2.7

PL-MNCs were prepared from inflammatory cells isolated from asymptomatic and symptomatic periapical lesions (both n = 4), as described in the section methods. Cytospins were stained with May-Grünwald Giemsa and analysed by light microscopy. The percentages of cell subsets were determined based on morphological criteria after calculation of 500 cells on each cytospin. Values are given as mean ± standard deviation (SD) (n = 4). No statistical significant differences in any cell subset were observed among the groups. GR – granulocytes; LY – lymphocytes; PC – plasma cells; MØ – macrophages; DC – dendritic cells; as – asymptomatic; sy – symptomatic.

Namely, both MSC lines inhibited the production of proinflammatory cytokines (IL-1 β and TNF- α) by as- and syPL-MNCs (Figures 1 and 2). In contrast, IL-8 was down-regulated only in the co-culture of PL-MSC lines with syPL-MNCs (Figure 3).

The PL-MSC lines were up-regulated the production of immunoregulatory cytokines by asPL-MNCs (IL-10 and TGF- β) and syPL-MNCs (TGF- β) (Figures 4 and 5). However, the levels of IL-10 in the co-cultures between PL-MSC lines and syPL-MNCs were not significantly modulated.

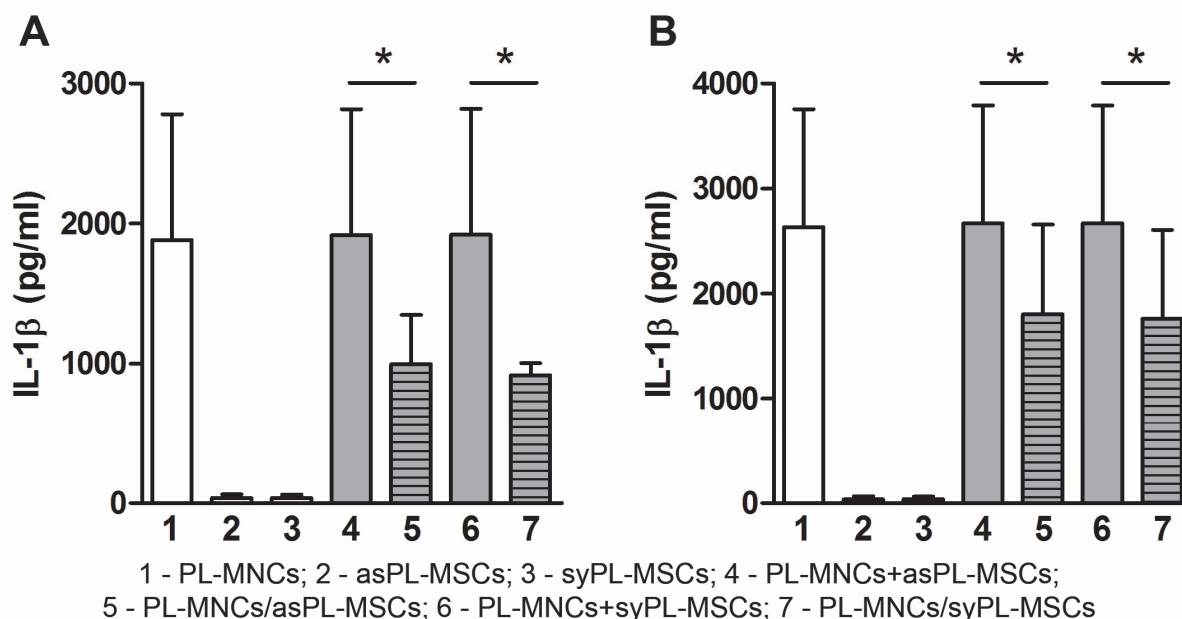


Fig. 1 – The effect of PL-MSCs on the production of IL-1 β by PL-MNCs in co-culture. PL-MSCs were co-cultivated with 4 different allogenic PL-MNCs for 48 h in direct co-culture system (PL-MSCs: PL-ICs cell ratio 1 : 10). The separate cultures of PL-MSCs and PL-MNCs (controls) were treated identically as co-cultures. A) Co-culture of PL-MSCs with asPL-MNCs. B) Co-culture PL-MSCs with syPL-MNCs. Values are given as mean ± SD (n = 4). *p < 0.05 compared to the sum of cytokines in the separate control cultures (PL-MNCs + PL-MSCs) (Mann-Whitney's test). PL – periapical lesion; MSCs – mononuclear cells; sy – symptomatic; as – asymptomatic.

