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Original article

Production of chemokines by mesenchymal stromal cells established from inflamed human gingiva and its modulation by lipopolysaccharide

Marina Radanović[1], Miodrag Čolić[1,2]

[1]University of East Sarajevo, Faculty of Medicine Foča, Republic of Srpska, Bosnia and Herzegovina

[2]Serbian Academy of Sciences and Arts, Belgrade, Serbia

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Corresponding author:
Marina Radanović, PhD
Studentska 5, 73300 Foča
e-mail: marinamilinkovic7@gmail.com

Summary

Introduction. The role of gingiva-derived stromal cells (GMSCs) in chronic periodontitis is poorly understood. Since GMSCs modulate the inflammatory and immune responses this study aimed to investigate the production of chemokines by GMSCs established from chronic periodontitis tissue under basal conditions and after stimulation with lipopolysaccharide (LPS), which is a main trigger of gingivitis/periodontitis.

Methods. GMSCs, established from periodontitis-affected gingival tissue, were characterized by flow cytometry and their differentiation capability. GMSCs, from the 5th passage, were cultivated either alone or with LPS for 24 hours followed by the determination of chemokine levels in culture supernatants by a sandwich ELISA assay.

Results. More than 95% of GMSCs expressed all three (CD90, CD73, and CD105) MSC markers, with a relatively high potential to differentiate into osteoblasts and chondroblasts and a low adipogenic differentiation capability. Of all chemokines, the basal production of interleukin-8 (IL-8) (186.2 ± 23.4 pg/mL) and Monocyte Chemoattractant Protein-1 (MCP-1) (103.5 ± 12.7 pg/mL) was highest, followed by the levels of Growth-Related Oncogene (GRO)-α (16.2 ± 2.4 pg/mL) and Regulated Upon Activation, Normal T-cell Expressed and Secreted (RANTES) (10.3 ± 1.4 pg/mL). The basal production of Interferon (IFN)-γ-Induced Protein 10 kDa (IP-10) was undetectable. When stimulated with LPS, GMSCs significantly up-regulated the production of all chemokines. Their levels (pg/mL) were as follows: IL-8 – 926.4 ± 84.0; MCP-1 – 424.0 ± 33.2; GRO-α – 109.3 ± 12.2; RANTES – 154.9 ± 6.6; IP-10 – 211.6 ± 15.7.

Conclusion. GMSCs may promote or suppress periodontal inflammation depending on produced chemokines and their balance.

Key words: gingiva, mesenchymal stromal cells, periodontitis, chemokines

Introduction

Gingiva-derived mesenchymal stromal cells (GMSCs) represent a unique subpopulation of dental mesenchymal stromal cells (MSC) with stem cell characteristics [1]. Similar to MSCs derived from other organs such as bone marrow, adipose tissue, umbilical cord, or other dental tissues like human dental pulp tissues (DPSC), apical papilla (SCAP), dental follicle (DFSC), exfoliated

deciduous teeth (SHED), periodontal ligament (PDLSC), and tooth germ stem cells (TGSC), GMSCs exhibit potent self-renewal, multipotent differentiation, immunomodulatory and anti-inflammatory properties [1, 2]. GMSCs, including other dental MSCs, are heterogeneous in their phenotypic and functional characteristics. This was the reason why the International Society of Cell & Gene Therapy (ISCT) defined human MSCs based on three minimal criteria, such as adherence to a plastic substrate, minimal expression of at least three markers (CD73, CD90, and CD105 but negative for hematopoietic cell markers) and the trilineage differentiation potentials (osteogenic, adipogenic, and chondrogenic) [3]. GMSCs possess a higher proliferative rate in culture than other MSCs and have additional potential to differentiate into neural cell lineages due to their neural crest origin [1, 2, 4].

GMSCs act on other cells in the gingival tissue by direct contact with multiple receptor-ligand interactions or indirectly with soluble products containing different biologically active components, including cytokines, prostaglandins, microRNA (miRNA), and exosomes. GMSC-derived exosomes possess biological functions and therapeutic potential similar to GMSCs, thus representing a promising tool for regenerative therapy, acting as a cell-free platform [5]. The therapeutic potential of GMSCs has been tested in many preclinical models, mostly in regenerative dentistry, including repairing periodontal tissue caused by chronic periodontitis [1, 6–8]. Similar effects were obtained in periodontitis models in rats [9] and mice [10] when exosomes or conditioned medium from GMSCs were used as a local treatment. The beneficial roles of GMSCs during periodontitis include not only the capability to repair hard and soft periodontal tissue but also immunomodulation, stimulation of angiogenesis, and anti-bacterial effects [1, 11].