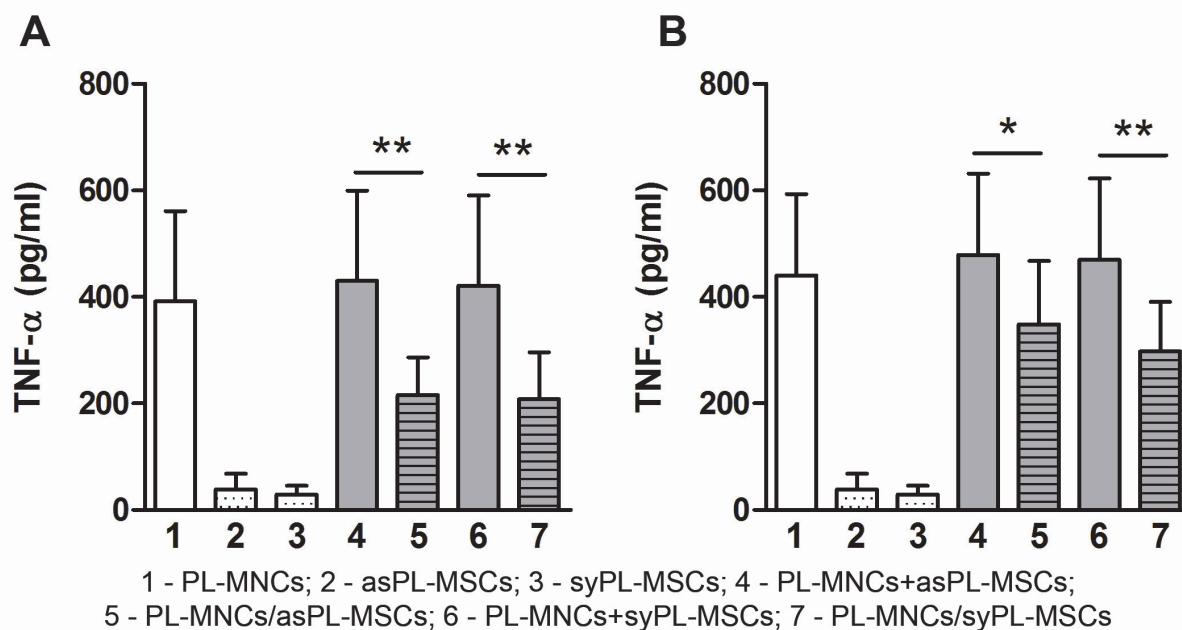


Fig. 2 – The effect of PL-MSCs on the production of TNF- α by PL-MNCs in co-culture. PL-MSCs were co-cultivated with 4 different allogenic PL-MNCs for 48 h in direct co-culture system (PL-MSCs: PL-ICs cell ratio 1 : 10). The separate cultures of PL-MSCs and PL-MNCs (controls) were treated identically as co-cultures. A) Co-culture of PL-MSCs with asPL-MNCs.

B) Co-culture PL-MSCs with syPL-MNCs.

Values are given as mean \pm SD (n = 4). * p < 0.05, ** p < 0.01 compared to the sum of cytokines in the separate control cultures (PL-MNCs + PL-MSCs) (Mann-Whitney's test).

PL – periapical lesion; MSCs – mononuclear cells; sy – symptomatic; as – asymptomatic.

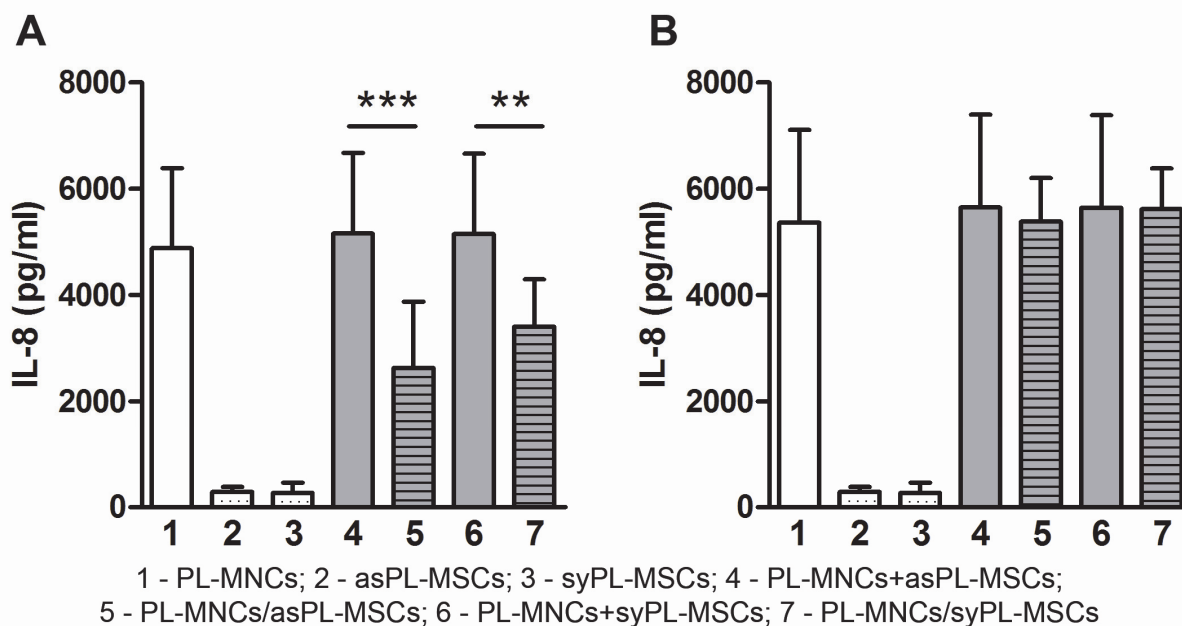


Fig. 3 – The effect of PL-MSCs on the production of IL-8 by PL-MNCs in co-culture. PL-PL-MSCs were co-cultivated with 4 different allogenic PL-MNCs for 48 h in direct co-culture system (PL-MSCs: PL-ICs cell ratio 1 : 10). The separate cultures of PL-MSCs and PL-MNCs (controls) were treated identically as co-cultures. A) Co-culture of PL-MSCs with asPL-MNCs.

B) Co-culture PL-MSCs with syPL-MNCs.

Values are given as mean \pm SD (n = 4). ** p < 0.01, *** p < 0.001 compared to the sum of cytokines in the separate control cultures (PL-MNCs + PL-MSCs) (Mann-Whitney's test).

sy – symptomatic; as – asymptomatic.

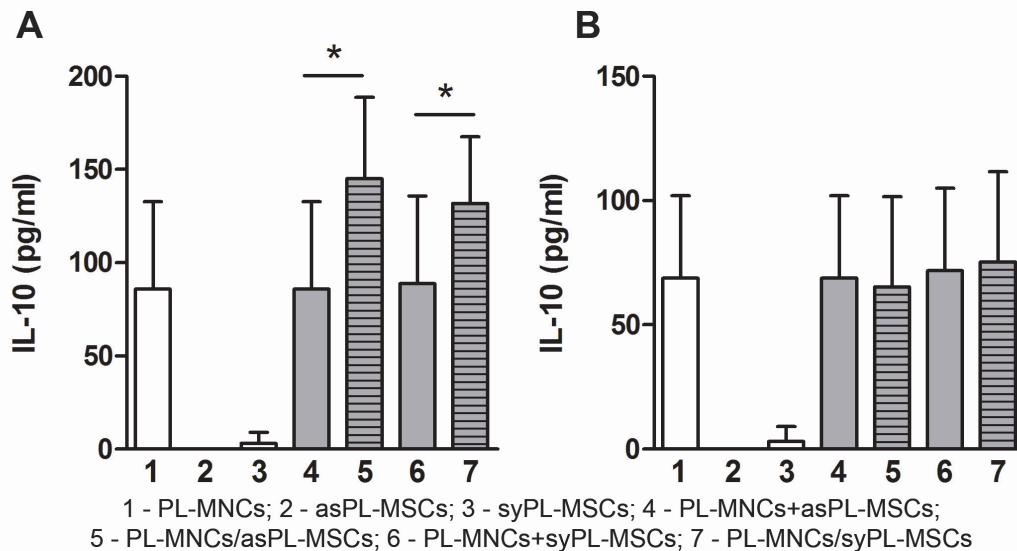


Fig. 4 – The effect of PL-MSCs on the production of IL-10 by PL-MNCs in co-culture. PL-MSCs were co-cultivated with 4 different allogenic PL-MNCs for 48 h in direct co-culture system (PL-MSCs: PL-ICs cell ratio 1 : 10). The separate cultures of PL-MSCs and PL-MNCs (controls) were treated identically as co-cultures. A) Co-culture of PL-MSCs with asPL-MNCs.

B) Co-culture PL-MSCs with syPL-MNCs.