The tissue destruction caused by periodontitis is triggered dominantly by periopathogenic bacteria originating from the dental plaque. The key mechanisms involved in these complex processes are associated with overactivated immune responses and subsequent promotion of inflammation due to the inability of the host to eradicate infection. One of the main triggers of the immune response is lipopolysaccharide (LPS) originating from Porphyromonas gingivalis, a dominant bacterial strain associated with chronic periodontitis [12, 13]. LPS is a well-known stimulator of Toll-like receptor-4 (TLR4) expressed on many immune cells, including MSCs [14]. However, once triggered, the inflammatory response is difficult to stop. Constant stimulation of the immune system not only influences the local destruction of periodontal ligament and alveolar osteolysis but also impacts systemic health [12, 13, 15]. Therefore, understanding the mechanisms controlling these processes is important for new therapeutic options for chronic periodontitis.

Our previous paper presented the results of GMSCs established from periodontitis-affected gingiva [16]. Although we showed certain heterogeneity of GMSC lines of different donors, all of them fulfilled already defined criteria for cells to be characterized as mesenchymal stromal cells. We showed that such GMSCs differed from those of healthy gingiva based on the expression of many genes involved in inflammation and immunomodulation. However, the relationship between the gene expressions and the expression of their products at the protein levels was not investigated, and it was the primary aim of this study. We have focused on pro-inflammatory mediators, especially chemokines because they are potent stimulators for the recruitment of different cells from blood circulation to inflamed periodontium. Interleukin-8 (IL-8) and Growth-Related Oncogene (GRO)-α are potent mobilizators for circulating neutrophils to the inflamed/injured tissue [17]. Monocyte Chemoattractant Protein-1 (MCP-1) is a chemoattractant for monocytes rapidly transforming into tissue macrophages [18]. Regulated Upon Activation, Normal

T-cell Expressed and Secreted (RANTES) and Interferon (IFN)-γ-Induced Protein 10 kDa (IP-10) are dominantly chemokines for subsets of T cells [19, 20]. However, most of these biomolecules are involved in many other immune functions [21]. Upon migration to the inflamed tissue, immune cells orchestrate all immune/inflammatory steps characteristic of chronic periodontitis. The role of GMSCs in these processes is not sufficiently known [22]. One approach to better mimic the inflammatory milieu present in chronic periodontitis is treating GMSCs with LPS. Therefore, the concrete aim of this study was to check the production of chemokines by GMSCs in culture under basal conditions and the modulation of their production by LPS.

Methods

Establishment of GMSCs

GMSCs were established from the inflamed gingival tissue in the Centre for Biomedical Sciences, Medical Faculty Foča (MFF), University of East Sarajevo (UES), Republic of Srpska, Bosnia and Herzegovina. The donor, male, 51 years old, non-smoker, without systemic diseases (except for hypertension), had chronic slow progressive periodontitis (grade A) according to the American Academy of Periodontology (AAP) Classification of Disease. Staging and gradation of the disease were performed according to the Consensus report of workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions [1, 23]. The patient had clinical attachment loss (CAL) of 4 mm at the site of greatest loss and a mean maximum probing depth of 4.6 mm. The gingival tissue was obtained during the flap debridement procedure.

The excised tissue was washed in phosphate-buffered saline (PBS), followed by the removal of the epithelial cell layer by a scalpel. The tissue was then minced with scissors and digested with collagenase type II (5 μg/mL) and DNAse (40 IU/mL) in serum-free α-MEM medium. All these components were obtained from Sigma-Aldrich, Darmstadt, Germany. The digestion lasted for two hours at 37°C and 5% CO2 in a cell incubator. The softened tissue was then gently pressed through a 30-40 μm nylon mesh and washed with α-MEM medium by centrifugation (1800 g/10 min). The released cells were counted and placed in 24-well cell culture dishes (Sarstedt, Numbrecht, Germany) at a density of 2,500/cm2 and cultured in a complete MSC medium, containing α-MEM, 10% fetal calf serum (FCS), 100 μM L-ascorbate-2-phosphate and standard antibiotics for cell cultures (all from Sigma-Aldrich). The cultivation of cells until reaching confluence lasted about two weeks. The medium was changed every three days. The propagation of cells in culture was up to seven passages, as described [16]. The morphology of cells was observed under an inverted microscope (Olympus, Hamburg, Germany). GMSCs were seeded at low density (10 cells/well of 96-well plates) and cultivated for 10 days to examine colony-forming potential. The cells were fixed with 4% paraformaldehyde, stained with hematoxylin and eosin, and analyzed under the inverted microscope (Olympus).

In Vitro Differentiation of GMSCs

Osteogenic, adipogenic, and chondrogenic differentiation of GMSCs was tested by incubating the cells with the complete α-MEM medium with corresponding supplements (all from Sigma-Aldrich). The detailed procedure was published in our previous publication (15). The protocol for osteogenic and adipogenic differentiation of GMSCs included cell cultivation on plastic coverslips placed in wells of a 24-well plate, whereas chondrogenic differentiation was performed in an Eppendorf tube, volume 2 mL (Sarstedt) where the cells were grown in a pelleted form. The cultivation of GMSCs in all three induction

media lasted for 21 days. The replacement of the culture medium with the fresh medium was every 3–4 days. The osteogenic and adipogenic differentiation was detected by staining the cultures with 2% Alizarin Red and 0.3% Oil Red O, respectively. Cryostat sections of pelleted chondroblast cultures were air-dried, fixed with 4% paraformaldehyde, and stained with Alcian blue, followed by counterstaining of nuclei with 0.1% Nuclear Fast Red solution. All dies were from Sigma-Aldrich. Negative controls for all differentiation procedures were GMSCs cultured in the complete basal α-MEM medium. The stained cells/sections were observed under a light optical microscope (Olympus). All images were analyzed offline by ImageJ software (National Institutes of Health, Bethesda, MD, USA). The differentiation potential of GMSCs was quantified semiquantitatively according to previously defined indices [16].

Flow Cytometry

Phenotypic analysis of GMSCs was performed by flow cytometry using monoclonal antibodies (mAbs) specific for markers of human MSCs after 3rd and 6th passages. The mAbs included anti-CD90-PE (5E10), anti-CD73-biotin (AD2), and anti-CD105-APC (43A3), all obtained from BioLegend, Basel, Switzerland. To exclude the contamination with hematopoietic cells, anti-CD14-FITC (63D3) (BioLegend), anti-CD34-FITC (581) (Elabscience, Wuhan, China), and anti-CD45-APC (HI30) (BioLegend) were used. Negative controls were isotype-matched mAbs conjugated with corresponding fluorochromes (BioLegend). GMSCs (1 x105/tube) were incubated with mAbs, appropriately diluted in 2% FCS/0.01% NaN3 in PBS, for 30 min at 4°C. The analysis of labeled cells was performed on an Attune flow cytometer (Thermo Fisher Scientific, Dreieich, Germany). The doublets of cells were excluded according to the appropriate forward scatter (FSC)-A/FSC-H profile. More than 5000 gated cells were analyzed according to the FSC-A/side-scatter (SSC)-A plots. Signal overlaps between the channels were compensated using single labeled cells. Non-specific fluorescence was determined using isotype controls. The flow cytometric data were analyzed offline in the FlowJoVX program (BD Biosciences, Franklin Lakes, NJ, USA). The results are expressed as the percentage of positively labeled cells.

Cell cultures and chemokine measurement

GMSCs (0.5x105) were cultured in an incubator (37°C and 5% CO2) in 24-well plates (triplicates) with or without E. coli LPS (100 ng/mL) (Sigma-Aldrich) for 24 hours. The medium was the complete α-MEM. After that, supernatants were collected and frozen at -80°C until the levels of chemokines were determined. The LEGEND MAX pre-coated ELISA kits for human IL-8, MCP-1, RANTES, and IP-10 were obtained from Biolegend (Basel, Switzerland), whereas the Quantikine ELISA kit for GRO-α was purchased from R&D Systems (Minneapolis, MN). The chemokines in culture supernatants were detected by ELISA following the manufacturer's instructions. The duplicates of each triplicate were assayed. The chemokine concentrations were determined according to standard curves constructed based on known concentrations of chemokines.