Values are given as mean \pm SD (n = 4). * p < 0.05 compared to the sum of cytokines in the separate control cultures (PL-MNCs + PL-MSCs) (Mann-Whitney's test).

PL – periapical lesion; MSCs – mononuclear cells; sy – symptomatic; as – asymptomatic.

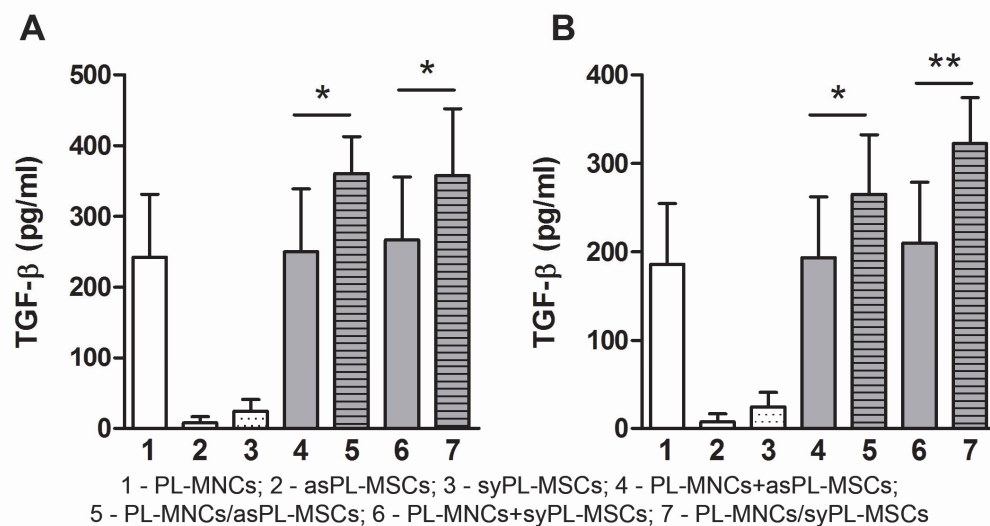


Fig. 5 – The effect of PL-MSCs on the production of TGF-β by PL-MNCs in co-culture. PL-MSCs were co-cultivated with 4 different allogenic PL-MNCs for 48 h in direct co-culture system (PL-MSCs: PL-ICs cell ratio 1 : 10). Separate cultures of PL-MSCs and PL-MNCs (controls) were treated identically as co-cultures. A) Co-culture of PL-MSCs with asPL-MNCs.

B) Co-culture PL-MSCs with syPL-MNCs.

Values are given as mean \pm SD (n = 4). * p < 0.05, ** p < 0.01 compared to the sum of cytokines in the separate control cultures (PL-MNCs + PL-MSCs) (Mann-Whitney's test).

PL – periapical lesion; MSCs – mononuclear cells; sy – symptomatic; as – asymptomatic.

Discussion

This study, together with our previous results^{9, 16, 18}, presents the rare publication dealing with characterization of MSCs from inflamed dental/periodontal tissues^{7, 8}. We showed that PL-MSC lines possess all properties similar to other MSCs, including typical phenotype¹⁹, capability to proliferate and form colonies *in vitro* as well as to differentiate into

osteoblasts, chondrocytes and adipocytes^{7, 9, 20}. The phenotypic analysis of new established PL-MSC lines demonstrated the expression of high levels of CD29, CD44, CD90, CD105 and CD166, moderate expression of CD46 and CD146 and low expression of STRO-1, regardless whether PLs were asymptomatic or symptomatic. This phenotype is similar to our previous finding with PL-MSCs, generated from several asPLs⁹.

It is known that PLs are heterogenous in terms of their cellular composition¹⁵, because PL development and persistence are a dynamic process in which many host and microbial factors play a role¹². We confirmed here that asPLs differ from syPLs by higher proportion of lymphocytes and plasma cells and lower percentages of granulocytes¹⁵. Such cellular composition is associated with the established chronicity, where anti-inflammatory mediators predominate (asPLs), or with the exacerbation of inflammation, followed by up-regulation of proinflammatory and osteo destructive mechanisms (syPLs). Due to these differences, we postulated that PL-MSCs from clinically different PLs may have different influence on PL-MNCs and that, due to the same reasons, MNCs from such PLs respond differently to the action of the PL-MSCs. Such hypothesis has not been tested so far. To prove this, we established a co-culture assay by incubating PL-MSC lines from different sources with as- and syPL-MNCs and measured cytokine production. The cultures were additionally treated with PMA/Ca²⁺ ionophore, as a recommended approach to enhance the cytokine production *in vitro*²¹. These MNCs had a very similar composition and this was the reason why they were used instead of total, very heterogenous, PL-ICs.