Statistics

To assess differences in chemokine concentrations between control and LPS-stimulated GMSC cultures, the Mann–Whitney test was used. Values at $p < 0.05$ were considered to be statistically significant. The statistical analysis and graphs were performed in GraphPad Prism version 8.0.0 (GraphPad Software, San Diego, CA, USA).

Results

Characterization of GMSCs

GMSCs showed typical fibroblastoid morphology capable of forming colonies when plated at low density (Figure 1).

When induced to differentiate, GMSCs transformed into osteoblasts and chondroblasts (high potential), and adipocytes (low potential) (Figure 2). The mean indices (mean ± SD of 6 different image analyses) were: 4.2 ± 0.3 (osteoblasts), 3.6 ± 0.3 (chondroblasts) and 1.1 ± 0.2 (adipocytes).

Flow cytometry showed the positivity of GMSCs over 95% to CD95, CD73, and CD105. The percentages of hematopoietic markers (CD45, CD14, and CD34) were below 2%, regardless of the number of passages (Table 1).

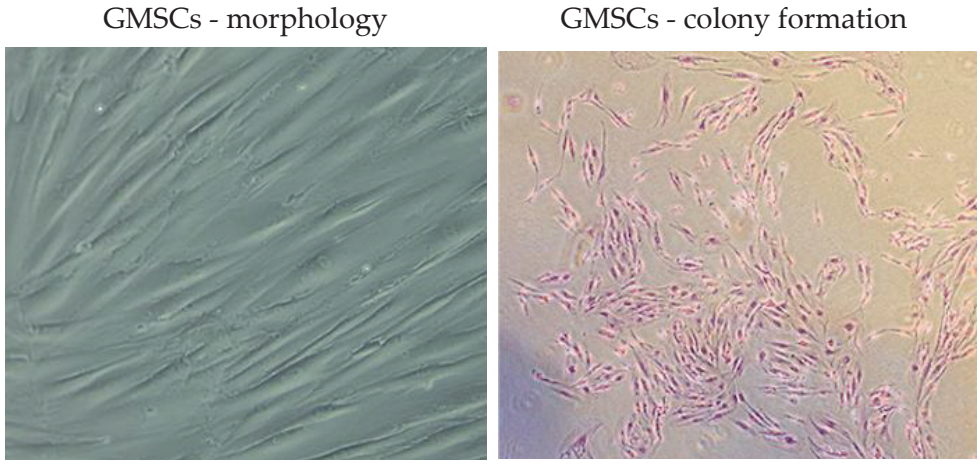

Figure 1. Fibroblastoid morphology and colony formation of GMSCs in culture. Magnification: x 400 (left); x 100 (right). The pictures were taken from the PhD thesis of Marina Radanović (UIS, MFF, 2023).

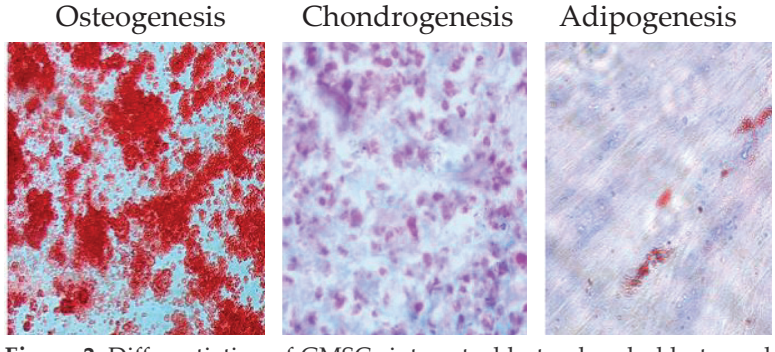

Figure 2. Differentiation of GMSCs into osteoblasts, chondroblasts, and adipocytes. Magnifications: x 100 (osteogenesis); x 200 (chondrogenesis and adipogenesis). The pictures were taken from the PhD thesis of Marina Radanović (UIS, MFF, 2023).

Table 1. The expression of MSC and hematopoietic markers on GMSCs

Passages (number)	Markers (% expression)					
	CD90	CD73	CD105	CD45	CD14	CD34
3	97.30	98.24	96.64	1.45	1.32	0.98
6	96.00	99.22	95.80	1.12	1.08	1.22

Results present the percentages of positive cells to a given marker studied on GMSCs after 3rd and 6th passages in culture.

Table 2. The levels of chemokines in supernatants of control and LPS-treated GMSC cultures

	IL-8	MCP-1	GRO-α	RANTES	IP-10
Control	186.2 ± 23.4	103.5 ± 12.7	16.2 ± 2.4	10.3 ± 1.4	ND
LPS	926.4 ± 84.0	424.0 ± 33.2	109.3 ± 12.2	154.9 ± 6.6	211.6 ± 15.7

LPS (100 ng/mL) was incubated with GMSCs of the 5th passage for 24 hours. Results are presented as the mean values (ng/mL) of triplicates ± standard deviation of a given chemokine. ND - Non Detectable

Production of chemokines by GMSCs with and without LPS

The main aim of this study was to examine the production of five chemokines by GMSCs in culture and its modulation by LPS. As presented in table 2, GMSCs produced low levels of IL-8, MCP-1, GRO-α, and RANTES, whereas the level of IP-10 was below the detection limit.

The treatment of GMSCs with LPS resulted in significant up-regulation of all cytokines. The differences in chemokine levels between control and LPS-treated cultures were statistically significant ($p < 0.005$).

Discussion

GMSCs, like other dental MSCs, have been shown to possess potent regenerative and therapeutic potentials, as concluded from different preclinical models of human diseases [1]. One of these is periodontitis, a dental plaque-induced chronic inflammatory disease affecting periodontal tissue. The therapy with GMSCs for periodontal tissue regeneration includes the direct application of cells in the form of cellular therapy or the application of their secretory products and exosomes [5]. However, to better understand the function of GMSCs in these processes, it is necessary to investigate their functions under inflammatory conditions. This was the reason why we isolated and propagated GMSCs from periodontitis-affected gingiva to mimic inflammatory conditions by treating the cells with LPS.

Our previous paper [22] showed that LPS enhanced the expression of several genes in GMSCs involved in inflammation, immunomodulation, tissue destruction/remodeling, and osteogenesis. This study aimed to extend previous results on protein expression, particularly on chemokines. These cytokines are not only responsible for the mobilization of leucocytes but also participate in different inflammatory and immune mechanisms.

As mentioned in the Introduction of this paper, dental tissues, including gingiva, are a source of different types of MSCs. Gingiva is a specific keratinized mucosal tissue and a key component of the periodontium. Besides its supporting masticatory functions, rapid wound healing with minimal scar formation makes gingiva unique among other tissues [1, 2, 5]. The other specificity is the dual origin of gingiva from both the neural crest and the mesenchyme [1, 5]. Due to the easy accessibility of the gingiva through minimally invasive surgical techniques, GMSCs are recognized as promising therapeutic potential. However, the initial step involves proving the ability of MSCs to differentiate into at least three lines of mesenchymal cells along with the expression of typical markers of MSCs [3]. In this context, we confirmed the MSC characteristics of our gingival cells based on the positivity over 95% of CD90, CD73, and CD105 and their capability to differentiate into osteoblasts, chondroblasts, and adipocytes. The adipogenesis was the lowest, which agrees with another study [24]. However, considerable variability in the differentiation potential of GMSCs isolated from healthy versus inflamed gingiva was published [16, 25, 26], suggesting that tissue microenvironmental factors and culture conditions could be relevant.

The main aim of our study was to check the production of chemokines by GMSCs in culture under basal conditions and after treatment with LPS. It has been shown that MSCs are potent producers of different chemokines of both the CCL and CXCL families [27]. Chemokines are a family of structurally related peptides (7,5–12,5 kDa) having dominantly chemoattractive properties [21]. They are produced and secreted by various cell types as a response to pro-inflammatory stimuli [27]. By mobilizing different types of immune cells (neutrophils, monocytes, lymphocytes, eosinophils, dendritic cells, and other effector cells) to the site of infection or tissue damage, chemokines play important roles in inflammation, immunoregulation, cell differentiation, and angiogenesis [21].