Some of examined cytokines were produced also by PL-MSCs, themselves, but their levels were significantly lower than in cultures with PL-MNCs due to much lower number of MSCs. Therefore, to avoid any mistake in conclusion, we compared the levels of cytokines in PL-MSCs/PL-MNCs co-culture with the sum of cytokines produced in separate cultures.

Generally, our results showed that both PL-MSC lines had similar modulatory activity on cytokine production, but the response of MNCs was different. Suppression of TNF- α and IL-1 β production was an expected phenomenon, shown already for other MSCs and it is in accordance with well-proven anti-inflammatory properties of MSCs^{22, 23}. This finding also suggests that local MSCs in PLs could control an excessive inflammation and, in this context, their clinical use may be beneficial for the restriction of inflammation-induced osteo destructive processes and the reparation of local tissues. It is known that both TNF- α and IL-1 β , through their action via specific receptors on infiltrating and stromal cells in PLs, stimulate inflammation in order to resolve infection. Additionally, the cytokines, indirectly by acting on the receptor activator of nuclear factor kappa-B (RANK)/RANK-ligand (L) signalling pathway, promote osteolytic processes, a hallmark of periodontal diseases, including apical periodontitis²⁴.

It seems that the regulation of IL-8 by PL-MNCs was different. Namely, although this chemokine was down-regulated by asPL-MNCs, similarly as we have already shown by total PL-ICs⁹, the production of IL-8 was not significantly changed by syPL-MNCs. It is known that IL-8 is a neutrophil recruiting chemokine²⁵ and its secretion is enhanced by IL-17²⁶. Our previous results^{15, 26} demonstrated that IL-17 is one of the key cytokines responsible for the exacerbation of inflammation in PLs. Furthermore, we showed that PL-MSCs were not able to down-modulate

significantly the production of IL-17 by both periapical blood MNCs and PL-ICs⁹, which could explain the absence of IL-8 down-regulation by syPL-MNCs observed in this study.

It is obvious that, although the composition of MNCs from syPLs and asPLs are similar, their functional state and subset content, especially within T cells¹⁵, is different. Such observations are also supported by the modulatory effect of PL-MSCs on the IL-10 production in the co-cultures. The production of this cytokine was augmented by asPL-MNCs but this was not the case with syPL-MNCs. It is well-known that IL-10, as a key immunoregulatory cytokine, suppresses the production of cytokines by T helper (Th)1, Th2 and Th17 cells as well as IL-6, IL-1 β , IL-8, IL-12 and many others²⁷. T regulatory cells (Tregs) are the main source of IL-10²⁷ and this finding was also confirmed for PLs in our previous papers²⁸. Since the counterbalance between Tregs and IL-17 exists^{28, 29}, it can be postulated that these interactions are less pronounced in syPLs. This is another fact supporting the hypothesis that the suppression of inflammation/immune response by PL-MSCs during the exacerbation of inflammation is not a desirable mechanism. Therefore, it will be of interest to know which factors control the down-modulatory functions of PL-MSCs under such conditions.

However, PL-MSCs were able to up-regulate another immunoregulatory cytokine, TGF- β , by both asPL-MNCs and syPL-MNCs. This cytokine is produced by leukocytes, macrophages, fibroblasts, osteoblasts and, most importantly Tregs³⁰. TGF- β stimulates migration of lymphocytes and monocytes to the inflammatory site, but inhibits their proliferation subsequently³¹. In addition, together with IL-10, it suppresses the production of different pro-inflammatory cytokines^{30, 31}. TGF- β is also expressed in PLs^{15, 32}, irrespective of clinical presentation of PLs, and represents a key down-modulator of inflammation and osteodestructive processes. In this context, it is not only that PL-MSCs produce TGF- β , but also stimulate its production by other cells in PLs.

Conclusion

Cumulatively, our results showed that PL-MSCs could contribute substantially to the restriction of local inflammation and immune response during chronic, asymptomatic course of PLs development by down-regulating the expression of pro-inflammatory cytokines and up-regulating immunoregulatory cytokines. It can be postulated that during exacerbation of inflammation within PLs, PL-MSCs allow the recruitment of granulocytes and maybe other cells, through the balance between IL-17/IL-10 axis and IL-8. However, the cells are still potent to control excessive inflammation through down-regulation of IL-1 β and TNF- α and up-regulation of TGF- β . Further studies are needed to understand how these processes are controlled.

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