LPS, which is a trigger of the inflammatory response during periodontitis [28], is one of the most potent activators of MSCs [29]. According to the concept of dual polarization property of MSCs, stimulation of TLR4 by LPS primes MSCs to the pro-inflammatory profile (MSC1 type). In contrast, stimulation of MSCs with TLR3 agonists promotes the development of the anti-inflammatory (MSC2 type) profile. Such plasticity of MSCs may explain their contradictory roles in inflammation. The key dilemma that still attracts researchers is whether LPS-primed MSCs retain or enhance their immunosuppressive capabilities, bearing in mind that LPS-stimulated MSCs can up-regulate the expression of indoleamine 2,3-dioxygenase (IDO1) and other immunosuppressive molecules, such as prostaglandin E2 (PGE2), tumor necrosis factor-alpha stimulating gene-6 (TSG-6), programmed death ligand (PD-L1), PD-L2, and transforming growth factor -β (TGF-β) [29, 30]. In this context, analyzing chemokine production with different functions in inflammation and immunity may be helpful [31].

The first step in the promotion of inflammation, which is also characteristic of periodontitis, involves the mobilization of neutrophils at the inflammatory site. Several chemokines participate in these processes such as IL-8, GRO-α, and macrophage migration inhibitory factor (MIF). Many resident cells, such as macrophages, endothelial cells, and MSCs are involved in the production of these chemokines and other cytokines like IL-6 and granulocyte-macrophage colony-stimulating factor (GM-CSF) [29, 32, 33].

We showed that basal production of IL-8 and GRO-α by GMSCs was significantly enhanced by LPS. IL-8, also known as CXCL8, and GRO-α, also known as CXCL1, are potent chemoattractants for neutrophils and activators of other cells participating in the inflammatory/immune response [17]. IL-8 is involved in the stimulation of cell proliferation, inflammation, neutrophil degranulation, migration of basophils and T cells, leukocyte activation, calcium homeostasis, and MSC migration. However, IL-8 promotes the growth of several types of cancer [34]. It is known that MSCs constitutively secrete GRO chemokines including GRO-α, which in turn regulate different functions of MSCs through binding to their cell-surface receptor, CXCR2. Besides, GRO production from MSCs can be significantly increased by the stimulation of IL-1β and TNF-α [35]. Both chemokines are involved in angiogenesis (stimulation of new blood vessel formation) which is an important pathophysiological mechanism in chronic periodontitis [17, 35].

MCP-1 (CCL2) is a chemokine playing a significant role in the recruitment of monocytes and their activation when transformed into macrophages. Therefore, MCP-1 is considered a pro-inflammatory chemokine participating in the early stages of the inflammatory response [35]. MCP-1 is also an angiogenic biomolecule. In addition, the chemokine modulates the T cell immune response, inducing a switch from the undifferentiated T helper (Th) response to Th2 with the predominant secretion of IL-4, IL-5, and IL-13 [27, 36]. Similarly to IL-8 and GRO-α, the basal production of MCP-1 is low but was significantly enhanced by LPS.

Based on these findings, our results support the hypothesis that LPS-stimulated GMSCs may have a beneficial proinflammatory role in the early phase of inflammation and thus help to develop a proper immune response [37].

The function of the next two chemokines is dominantly associated with T-cell-mediated immunity. RANTES (CCL5) was discovered as a biomolecule secreted by activated T cells with chemotactic activity to lymphocytes, monocytes, and dendritic cells. Later, this chemokine was shown to be a potent activator of leucocytes, angiogenesis promoter, and wound-healing supporter [27, 38]. RANTES suppresses the T-cell response by inducing cell apoptosis through the Bcl-2-dependent, caspase-independent pathway. In addition, RANTES is involved in developing CD8+ FoxP3+ T regulatory cells and inhibiting memory Th1 cell development [27]. It has been shown that MCP-1 and RANTES stimulate the migration of MSCs to sites of tissue damage in an autocrine manner, and this function may be relevant for the migration of GMSCs during chronic periodontitis [27, 33, 38]. We showed low basal production of RANTES by GMSCs, and its production was significantly enhanced by LPS. These results agree with our previous findings at the level of mRNA expression.

IP-10, also known as CXCL10, is a chemokine playing the significant role in the immune system, particularly in inflammation and the immune response to infections. IP-10, which is produced in response to IFN-γ, acts as a chemoattractant for various immune cells including T cells. IP-10 is a versatile chemokine involved in various aspects of the immune response, including activation of T cells, inflammation, antiviral defense, and suppression of angiogenesis. Its regulation is critical for maintaining a balanced and effective immune response. Dysregulation of IP-10 has been implicated in various diseases, including autoimmune disorders and chronic inflammatory conditions [39]. The studies related to the modulation of IP-10 in MSCs are relatively scarce. One paper published by Vereb et al., 2020, [40] compared the phenotypical, differentiation, and functional capabilities of MSCs from bone marrow (BM) and saphenous vein (SV). They showed that both types of MSCs secreted IP-10 chemokine upon TLR stimulation (LPS or PolyI: C) and cytokine receptor ligation (IFN-γ). However, the response of SV-MSCs was more pronounced especially when TLR agonists were used. Similarly, as in our study, basal production of this chemokine was under the limit of detection.

Conclusion

In this study, GMSCs, isolated from periodontitis-affected gingiva, were additionally treated with LPS to enhance the inflammatory response. The obtained results showed that LPS was a potent stimulator of chemokine production by GMSCs with the capability to attract various cells of innate and adaptive immunity. The findings suggest that GMSCs may have a pro-inflammatory role, at least in the early stage of periodontitis. However, at the same time, their immunosuppressive properties, in which subsets of T cells play a crucial role, can be enhanced. In addition, GMSCs could act in an autocrine manner to mobilize new MSCs to the inflamed gingiva and periodontium. Each of these opposite functions deserves to be studied in the future using appropriate in vitro models.

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Ethical approval. The Ethics Committee of the University of East Sarajevo, Faculty of Medicine Foča, Republic of Srpska, Bosnia and Herzegovina, approved the study and informed consent was obtained from all individual respondents. The research was conducted according to the Declaration of Helsinki.

Conflicts of interest. The authors declare no conflict of interest.

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Produkcija hemokina od strane mezenhimalnih stromalnih ćelija uspostavljenih iz inflamirane humane gingive i njena modulacija lipoplisaharidom

Marina Radanović[1], Miodrag Čolić[1,2]

[1]Univerzitet Istočno Sarajevo, Medicinski fakultet Foča, Republika Srpska, Bosna i Hercegovina

[2]Srpska akademija nauka i umetnosti, Beograd, Srbija

Uvod. Uloga mezenhimalnih stromalnih ćelija iz gingive (GMSCs) u hroničnom periodontitisu je nedovoljno poznata. Pošto GMSCs modulišu inflamatorne i imunološke odgovore, ova studija je imala za cilj izučavanje produkcije hemokina od strane GMSCs uspostavljenih iz tkiva zahvaćenog hroničnim periodontitisom u bazalnim uslovima u kulturi, kao i nakon njihove stimulacije lipopolisaharidom (LPS), koji je glavni stimulus patogeneze gingivitisa/periodontitisa.

Metode. GMSCs, uspostavljene iz tkiva zahvaćenog periodontitisom su okarakterisane pomoću protočne citometrije i njihove sposobnosti diferencijacije. GMSCs, iz pete pasaže, su kultivisane sa ili bez LPS-a tokom 24 sata, nakon čega su nivoi hemokina u supernatantima kultura određivani pomoću ELISA testa.

Rezultati. Više od 95% GMSCs je ispoljavalo sva tri markera MSC (CD90, CD73 i CD105) sa relativno visokim potencijalom za diferencijaciju u osteoblaste i hondroblaste, i malom sposobnošću za adipogenezu. Od svih hemokina, bazalna produkcija interleukina-8 (IL-8) (186,2 ± 23,4 pg/mL) i monocitnog hemotaktičkog proteina-1 (MCP-1) (103,5 ± 12,7 pg/mL) je bila najveća, zatim Growth-Related Oncogene (GRO)-α (16,2 ± 2,4 pg/mL) i Regulated Upon Activation, Normal T-cell Expressed and Secreted (RANTES) (10,3 ± 1,4 pg/mL). Nasuprot tome, bazalna produkcija interferona (IFN)-γ-Induced Protein 10 kDa (IP-10) bila je neprenosiva. Nakon stimulacije LPS-om, produkcija svih citokina od strane GMSCs je bila značajno povećana. Njihovi nivoi (pg/mL) bili su sljedeći: IL-8 – 926,4 ± 84,0; MCP-1 – 424,0 ± 33,2; GRO-α – 109,3 ± 12,2; RANTES – 154,9 ± 6,6; IP-10 – 211,6 ± 15,7.

Zaključak. GMSCs mogu da stimulišu ili zaustavljaju periodontalnu inflamaciju u zavisnosti od produkovanih citokina i njihovog međusobnog balansa.

Ključne riječi: gingiva, mezenhimalne stromalne ćelije, periodontitis, hemokini